

**WESTERN SECTION**  
**AMERICAN SOCIETY OF ANIMAL SCIENCE**  
**COMMITTEE APPOINTMENTS (2003-2004)**

**\* Denotes Committee Chair**

**EXECUTIVE**

1. J.C. Whittier\*, President (05, Colorado State Univ.)
2. E.E. Grings, Pres Elect (06 USDA-ARS, Miles City, Montana)
3. Jim Thompson, Sec-Treas. (07 Oregon State Univ.)
4. P.G. Hatfield, Past-President (04, Montana State Univ.)
5. J.J. Reeves, ASAS Board Direct. (04, Washington State Univ.)
6. B.L. Christensen (05, Alltech)
7. C.P. Mathis (05, New Mexico State Univ.)

**AWARDS**

1. E.E. Grings\* (04, USDA-ARS, Miles City, Montana)
2. R. P. Ansotegui (05, Montana State Univ.)
3. D. E. Hawkins, (05, New Mexico State Univ.)
4. T. DelCurto, (06, Oregon State Univ.)
5. M.K. Petersen (06, New Mexico State Univ.)

**ADVISORY & COORDINATING**

1. C.P. Mathis\* (05, New Mexico State Univ.)
2. R.A. Battaglia (04, Univ. Idaho)
3. C.T. Gaskins (04, Washington State Univ.)
4. T.G. Field (04, Colorado State Univ.)
5. S.B. LeValley (04, Colorado State Univ.)
6. L.H. Baumgard (04, Univ. Arizona)
7. C.J. Ackerman (04, Oregon State Univ.)
8. G.E. Moss (04, Univ. Wyoming)
9. R.D. Sainz (04, Univ. California Davis)
10. F.M. Mitloehner, (05, Univ. Calif. Davis)
11. G.E. Sides, (05, Pfizer)
12. J.B. Taylor (05, USDA-ARS, Dubois, ID)
13. K. Olson (05, Utah State Univ.)
14. J.M. Rumph (06, Montana State Univ.)
15. S. J. Filley, (06, Oregon State Univ.)

**PAPER COMPETITION**

1. P. A. Ludden\* (06, Univ. of Wyoming)
2. R.W. Silcox (04, Brigham Young Univ.)
3. M.E. Wise (04, New Mexico State Univ.)
4. J. Berardinelli (05, Montana State Univ.)
5. D. McLean (05, Washington State Univ.)
6. C.A. Loest (05, New Mexico State Univ.)
7. D.H. Crews (06, Lethbridge, Canada)
8. R.M. Enns (06, Colorado State Univ.)

**ACADEMIC QUADRATHLON**

1. L.M. Surber\* (Montana State Univ.)
2. D.C. Rule (Univ. Wyoming)
3. J.B. Lamb (BYU-Idaho)
4. R. Townsend (Univ. of Wyoming)
5. C.A. Loest (New Mexico State Univ.)
6. C.W. Hunt (Univ. Idaho)
7. N.A. Irlbeck (Colorado State Univ.)
8. W.E. Plummer (Cal Poly State Univ.)
9. S. Wickler (Cal Poly, Pomona)
10. R.D. Wiedmeier (Utah State Univ.)
11. P.D. French (Oregon State Univ.)

**EXTENSION**

1. J.B. Glaze\* (03-04, Univ. Idaho)
2. J.M. Harper (04, Univ. California Coop. Exten.)
3. S.I. Paisley (04, Univ. Wyoming)
4. R.M. Kattnig (04, Univ. Arizona)
5. J.A. Scanga (05, Colorado State Univ.)
6. W.F. Gipp (05, Montana State Univ.)
7. T. Patterson (05, South Dakota State University)
8. D. Zobell (06, Utah State Univ.)
9. R. Zinn (06, Univ. California, Davis)

**NECROLOGY**

- J. W. Oltjen\* (03, Univ. California, Davis)

**NOMINATING**

1. P.G. Hatfield\* (04, Montana State Univ.)
2. J.A. Paterson (04, Montana State Univ.)
3. B.L. Christensen (04, Alltech)

**SYMPOSIUM**

1. G.D. Pulsipher\* (03-04, Oregon State Univ.)
2. K.E. Belk (04, Colorado State Univ.)
3. J.G.P. Bowman (04, Montana State Univ.)
4. P.D. Burns (05, Colorado State Univ.)
5. T.W. Geary (05, USDA-ARS, Miles City, MT)
6. J.E. Sawyer (05, New Mexico State Univ.)

**Minutes of the Western Section of the  
American Society of Animal Science  
Business Meeting**

June 25, 2003  
Wyndam Hotel Ballroom  
Phoenix, Arizona

President Pat Hatfield called the meeting to order at 1:50 p.m.

**Acceptance of the minutes of the 2002 business and awards meeting**

The minutes of the 2002 business meeting were approved as printed in the 2003 Proceedings of the Western Section of the American Society of Animal Science.

**Committee Reports  
Necrology**

John Paterson  
James Meyers  
Wade Rollins  
Clifton Blincoe

**Nominating**

Jim Oltjen, Chair

Nominations for the 2003 WSASAS elections were as follows:

President-Elect: Elaine Grings, USDA/ARS, Miles City, Montana

Secretary-Treasurer: James Thompson, Oregon State University

Industry Representative: Bret Christensen, Alltech

All three candidates were elected.

**Symposium Committees**

Neither the Beef nor Extension Symposia were held in 2003 while WSASAS meeting was held in conjunction with ASAS and ADSA. Both symposia will be held in 2004.

**Advisory and Coordinating Committee Report**

Tim Ross, Chair

***Proposed Constitutional Amendments***

Motion: Change the term of office for the chair of the Advisory and Coordinating Committee from 3 years to 1 year (Article III, Section 2).

Rationale: Spread the responsibility of this position to more members and eliminate the potential for committee service to exceed the time of an appointed committee member. The normal term on committee membership is 3 years. If a committee member is appointed chair, then the time of service will exceed the original 3 year term.

Motion: Include wording for electronic balloting in Article IV and Article VII.

Rationale: Electronic media is an efficient and fast method of communication. This could reduce costs associated with mailing and speed up the balloting process.

***This motion was voted upon and passed. The other motions will be put to a vote of the membership by electronic ballot.***

Motion: Include in Article II, Section 2 the following: The Western Section Applied Animal Science Award will be open to those papers published in the Western Section Proceedings.

Rationale: The industry groups supporting and funding this award intended to award research with applicability to production systems, economic return, and introducing new and innovative techniques supporting production systems without other restrictions.

**Other Recommendations:**

The Western Section should establish a committee membership structure for the Western Section Applied Animal Science Award that would approximate a 70% industry and 30% academic representation. In addition, the Advisory and Coordinating Committee suggests that the award should not exceed the dollar value of the Graduate Competition Paper award with 50% of the prize money going to first place, 30% to second place and 20% to third.

**Academic Quadrathlon**

Lisa Surber, Chair

The 2003 Regional Academic Quadrathlon contest was hosted by Oregon State University on March 28 & 29, 2003. Ten teams participated in this year's contest and they were as follows; Brigham Young University - Provo, Brigham Young University - Idaho, Cal Poly Pomona, Colorado State University, Montana State University, New Mexico State University, Oregon State University, University of Idaho, University of Wyoming, and Utah State University. Dr. Carl Hunt and was responsible for the written examination. Dr. Randy Weidmeier was in charge of the discussion group. Randy Townsend was

responsible for the quiz bowl portion of the contest. Dr. Patrick French secured over \$2,132 in book awards. Monsanto Company provided \$5,000 in scholarships for this year's contest; first place team members received \$600, second place team members received \$400 and third place team members received \$250. The contest results were as follows:

#### *Quiz Bowl*

- 1<sup>st</sup> Place Colorado State University
- 2<sup>nd</sup> Place Oregon State University
- 3<sup>rd</sup> Place University of Idaho

#### *Written Examination*

- 1<sup>st</sup> Place University of Idaho
- 2<sup>nd</sup> Place Cal Poly Pomona
- 3<sup>rd</sup> Place Brigham Young University – Idaho

#### *Discussion*

- 1<sup>st</sup> Place Brigham Young University – Provo
- 2<sup>nd</sup> Place Colorado State University
- 3<sup>rd</sup> Place University of Wyoming

#### *Practicum*

- 1<sup>st</sup> Place Oregon State University
- 2<sup>nd</sup> Place Colorado State University
- 3<sup>rd</sup> Place Brigham Young University – Idaho

#### *Overall Placing*

- 1<sup>st</sup> Place Colorado State University
- 2<sup>nd</sup> Place University of Idaho
- 3<sup>rd</sup> Place Oregon State University

The AQ Advisor Recognition Award was given to Dr. Richard Kellems of BYU – Provo for his support and commitment to the AQ program. Dr. Kellems served as BYU advisor for many years. At the regional level, from 1999-2001 served as the Western Section AQ committee chairman.

Last year's first place team was from University of Wyoming and they competed in the National Collegiate Quiz Bowl during the National Cattlemen's Beef Association convention in Nashville, Tennessee and placed 2<sup>nd</sup>. The NCBA has discontinued the National Collegiate Quiz Bowl so there is now no national contest for winners in 2004.

The 2004 Regional Academic Quadrathlon contest will be hosted by New Mexico State University and will be held on March 26 & 27, 2004.

#### **Awards**

Jack Whittier, Chair

Listed below are the award categories, awardees, and sponsors.

Distinguished Service Award: Dr. Temple Grandin, Colorado State University. Award sponsored by Roche Vitamins Inc.

Distinguished Teacher Award: Dr. Tim Ross, New Mexico State University. Award sponsored by Elanco Animal Health

Extension Award: Dr. John Paterson, Montana State University. Award sponsored by Fort Dodge Animal Health

Young Scientist Award: Dr. Bret Hess, University of Wyoming. Award sponsored by Cargill Animal Nutrition

#### **Applied Animal Science Award**

Jim Killen, Chair

The Applied Animal Science Awards are selected from papers accepted for publication in the WSASAS proceedings based on the criteria of 1) Applicability to current production systems, 2) Potential for economic return on investment, 3) New and innovative approaches to existing production problems. Twelve papers were submitted to the committee (per instructions on the call for WSASAS papers). The papers were evaluated and selected by a committee of industry and academic animal agriculture consultants appointed and chaired by the WSASAS Industry Representative. After two selection rounds the following papers were chosen:

1<sup>st</sup> Place, Certificate and check for \$400:  
H. H. Patterson, P. S. Johnson, and W. B. Epperson. South Dakota State University, Brookings, SD, "EFFECT OF TOTAL DISSOLVED SOLIDS AND SULFATES IN DRINKING WATER FOR GROWING STEERS"

2<sup>nd</sup> Place, Certificate and check for \$300:  
T. L. Lawler, J. B. Talyor, J. W. Finley, and J. S. Caton. North Dakota State University, Fargo, ND, USDA-ARS, Dubois, ID, USDA-ARS, Grand Forks, ND "EFFECT OF FEEDS NATURALLY HIGH IN SELENIUM OF PERFORMANCE AND SELENIUM CONCENTRATIONS IN VARIOUS TISSUES OF FINISHING BEEF STEERS"

3<sup>rd</sup> Place, Certificate and Check for \$200  
R. C. Waterman, W. D. Bryant, C. A. Loest, and M.K. Petersen, New Mexico State University, Las Cruces, NM, "METHIONINE IMPROVES NITROGEN RETENTION OF YOUNG GESTATING BEEF COWS CONSUMING LOW QUALITY FORAGES"

**Financial Report**

Elaine Grings, Secretary- Treasurer

**American Society of Animal Science  
Western Section  
Financial Report as of December 31, 2002**

**Cash on Hand at December 31, 2001****36,706.48****Revenue and Support**

Donations - General	1,500.00
Donations - Awards	550.00
Meeting Registrations	
Ticketed Events	
Proceedings	13,771.00
ASAS-Speaker Support	1,500.00
ASAS-Dues	1,205.00
Interest Income	1,771.75
Miscellaneous Income	3,300.00

**Total Revenue and Support** 23,597.75**Expense**

Programs/ Registration	1,008.84
Call for Papers/Abstracts	1,029.18
Awards/Plaques	3,109.58
Quadrathlon	4,500.00
Convention Fees	350.00
Convention Liability Insurance	
Proceedings	6,574.27
Travel- Speaker	1,842.42
Travel- Board	1,271.36
Postage/Supplies	152.97
Miscellaneous	1,794.68
Shipping	
Telephone	30.51
General Printing	
Staff Support	2,624.16

**Total Expenses** 24,287.97**Net Revenue over Expense** (690.22)**Cash on Hand as of December 31, 2002****36,016.26**

**Graduate Student Competition Committee Report  
Denny Crews, 2003 Chair**

Eighteen manuscripts and oral presentations by graduate students from 7 institutions were evaluated

by the 8-member committee. Committee members were: Dr. Denny Crews, AAFC-LRC; Dr. Paul Ludden, University of Wyoming; Dr. Roy Silcox, Brigham Young University; Dr. Clint Loest, New Mexico State University; Dr. Derek McLean, Washington State University; Dr. Jim Berardinelli, Montana State University; Dr. Mark Wise, New Mexico State University and Dr. Greg Lewis, USDA-ARS.

The individual placing awards were:

1<sup>st</sup> place: E. J. Scholljegerdes, University of Wyoming  
2<sup>nd</sup> place: C. S. Schauer, Oregon State University  
3<sup>rd</sup> place: S. L. Lake, University of Wyoming

Each of these were awarded certificates and \$500 for first place, \$300 for second place, and \$200 for third place.

2003 was the first year of the institutional award, sponsored by Zinpro. A check for \$2,000 was presented to the five graduate student competitors representing the University of Wyoming by Connie Swenson of Zinpro. The University of Wyoming had the highest average score among institutions with at least three competitors.

By unanimous vote, the committee makes the following recommendations. Dr. Paul Ludden, University of Wyoming, will serve as chair for the 2004 meetings in Corvallis, Oregon. Additionally, Drs. Ludden, Crews, and Lewis volunteered to serve for another three-year term on the committee. Dr. Roy Silcox volunteered to remain on the committee for one additional year. A vacancy left by Dr. Mark Wise, whose term on the committee expired in 2003, needs to be filled.

Thank yous were given to Zinpro for providing the monies for the new Institution Award.

A discussion was held about the pros and cons of limiting the number of competition papers to fit a scheduled time slot. No action was taken.

**OLD BUSINESS:**

None

**NEW BUSINESS:**

Pat Hatfield turned the gavel over to President-Elect Jack Whittier. Jack Whittier then presented President Hatfield a plaque in appreciation of his service. The meeting was adjourned at 2:15 pm.

# FERTILITY IN BEEF HEIFERS SYNCHRONIZED USING A MODIFIED CO-SYNCH PLUS CIDR PROTOCOL WITH OR WITHOUT GnRH AT TIMED AI

R. S. Walker<sup>1</sup>, R. M. Enns<sup>2</sup>, T. W. Geary<sup>3</sup>, N. W. Wamsley<sup>2</sup>, E. R. Downing<sup>2</sup>, R. G. Mortimer<sup>2</sup>, B. A. LaShell<sup>1</sup> and D. D. Zalesky<sup>1</sup>

<sup>1</sup>San Juan Basin Research Center, Hesperus, CO, <sup>2</sup>Colorado State University, Fort Collins, CO, <sup>3</sup>USDA-ARS, Miles City, MT

**ABSTRACT:** The objectives of this study were to determine if a second injection of GnRH at timed AI (TAI) increases the percentage of induced ovulations and improves pregnancy rates in beef heifers synchronized with the CO-Synch plus CIDR protocol. Nulliparous crossbred beef heifers (n = 375, BW = 362.7 kg, body condition score, BCS = 5.6) from three locations (Colorado [CO], Wyoming [WY] and South Dakota [SD]) were stratified by BW within BCS and randomly allotted to one of two treatments. All heifers received 100 µg of GnRH with a CIDR insert on day 0, followed by CIDR removal and 25 mg of PGF<sub>2α</sub> on day 7. At 54 hours post PGF<sub>2α</sub>, heifers in the control (CON) and treatment (TRMT) groups were mass mated and heifers in the TRMT group were given a second injection of GnRH at that time. Blood samples were collected in heifers at d -10 and 0 to determine cyclicity status at CO and WY. Ultrasonography was used to determine percentage of heifers ovulating 40 h after TAI at the CO and WY locations. Cyclicity rates were higher ( $P < 0.01$ ) for heifers at CO (97.4 %) vs WY (46.4 %). Pregnancy rates were similar ( $P > 0.10$ ) between treatment groups and for cycling and non-cycling heifers at CO and WY; however, pregnancy rates were higher ( $P < 0.05$ ) for heifers in the TRMT (54.2 %) vs CON group (40.4 %) at SD. Body weight did not affect pregnancy rates for either treatment group across all locations ( $P > 0.10$ ); however, pregnancy rates tended to decrease ( $P = 0.08$ ) for heifers with body weights greater than 409.1 kg (39 %) vs heifers with body weights less than 409.1 kg (53.2 %) at SD. The percentage of heifers ovulating were similar ( $P > 0.10$ ) between CO and WY and ovulation rates tended to be higher ( $P = 0.10$ ) for heifers in the TRMT (81.3 and 73.9 %) vs CON (62.5 and 66.7 %) groups at CO and WY. We conclude that synchronizing beef heifers with a modified CO-Synch plus CIDR protocol induces ovulation in cycling and non-cycling heifers and produces acceptable pregnancy rates at 54 h TAI. The value of incorporating a second injection of GnRH at timed AI remains questionable.

**Key Words:** Estrous Synchronization, GnRH, CIDR

## Introduction

Past approaches to synchronization have included the use of both gonadotropins and prostaglandins; however, variations in estrous response (Moreira et al., 2000), interval to standing estrus (Geary et al., 2001) and missed standing heats (Hixon et al., 2001) influence pregnancy outcomes from fixed-time AI protocols. Some of these responses are dependent upon the stage of the estrous cycle

when GnRH and/or PG are given (Geary et al., 2000). Schmitt et al. (1996) reported no differences in a 4-day estrous response period, but conception rates in beef heifers were reduced by 16 % when GnRH was removed from the beginning of a 9 d CIDR insert plus PG. Schmitt also reported that the average interval from PG to standing estrus was 47.3 h. Twagiramungu et al. (1995) reported higher pregnancy rates in beef heifers synchronized with a modified Select Synch protocol with the addition of a second injection of GnRH at 54 h timed AI vs no GnRH at TAI. With respect to GnRH, 75 % of anestrus cows formed new luteal tissue and more than 85 % ovulated in response to a second injection of GnRH 48 h after a Select Synch protocol (Thompson et al., 1999). When combining progestin with a gonadotropin and prostaglandin, estrous response was high with a reported 29 % increase in pregnancy rates for beef heifers synchronized with a CO-Synch + CIDR vs CO-Synch protocol alone (Martinez et al., 2002). The objective of this study was to determine if a second injection of GnRH increases the percentage of induced ovulations and improves fertility in beef heifers synchronized with a CO-Synch + CIDR protocol and mass mated at 54 h post PG injection.

## Materials and Methods

*Experimental Design.* Nulliparous crossbred beef heifers from one cooperators herd in South Dakota (SD; n = 211, BW = 392.3 kg, body condition score, BCS = 5.7) and two research station herds in Colorado (CO; n = 39, BW = 324.5 kg, BCS = 5.7) and Wyoming (WY; n = 125, BW = 325 kg, BCS = 5.4) were used to determine if a second injection of GnRH 54 h following CIDR removal increases the percentage of induced ovulations and improves timed AI (TAI) pregnancy rates. Heifers were synchronized with the CO-Synch plus EAZI BREED CIDR® (CIDR; 1.38 g of progesterone) protocol and stratified by BW within BCS to be randomly allotted to one of two treatment groups. All heifers received 100 µg (i.m.) of GnRH with a CIDR insert on day 0, followed by CIDR removal and 25 mg (i.m.) of prostaglandin F<sub>2α</sub> (PG) on day 7. At 54 hours post PG administration (d 9), heifers in the control (CON) and treatment (TRMT) groups were mass mated and heifers in the TRMT group were given a second injection of GnRH at that time. Body condition scores (1 to 9; 1 = emaciated and 9 = obese) and body weights were assessed on all heifers at time of CIDR insertion (day 0). Heifers were then assigned by weight class (90.9 kg increments) as either 1 (227.3 to 317.7 kg), 2 (318.2 to 408.6 kg) or 3 (409.1 to 499.5 kg) for each location. All heifers were diagnosed for pregnancy to

AI via transrectal ultrasonography 45 d post TAI. Cleanup bulls were turned out 8-14 d after mass mating and left in for 45 d.

Prior to synchronizing, two jugular vein blood samples were collected from all heifers at CO and WY 10 days apart (d -10 and 0) to determine cyclicity status. Heifers were assumed to be cyclical before the onset of treatments if any one of the two samples contained concentrations of serum progesterone  $\geq 1$  ng/mL. Serum was collected and stored at  $-20^{\circ}\text{C}$  until analyzed for progesterone by solid-phase radioimmunoassay (RIA; Diagnostic Products Corp., Los Angeles, CA). Serum samples were assayed in duplicate and sensitivity of the assay was 0.08 ng/mL. Within and between assay CV for serum samples was 12.86 and 9.6 % across two assays, respectively.

Ovaries from a subset of heifers at CO (n = 19) and WY (n = 49) were examined by transrectal ultrasonography to characterize incidence of ovulation relative to treatment groups 40 h following TAI. While ultrasounding heifers at TAI, follicular cysts were detected in one heifer from CO and 3 heifers from WY. These animals were removed from the study. Follicles were classified as cystic if the diameter of the follicle was  $> 20$  mm. Ovulation was defined as the disappearance of a large dominant follicle present on the ovary at time of insemination. Ovaries were scanned using transrectal ultrasonography (5-MHz intrarectal transducer, Aloka 500V, Corometrics, Wallingford, CT).

Heifers from CO were visually observed three times daily from day 6 (24 h prior to PG) to 10 (72 h after PG) to characterize estrous response. Estrous response was used to calculate pregnancy rates for specific times of estrus relevant to TAI.

**Statistical Analysis.** Preliminary analysis revealed a location effect on treatment, therefore data were not pooled and the main effects were evaluated within each location. Effects of treatment, AI technician, sire, cyclicity status and weight class on pregnancy rates were analyzed using Proc GENMOD procedure in SAS (1996). Differences in least squares means were used to compare pregnancy rates between treatments, sire and weight class. AI sire was then included in all models as a random effect with BW and BCS analyzed as covariates. Effects of BCS and BW on percent of heifers cycling were determined using Proc GENMOD. Significance was determined using Chi-square at  $P < 0.05$ .

All follicle data for heifers at CO and WY were analyzed using Proc GLM in SAS (1996). Effects of treatment, BCS and BW on incidence of ovulation were analyzed. Pregnancy rate differences based on treatment, incidence of ovulation, BCS and BW were analyzed. Significance was determined at  $P < 0.05$ .

## Results and Discussion

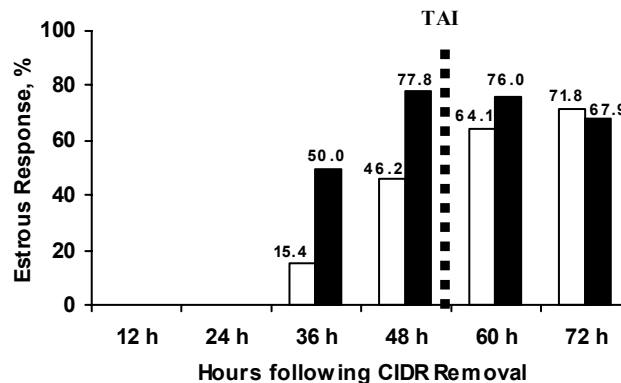
**Estrous Response.** There were no heifers observed in standing estrus until 36 h after CIDR removal and average interval from PG to first observed standing estrus was 51 h (Figure 1). Estrous response was 64.1 and 71.8 % within a 24 and 36 h period beginning 36 h after CIDR

removal and pregnancy rates for heifers exhibiting estrus within 36 h was 67.9 %. The percentage of heifers pregnant not exhibiting a standing estrus by 72 h after CIDR removal was 18.2 %. In heifers, estrous response rate was 65 % within a 72 h period when CIDR inserts were incorporated into a CIDR plus PG protocol (Lucy et al., 2001). Schmitt et al. (1996) and Martinez et al. (2002) reported that the average interval from PG to standing estrus in heifers was 47.3 and 47.8 h when a CIDR insert or MGA feeding was incorporated with GnRH at the time of insert/beginning of MGA; however, Richardson et al. (2002) reported average interval to estrus was 68 h in beef heifers after a CIDR+PG protocol. These responses are largely dependent upon the stage of the estrous cycle when GnRH and/or PG is given (Geary et al., 2000). However, incorporating a CIDR insert with GnRH injection at the time of insert synchronized a tight estrous response in the current study within 54 h to allow for TAI.

**Cyclicity Status.** The proportion of heifers cycling was lower ( $P < 0.01$ ) for WY (46.4 %) vs CO (97.4 %) heifers (Table 1). Heifers from WY had low cyclicity rates and a 55.2 % pregnancy rate to AI which indicated the effectiveness of GnRH and a CIDR insert to induce puberty in prepubertal heifers. The progesterone insert is known to increase LH secretion which increases follicular growth and development resulting in ovulation following its removal (Anderson et al., 1996). The proportion of heifers cycling was similar ( $P > 0.10$ ) between CON and TRMT heifers at both CO (100 and 95 %) and WY (47.6 and 45.2 %). Lucy et al. (2001) reported a 48 % synchronization rate in prepubertal beef heifers using a 7 d CIDR insert plus PG.

**Fertility.** Pregnancy rates to TAI were similar ( $P > 0.10$ ) for both cycling and non-cycling heifers at CO and WY (Table 1). The proportion of cycling and non-cycling heifers pregnant to AI did not differ ( $P > 0.10$ ) between CON vs TRMT heifers for CO and WY. Incorporating a norgestomet implant prevented short estrous cycles from naturally occurring after the first pubertal ovulation in beef heifers (Gonzalez-Padilla et al., 1975), and may be the reason differences in pregnancy rates were not observed between cycling and prepubertal heifers at both locations.

The percentage of CIDRs lost during the 7 d CIDR insert was 0 % for all locations. Final pregnancy rates for

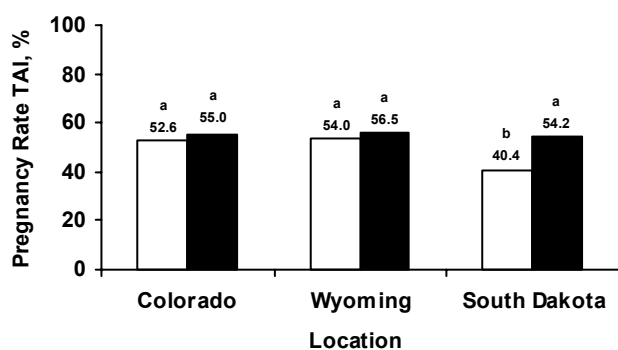


**Figure 1:** Cumulative estrous response (open bars) and pregnancy rates (shaded bars) for heifers detected in estrus at CO relative to TAI (54 h).

heifers at Colorado (97.4 %) and Wyoming (89.1 %) were not different ( $P > 0.10$ ) between the two locations and final pregnancy rates were not determined at SD. Timed AI pregnancy rates for heifers from both treatments combined did not differ ( $P > 0.10$ ) between CO (53.9 %; 21/39), SD (47.4 %; 100/211) and WY (55.2 %; 69/125). There was a treatment effect on AI pregnancy rates at the SD location, therefore data from all locations were not pooled and treatment effects were analyzed within each location. At SD, the proportion of pregnant heifers was lower ( $P < 0.05$ ) in the CON (40.4 %) vs TRMT group (54.2 %), but differences in pregnancy rates were not observed between treatments ( $P > 0.10$ ) for both CO and WY locations (Figure 2). The reason for decreased fertility among CON heifers at SD is unclear. While we did not determine cyclicity rate at SD, cyclicity did not affect response to treatment from heifers at CO and WY locations and thus, would not be expected to have contributed to fertility at SD.

Effects of BCS and body weight on heifer pregnancy rates were not significant ( $P > 0.10$ ); however, average BW was heavier for SD heifers compared to CO and WY heifers. Anderson et al. (1987) reported reduced fertility in beef heifers that carried a condition score greater than 6. The subjectivity of body condition scores, within locations, may have prevented us from detecting differences in pregnancy rates. Weight differences may explain more variation in pregnancy rates, so we separated body weights for heifers at all locations into three weight classes and analyzed for differences in pregnancy rates as a fixed variable (Table 1). While 96 % of the heifers from CO and WY and 72 % of the heifers from SD weighed between 272 to 408 kg, the remaining 28 % from SD weighed between 409 to 499 kg. Weight class tended ( $P = 0.08$ ) to affect pregnancy rates for heifers at SD, but no differences ( $P > 0.10$ ) in pregnancy rates were observed for heifers at CO and WY locations within weight class.

The number of service sires varied within location. Pregnancy rate differences were not observed ( $P > 0.10$ ) for CO heifers inseminated to sires A (57.9 %) and B (50 %), but 3 out of 13 AI sires used at SD and 1 out of 4 sires used at WY resulted in lower pregnancy rates within both locations ( $P < 0.05$ ). Pregnancy rates from sires used at WY and SD ranged from 45.2 to 73 % and 31.8 to 72.2 %.



**Figure 2:** Effect of treatment (CON [open bars] vs TRMT [shaded bars]) on pregnancy rates to 54 h fixed time AI within location. Percentages within location without a common letter (a,b) differ ( $P < 0.05$ ).

Dalton et al. (2001) reported significant differences between AI bulls on fertility rates when used for insemination at hours 0, 12 and 24 after first standing estrus in dairy cows.

**Incidence of Ovulation after Treatments.** The incidence of ovulation did not differ ( $P > 0.10$ ) between CO and WY heifers (66.7 % and 73.9 %), however ovulation rates tended to be lower ( $P = 0.10$ ) for heifers in the CON (62.5 and 66.7 %) vs TRMT (81.3 and 73.9 %) groups, respectively. Heifers ovulating by 40 h after TAI at CO and WY, regardless of treatment, had higher ( $P < 0.01$ ) pregnancy rates (50 and 61.8 %) than heifers that had not ovulated (16.7 and 16.7 %). Incorporating a second injection of GnRH at timed AI in dairy cattle successfully induced ovulation 24 to 32 h after GnRH (Pursley et al., 1994), but ovulation rates in the current study from heifers receiving a CIDR, but not receiving an additional injection of GnRH (62.5 %) resulted in pregnancy rates that were not different from heifers receiving an additional GnRH at TAI. It would appear that incidence of ovulation is high without incorporating a second injection of GnRH at TAI and suggest that a tight estrous response was in close proximity to the 54 h TAI period.

## Implications

Currently, there is no consistent TAI synchronization protocol that exists for controlling ovulation in beef heifers. In the current study, pregnancy rates were not improved for heifers receiving an additional GnRH injection at TAI at two locations, but were improved at the third location. Producers may be able to achieve acceptable pregnancy rates in beef heifers using a synchronization protocol that utilizes 54 h TAI, without estrous detection, with a CIDR insert plus GnRH at CIDR insertion and PG. Administering the second GnRH injection at timed AI may not improve pregnancy rates to AI, but guard against low pregnancy rates.

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## Literature Cited

- Anderson, K. J., J. S. Brinks, D. G. LeFever, W. C. Asbury, D. W. Schafer, and J. L. Moon. 1987. Reproductive performance of Angus heifers. Colorado State Univ. Beef Program Rep., p. 71. Fort Collins.

**Table 1:** Characteristics of heifers bred TAI by location

Item	Location						
	Colorado (CO)		Wyoming (WY)		South Dakota (SD)		Overall
				(no.) %			
Cyclicity <sup>a</sup>							
% cycling	(38/39)*	97.4	(58/125)	46.4	n/a		(96/164) 58.5
PR, cycling	(21/38)	55.3	(32/58)	55.2	n/a		(53/96) 55.2
PR, noncycling	(0/1)	0.0	(35/67)	52.2	n/a		(35/68) 51.5
Wt class, kg <sup>b</sup>							
1 (227.3 – 317.7)	(8/16)	50.0	(27/51)	52.9	(0/1)	0.0	(35/68) 51.5
2 (318.2 – 408.6)	(13/23)	56.5	(42/74)	56.8	(77/151)	51.0	(132/248) 53.2
3 (409.1 – 499.5)					(23/59)	39.0	(23/59) 39.0

<sup>a</sup>Percentage of heifers cycling based on progesterone values > 1 ng/ml taken on d -10 and 0.

Cyclicity data was not available (n/a) for heifers at the SD location because they were a cooperator herd.

Pregnancy rates (PR) for the percent of heifers cycling and non-cycling.

<sup>b</sup>Combined weights of heifers separated into three classes for all locations.

\*Raw mean percentages within a row for % cycling lacking the common asterisks differ ( $P < 0.05$ ).

Anderson, L. H., C. M. McDowell, and M. L. Day. 1996. Progesterone-induced puberty and secretion of luteinizing hormone in heifers. *Biol. Reprod.* 54:1025-1031.

Dalton, J. C., S. Nadir, J. H. Blame, M. Noftsinger, R. L. Nebel, and R. G. Saacke. 2001. Effect of time of insemination on number of accessory sperm, fertilization rate, and embryo quality in nonlactating dairy cattle. *J. Dairy Sci.* 84:2413-2418.

Geary, T. W., E. R. Downing, J. C. Bruemmer, and J. C. Whittier. 2000. Ovarian and estrous response of suckled beef cows to the Select Synch estrous synchronization protocol. *Prof. Anim. Sci.* 16:1

Geary, T. W., J. C. Whittier, R. G. Mortimer, J. W. Young, and R. R. Salverson. 2001. Synchronization of estrus in beef cows using GnRH and PGF with estrus AI or timed AI 72 h after PGF with or without a second GnRH injection. *Proc. Western Section Am. Soc. Anim. Sci.* 52:369-371.

Gonzalez-Padilla, E., R. Ruiz, D. LeFever, A. Denham, and J. N. Wiltbank. 1975. Puberty in beef heifers. III. Induction of fertile estrus. *J. Anim. Sci.* 40:1110-1118.

Hixon, D. L., R. D. Landis, B. M. Alexander, D. W. Moore, D. D. Carr, and G. E. Moss. 2001. A MGA-Prostaglandin-GnRH estrus synchronization protocol suitable for timed artificial insemination. *Proc. Western Section Am. Soc. Anim. Sci.* 52:372-373.

Lucy, M. C., H. J. Billings, W. R. Butler, L. R. Ehnis, M. J. Fields, D. J. Kesler, J. E. Kinder, R. C. Mattos, R. E. Short, W. W. Thatcher, R. P. Wettemann, J. V. Yelich, and H. D. Hafs. 2001. Efficacy of an intravaginal progesterone insert and an injection of PGF<sub>2α</sub> for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. *J. Anim. Sci.* 79:982-995.

Martinez, M. F., J. P. Kastelic, G. P. Adams, R. B. Cook, W. O. Olsen, and R. J. Mapletoft. 2002. The use of progestins in regimens for fixed-time artificial insemination in beef cattle. *Theriogenology* 57:1049.

Moreira, F., R. L. de la Sota, T. Diaz, and W. W. Thatcher. 2000. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. *J. Anim. Sci.* 78:1568-1576.

Pursley, J. R., M. O. Mee, M. D. Brown, and M. C. Wiltbank. 1994. Synchronization of ovulation in dairy cattle using GnRH and PGF<sub>2α</sub>. *J. Anim. Sci.* 72(Suppl. 1) / *J. Dairy Sci.* 77(Suppl. 1):230 (Abstr.).

Richardson, A. M., B. A. Hensley, T. J. Marple, S. K. Johnson, and J. S. Stevenson. 2002. Characteristics of estrus before and after first insemination and fertility of heifers after synchronized estrus using GnRH, PGF<sub>2α</sub>, and progesterone. *J. Anim. Sci.* 80:2792-2800.

SAS. Institute Inc., SAS/STAT(R) Version 8.1, Cary, NC: SAS Institute Inc., 1996.

Schmitt, E. J.-P., M. Drost, T. Diaz, C. Roomes, and W. W. Thatcher. 1996. Effect of a gonadotropin releasing hormone agonist on follicle recruitment and pregnancy rate in cattle. *J. Anim. Sci.* 74:154-161.

Thompson, K. E., J. S. Stevenson, G. C. Lamb, D. M. Grieger, and C. A. Löest. 1999. Follicular, hormonal, and pregnancy responses of early postpartum suckled beef cows to GnRH, norgestomet, and prostaglandin F<sub>2α</sub>. *J. Anim. Sci.* 77:1823-1832.

Twagiramungu, H., G. L. Roy, G. Laverdière, and J. J. Dufour. 1995. Fixed-time insemination in cattle after synchronization of estrus and ovulation with GnRH and prostaglandin. *Theriogenology* 43:341(Abstr.).

**VALIDATION AND IMPROVEMENT OF THE TEXAS A&M GRAZINGLANDS  
ANIMAL NUTRITION LABORATORY NEAR INFRARED REFLECTANCE  
SPECTROSCOPY PREDICTION EQUATION**

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**ABSTRACT:** Near-infrared spectroscopy (NIRS) of fecal samples has been used to predict the CP and digestible organic matter (DOM) of forages consumed by the grazing animal. For NIRS predictions to be accurate, the prediction equation must be based on data from the same population as samples to be predicted. Beef cattle in California graze a unique rangeland composed mostly of non-native annual species. The predominance of annual species, along with a long dry summer during which annual grasses mature, die and decrease in quality, makes information from other systems difficult to apply. The Grazinglands Animal Nutrition (GAN) Laboratory at Texas A&M has a NIRS program based on forages in Texas, the Mid-west and the lower part of the prairie provinces of Canada. California's annual grasses do not fit into this system, but California producers have been using NIRS predictions from the GAN Lab even though those equations have never been validated under California conditions. Beef cattle digestibility trials were conducted on forages harvested from two California rangeland sites at six-week intervals from June to November of 2002. These trials produced forage-fecal pairs that were used to test the existing equation and to develop new equations if necessary. CP and DOM of the forages ranged from 4.1 to 6.0% CP and 50.4 to 56.2% DOM. On average, the original equation overpredicted CP by 3.4%-units. When California samples were added to the existing equation to form an improved equation, prediction bias was reduced to +0.2%-units. The original equation overpredicted DOM by 4.7%-units. Predictive capability for DOM decreased as CP and DOM content of the rangeland forages declined through the summer. The improved equation overpredicts DOM by 0.9%-unit. This study shows that the addition of forage-fecal paired data produced on California rangelands improves the predictive capability of the GAN Lab system for California samples. These data must be added for the system to accurately predict the CP and DOM content of California annual rangeland forages.

**Key Words** Beef Cattle, California Rangelands, Near-Infrared-Reflectance-Spectroscopy

### **Introduction**

California has a unique rangeland composed of non-native annual species which germinate with fall rains, grow through the rainy winter and senesce following the last spring rains. Forage quality declines through the long, dry summer. Inherent in the drying process is an increase in fiber content, a decrease in energy content, and an increase in shatter and bleaching. Of particular concern for many cattle producers is the loss of CP, which is important for

growth, lactation and gestation. Supplementation is often necessary to maintain production.

Cattle producers must determine how to supplement to meet the changing nutrient demands of their cattle. Traditional methods of determining when to supplement, such as tracking cow body condition and hand sampling of available forage, have not provided the accuracy producers need. Fecal near-infrared-reflectance spectroscopy is gaining attention as a method to measure dietary composition of grazing animals because it measures nutrients consumed rather than nutrients available.

Two predictive equations were developed by the Grazinglands Animal Nutrition (GAN) Lab at Texas A&M University in College Station, TX, using data sets from rangelands in Texas, the Midwest and the southern portion of the prairie provinces of Canada. These equations are commonly called the "warm season equation" and the "cool season equation." The "warm season" equation is used where native C3 and C4 forages are found, such as more extensive rangeland systems. (D. Tolleson, GAN Lab, personal communication). The cool season is rarely used to predict nutrient composition of California forages and was not analyzed in this study.

The annual forage species found in the cattle producing areas of California do not completely fit into either equation. California cattle producers are using this system despite the fact it has not been validated for use in California. Based on cattle performance, producers report mixed results in terms of perceived predictive accuracy.

### **Materials and Methods**

Starting in June of 2002, rangeland forage was harvested from two California sites to provide forage for *in vivo* digestibility trials. The first site was at the Sierra Field Research and Extension Center (SFREC), near Marysville, CA. and the second sites was near Petaluma, CA.

To minimize shatter losses, range forage was cut using a sicklebar mower at SFREC, or a rotocombine at Petaluma, to a stubble height of approximately 10 cm. Forage was raked into rows and placed on tarpaulins for transfer to a trailer. To minimize forage species variation among harvests, the total area to be harvested was divided and a portion of each section included in each harvest. Harvest occurred at 6 wk intervals at each site, with the sites offset by 3 wks. Harvest continued at each site until one harvest following the first germinating rain, defined by 12 to 25 mm of rainfall within 1 wk (George et al., 2001; George et al., 1988; Bentley and Talbot, 1951). The first harvest at the SFREC was on June 11, 2002 and the first

rain event of 40 mm occurred on November 7, with an additional 119 mm falling on November 12. The last harvest occurred at the SFREC on November 26, 2002, 2 wks following germination. Significant “green-up” was observed by the time of harvest. A total of 5 harvests at SFREC and 4 harvests at Petaluma were collected.

The main species at SFREC in late May and early June, 2003 were Wild oat (*Avena barbada*), Rose clover (*Trifolium hirtum*), Medusahead (*Taeniatherum caput medusae*) and Soft chess (*Bromus hordeaceus*). The main species at Petaluma were Annual rye (*Lolium multiflorum*), Ripgut brome (*Bromus diandicus*), Foxtail (*Hordium leporanium*) and Wild oat (*Avena fatua*).

The harvested range forage was chopped to an average length of 7.5 cm to increase voluntary intake by the cattle and to minimize sorting. An average of 5 cross-bred Angus steers were fed chopped forage every 8 h to meet predicted maintenance energy requirements. Immediately prior to forage feeding, soybean meal (**SBM**) was offered to bring total N in the diet to 3% of DM. Water was offered *ad libitum*.

Steers were housed in individual pens at a feedlot at the University of California, Davis, which is located approximately 3 km west of Davis, CA. All animal procedures were approved by the University of California, Davis Animal Care and Use Administrative Advisory Committee.

Steers were fed for a 14 d adjustment period, followed by a 5 d total fecal collection period using fecal harnesses. Fecal samples were composited on a percentage of total fecal output basis by day, steer and period. All composite samples were preserved in triplicate and frozen. One sample was sent by 2 d mail to the Texas A&M GAN Lab for near-infrared spectroscopy (**NIRS**) analysis, and a second sample was dried at 50°C for 72 h before being ground to pass a Wiley mill (Arthur H. Thomas, Philadelphia, PA) 1mm screen. Dried fecal samples were analyzed for CP, ADF, NDF, and ash by the Dairy One Forage Laboratory (Ithaca, NY). Dry matter was determined at the time of compositing by drying at 50°C for 72 h.

Forage samples were taken at chopping, and forage and soybean meal samples were taken once during the fecal collection period. Dry matter for forage and soybean meal was determined once during the fecal collection period throughout the dry season, then once daily from day 13 to day 18 once rains began by drying at 50°C for 72 h. Significant refusals, if they occurred, were collected and weighed daily from day 13 to day 18, and were composited in the same manner as fecal samples. Forage, SBM and refusal samples were dried, ground to pass a 1mm screen and analyzed for CP, ADF, NDF and ash, as described previously. All nutrient analyses were conducted by the Dairy One Forage Laboratory (Ithaca, NY).

Forage CP was determined by standard laboratory nutrient analysis. Digestible OM (**DOM**) of the forage was (forage DM intake x forage OM - (fecal DM output x fecal OM - fecal OM SBM)) / (forage DM intake) where fecal OM SBM was SBM DM intake x (1 -SBM ash) x 0.85 and SBM is assumed to be 85% digestible.

## Site Descriptions

The site in Petaluma was on privately owned land located 10 km west of the town of Petaluma. Forage was harvested from a relatively steep slope with a western aspect. The surrounding region is heavily influenced by coastal weather patterns, as it is located approximately 25 km from the Pacific Ocean and approximately the same distance from San Pablo Bay. Fog is common throughout the entire year. This moisture contributes to a decline in forage quality above what occurs further inland. The area receives about 64 cm of rain per year, primarily in the late fall to spring.

An additional site was located at the Sierra Foothill Research and Extension Center (SFREC), 25 km east of Marysville. Forage was harvested from mostly flat to rolling ground with an eastern exposure. The site was dominated by annual grasses and is typical of the land grazed by many cattle in California. It has a hot and dry climate, being on the eastern side of the Sacramento Valley in the Sierra foothills. Average rainfall is 71 cm per year, with rain events generally from late fall through spring.

## Statistical Analysis

Data were analyzed using the Proc GLM in SAS (SAS Inst. Inc.; Cary, NC) to determine the effect of equation (original vs. improved), time (date of harvest), and location (SFREC vs. Petaluma) on predictive capability. Interactions were also analyzed.

## Equation Compilation

Equations for forage CP and DOM were constructed using forage-fecal pairs whose predicted constituents fell within 1.5 standard deviations of the expected value based on similar spectra from the same population of samples. An equation was constructed using forage-fecal pair data from the day composites obtained from the digestibility trial. Day composites were used because they match what occurs when a cattle producer takes a sample on one day from several fecal pats.

## Results and Discussion

DOM values declined as the summer progresses and forage senesced, but CP did not seem to change significantly. (Table 1).

## Crude Protein

Addition of dry season forage-fecal pairs to the existing GAN Lab equation improved predictive capability for CP. All single factors were highly significant, and location by equation and location by time interactions were observed ( $P < 0.01$ ). Because there was a location by equation interaction, data were analyzed by location (Table 2).

Data from the Petaluma site show that both time and equation used were significant ( $P < 0.01$ ). Analysis of

the least-square means (prediction – laboratory value), shown in Table 3, demonstrates that predictive capability for CP was improved by more than 2 percentage units with the improved equation. ( $P < 0.01$ )

At the SFREC site, equation, time and an equation by time interaction were important sources of variation, but equation had the most effect on predictive capability. Ability to predict forage CP from SFREC fecal samples improved by more than 4 percentage units under the improved equation. (Table 3)

There was also a strong location by equation interaction ( $P < 0.01$ ). Both the original equation and the improved equation predict CP for Petaluma samples more correctly than CP for SFREC samples. Under the new equation, predictive ability for Petaluma samples is numerically more accurate (.21 percentage units) than predictions for SFREC samples. On average, CP was over predicted. The ability of the equations to better predict Petaluma samples may be due to the higher percentage of perennials at the Petaluma site. Another explanation is that there was greater specie variability at the SFREC site, indicating that the system may not have been exposed to all of the different species or that a single lab value may not be sufficient to reflect the variation seen at the SFREC site.

#### *Digestible Organic Matter*

Predictions of DOM were more accurate under the new equation than predictions with the original equation. In the statistical model including all data, only equation is highly significant ( $P < 0.01$ ). Accuracy of predictions of DOM for Petaluma samples were 4.39 percent better under the new equation than the old equation. For samples from the Sierra site, predictions of DOM by the new equation were 3 percent more accurate than the original equation.

Similar to the original equation, the new equation predicts Petaluma samples more accurately than SFREC samples by more than .92 percentage units. The ability of the GAN NIRS equation to better predict Petaluma samples may be a plant species effect, meaning that the plants found at the Petaluma site are more similar to the plants fed to create forage-fecal pairs in other trials whose data was also used to construct the equation. In addition, the Petaluma site had slightly more perennials and biennials than the SFREC site.

DOM was consistently over predicted with both the original and improved equation. The improved equation over predicts DOM of samples from Petaluma by nearly .74 percentage units, and samples from the SFREC site by more than 1.67 percentage units. The consistent over prediction of DOM indicates a systematic error either in the GAN Lab system or in the digestibility trial. However, because this over prediction was seen in the original equation as well as for CP, it is likely that the error lies within the GAN Lab NIRS system.

When DOM and CP are compared, the new equation is more accurate for DOM than CP. This difference in predictive accuracy is likely due to greater variation in measures of DOM due to animal and day variation.

## **Implications**

The addition of California rangeland forage-fecal pairs made a significant improvement to the existing GAN Lab NIRS system. Further improvements are necessary and could be made with additional digestibility trials on a wider variety of California rangelands. To increase usefulness of the system, these trials should include forages from the entire year rather than only the dry season.

Cattle producers should see an improvement in fecal NIRS predictions by late 2004, when the GAN Lab will add the California data set along with several other data sets from around the world to the original equation. The GAN Lab has further plans to use locally weighted regression to improve predictive ability. In this method, the computer obtains a spectral reading on a sample, which uses that reading to identify the 100 most similar spectral samples in the database. These 100 samples are used to develop an equation which predicts parameters for the specific unknown sample. Once available, this method is expected to yield much more accurate predictions (D. Tolleson, GAN Lab, personal communication).

## **Literature Cited**

- Bentley, J. T. and M. W. Talbot. 1951. Efficient use of annual plants on cattle ranges in the California foothills. USDA Circ. 870, Washington D.C.
- George, M., J. Bartolome, N. McDougald, M. Connor, C. Vaughn, and G. Markegard. 2001. Annual range forage production. University of California, Division of Agriculture and Natural Resources Publ. 8018.
- George, M., J. Clawson, J. Menke, and J. Bartolome. 1985. Annual grassland forage productivity. *Rangelands* 7:17-19.
- National Research Council. 1996. Nutrient requirements of beef cattle, 7th edition. Washington, D.C. National Academy Press.

Table 1. Laboratory CP and in vivo digestible organic matter (DOM) of California range forage from Petaluma and Sierra Foothill Research and Extension Center (SFREC) sites

Location	Date	Laboratory CP, %	In vivo DOM, %
Petaluma	July 1, 2002	5.2	53.96 ± 0.27
	August 12, 2002	5.2	54.03 ± 1.04
	September 23, 2002	5.8	50.36 ± 1.82
	November 4, 2002	6.0	50.55 ± 1.89
SFREC	June 1, 2002	4.3	56.17 ± 1.32
	July 23, 2002	4.1	53.01 ± 1.83
	September 3, 2002	4.5	52.95 ± 2.13
	October 15, 2002	4.8	52.59 ± 3.52
	November 26, 2002	5.1	50.89 ± 0.53

Table 2. Mean near-infrared reflectance spectroscopy predictions for CP and digestible organic matter (DOM) for original and improved Texas A&M Grazing Animal Nutrition Lab equations using feces from steers fed California range forage from Petaluma and Sierra Foothill Research and Extension Center (SFREC) sites. The improved equation is based on a database which includes the California data in addition to the original database, which was the basis for the original equation

Location	Date	Predicted CP, %		Predicted DOM, %	
		Original	Improved	Original	Improved
Petaluma	July 1, 2002	8.28 ± 0.33	6.62 ± 0.25	59.46 ± 0.26	58.49 ± 0.34
	August 12, 2002	7.12 ± 0.05	4.54 ± 0.28	56.54 ± 0.48	54.20 ± 0.15
	September 23, 2002	7.28 ± 0.29	5.23 ± 0.35	56.74 ± 0.17	49.99 ± 0.18
	November 4, 2002	7.60 ± 0.25	5.78 ± 0.40	56.60 ± 0.29	49.19 ± 0.43
SFREC	June 1, 2002	7.89 ± 0.42	4.50 ± 0.19	57.33 ± 0.21	55.19 ± 0.42
	July 23, 2002	7.71 ± 0.37	4.03 ± 0.14	58.28 ± 0.20	56.09 ± 0.48
	September 3, 2002	9.22 ± 0.29	4.58 ± 0.34	58.00 ± 0.05	54.14 ± 0.14
	October 15, 2002	9.94 ± 0.17	4.94 ± 0.11	58.64 ± 0.13	54.91 ± 0.19
	November 26, 2002	9.28 ± 0.16	5.83 ± 0.43	56.71 ± 0.22	53.63 ± 0.31

Table 3. Least square mean errors of near-infrared reflectance spectroscopy predictions for CP and digestible organic matter (DOM) for original and improved Texas A&M Grazing Animal Nutrition Lab equations using feces from steers fed California range forage from Petaluma and Sierra Foothill Research and Extension Center (SFREC) sites. The improved equation is based on a database which includes the California data in addition to the original database, which was the basis for the original equation

Location	Date	Error of CP Prediction, %-units			Error of DOM prediction, %-units		
		Original	Improved	P <sup>1</sup>	Original	Improved	P <sup>1</sup>
Petaluma	July 1, 2002	3.08	1.42	<0.01	5.5	4.53	0.64
	August 12, 2002	1.92	-0.66	<0.01	2.52	0.18	0.26
	September 23, 2002	1.48	-0.58	<0.01	6.38	-0.37	<0.01
	November 4, 2002	1.6	-0.22	<0.01	6.14	-1.36	<0.01
SFREC	June 1, 2002	3.59	0.2	<0.01	1.17	-0.98	0.48
	July 23, 2002	3.61	-0.07	<0.01	5.27	3.08	0.47
	September 3, 2002	4.72	0.08	<0.01	5.04	1.19	0.21
	October 15, 2002	5.14	0.14	<0.01	6.04	2.31	0.22
	November 26, 2002	4.18	0.73	<0.01	5.82	2.74	0.31

<sup>1</sup>Probability that the mean prediction errors are not different.

**Efficacy of an intravaginal progesterone insert and an injection of PGF<sub>2α</sub> to advance date of breeding in postpartum beef cows while utilizing natural service**

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**ABSTRACT:** The objective of this experiment was to compare conception date and overall pregnancy rates in beef cows given a single i.m. injection of 25 mg PGF<sub>2α</sub> (Lutalyse; control) on d 7 of a natural breeding season with or without 7-d pretreatment with an intravaginal progesterone insert (CIDR). Crossbred cows from two locations were stratified by age within location and randomly allotted to either treatment or control; CIDR were inserted on d 0 and removed on d 7; location 1:n=224, 3.36 ± .04 yr age, 62.5 ± 1.21 d postpartum (PP), 513.9 ± 4.6 kg BW, and 3.9 ± .04 BCS; location 2:n=73, 4.64 ± .15 yr age, 58.4 ± 1.17 d PP, 488.8 ± 6.4 kg BW, and 4.74 ± 0.57 BCS. Bulls were placed with cows in each herd from d 0 to d 60. Day of conception and cycle conceived (21 day periods) were estimated by ultrasonography on d 66 and 73; and again by rectal palpation on d 176 and 123 for locations 1 and 2, respectively. These response variables were analyzed with a model that included d PP, BCS and BW at d 0 as covariates and cow age (2, 3, 4+ yr), location, and treatment as fixed effects (location by treatment not significant). Days to conception (24 vs. 27 d for CIDR vs. control) and cycle (1.53 vs. 1.66 for CIDR vs. control) of conception tended ( $P < 0.13$ ) to be decreased with CIDR. The number of pregnancies lost between first and second pregnancy diagnosis, did not differ ( $P = 0.31$ ) between CIDR (7) and control (8). No beneficial affect of CIDR was observed ( $P = 0.22$ ) for the cycle that cows became pregnant and remained pregnant. Overall pregnancy rates were not affected by treatment ( $P = 0.32$ ) for CIDR (94.6%) or control groups (93.2%). In conclusion, treatment with a CIDR and an injection of PGF<sub>2α</sub> was not effective in increasing overall pregnancy rates or advancing breeding date compared to cows receiving PGF<sub>2α</sub> on day 7 of the breeding season.

**Keywords:** Beef cows, CIDR, Breeding date, Pregnancy rate

### Introduction

In both milked and suckled cows following parturition there is a period of anestrus of varying duration (Rhodes et al., 2003). In suckled beef herds an average of 23% of cows had not ovulated by the start of breeding (Lamb et al., 2001). If postpartum anestrus persists at initiation of breeding seasons, time of conception may be delayed or cows may fail to conceive during the breeding season,

which increases the culling rate in herds and decreases net income of producers (Bellows et al., 1979). Progestins have been reported to induce estrous cycles in anestrus cows and prepubertal heifers (Smith et al., 1987; Anderson and Day, 1994). Treatment with progesterone from an intravaginal progesterone insert (CIDR) has resulted in resumption of luteal function in suckled beef cows that were anestrus (Fike et al., 1997). Improved pregnancy rates have been achieved when a progestin was incorporated into GnRH + PGF<sub>2α</sub> protocols (Stevenson et al., 1997, 2000; Lamb et al., 2001). Patterson et al., (1995) also observed greater pregnancy rates when lactating beef cows were pretreated with MGA (melengesterol acetate) before administering PGF<sub>2α</sub> than controls treated with PGF<sub>2α</sub>. The objective of this study was to compare the conception date and overall pregnancy rates in beef cows given a single i.m. injection of 25 mg PGF<sub>2α</sub> (Lutalyse; control) on d 7 of a natural breeding season with or without 7-d pretreatment with a CIDR.

### Materials and Methods

**Cattle.** Postpartum lactating beef cows n=297 from two locations were stratified by age within location and then randomly allotted to one of two groups (CIDR or Control). Cows in the CIDR group were implanted with the progesterone releasing device on d 0 (beginning of a natural breeding season) and given an i.m. injection of 25 mg PGF<sub>2α</sub> (Lutalyse) coincident with CIDR removal on d 7. Control cows received the i.m. injection of PGF<sub>2α</sub> on d 7, without CIDR pretreatment. For age stratification, cows at each location that were 4 yr of age or older were combined into one class. The number of cows in each treatment by age classification is shown in Table 1. Cow BW, average BCS (determined from two independent scorers) and days postpartum were recorded on d 0. Table 2 depicts BCS, days postpartum, and BW of cows within treatment at each location. Calves were maintained with cows at all times and allowed to suckle without restriction. Natural service was used in this study with bulls being introduced into the herd on d 0 and removed on d 60.

Days to conception and number of estrous cycles to conception (21 d periods) were estimated by ultrasonography on d 66 and 73 and again by rectal palpation on d 176 and 123 for cows at locations 1 and 2,

respectively. In cases where pregnancies were lost between the first and second diagnosis, these losses were accounted for in a final estimate of number of days or 21-day cycles required for conception.

*Blood Collection and Progesterone analysis.* Two blood samples were collected from all cows ( $n=224$ ) on either d 0 and 7 for location 1 or d -10 and 0 for location 2. Serum from these samples was used to determine circulating concentrations of progesterone. Progesterone concentrations were determined by radioimmunoassay without extraction of serum, using commercially available antibody and tracer (ICN Pharmaceuticals, Inc. Costa Mesa, CA; Roberts and Jenkins, 2002). Samples with progesterone concentrations greater than 1 ng/mL were considered indicative of cyclicity for cows allotted to the control treatment at location 1 and for cows allotted to the control and CIDR treatment at location 2. Samples from cows allotted to the CIDR treatments at location 1 were taken the d CIDR were removed and thus confounded by progesterone released from the CIDR, preventing determination of cyclicity in this group. The difference in blood sampling dates between location 1 and location 2 was due to time constraints when the cattle were available at location 1.

*Bulls and Pasture.* Breeding pasture size was approximately 260 acres for location 1 and approximately 220 acres for location 2. Bulls used at both locations passed breeding soundness examinations by a local veterinarian before initiation of the breeding season. Observations were made daily for the physical condition of the bulls for the duration of the first synchronized estrus. At location 1 a yearling bull was used from d 0 to d 7 and eleven bulls were placed with cows on d 7. The total number of bulls used was 12 allowing the cow to bull ratio to be approximately 19:1. Cows at location 1 were transported in small groups over three days to summer pasture from June 9<sup>th</sup>–12<sup>th</sup>, 2003. At location 2, three, 3-yr old bulls were used throughout the breeding season, and an additional yearling bull was placed with the cows on d 3 of the breeding season. The cow to bull ratio at location 2 was also approximately 20:1. Cows at this location were not transported throughout the duration of the experiment.

*Statistical Analyses.* Cow was the experimental unit. To ensure the randomization of cows was not biased with respect to BCS, BW, or PP on d 0, these traits were analyzed using GLM procedures (SAS Inst. Inc., Gary NC 2001) with a model that included location, treatment and location x treatment interaction. Treatment and interaction effects were not significant ( $P > 0.60$ ) verifying acceptable randomization. The number of days and estrus cycles to initial conception (determined by the first pregnancy diagnosis) and final pregnancy (determined by the second pregnancy diagnosis) were analyzed using GLM procedures with an initial model that included treatment, location, and cow age (2, 3, 4+) as

class variables, plus covariates for BCS, BW, and days postpartum, and all two-way interactions. A step down approach was used to delete variables from the model with F values  $<1$ . The final reduced model included treatment, location, days postpartum, BCS, BW, and the interactions of location with days postpartum and BW. Chi Square procedures were used to evaluate treatment effects on the number of cattle pregnant in the first, second, and third estrous cycles, and for overall pregnancy rates pooled across locations.

## Results and Discussion

*Cyclicity.* Blood samples collected at d 0 and 7 from cows in the control group at location 1 indicated that an average of 76% of cows were cycling before the onset of treatment. Samples collected at d -10 and 0 from cows at location 2 indicated that an average of 54% of cows were cycling before the onset of treatment.

*Breeding and Conception Date.* Days to conception ( $P = 0.13$ ) and estrous cycle of conception tended ( $P = 0.11$ ) to be decreased with CIDR, when evaluated before accounting for pregnancy losses between the first and second pregnancy diagnosis (Table 3.). The number of pregnancies lost between first and second pregnancy diagnosis, did not differ ( $P = 0.31$ ) between CIDR (7) and control (8). After accounting for these losses, no beneficial affect of CIDR was observed to day of final pregnancy ( $P = 0.21$ ) or cycle of pregnancy ( $P = 0.22$ ; Table 3). Overall pregnancy rates were not affected by treatment ( $P > 0.10$ ) for CIDR (94.6%) and control (93.2%) groups.

In conclusion, pretreatment of postpartum beef cows with CIDR did not advance date of conception or improve pregnancy rates compared to a single injection of PGF<sub>2α</sub> 7d after initiation of the breeding season. Similar results were reported by Lucy et al., (2001) where postpartum beef cows had pregnancy rates of 50, 55, and 58% for control, PGF<sub>2α</sub>, and CIDR + PGF<sub>2α</sub> treated cows during a 31 d breeding period. Economically, using the CIDR + PGF<sub>2α</sub> treatment to synchronize cattle is more expensive than using a single injection of PGF<sub>2α</sub>, and more labor intensive. Further studies are needed to determine if CIDR would be beneficial in beef cattle herds with a higher incidence of anestrus.

## Implications

While numerous studies indicate that exogenous progestins may hasten return of estrus in postpartum cows and thereby advance date of breeding and over all conception rate, application of a CIDR + PGF<sub>2α</sub> protocol to postpartum cows subjected to natural breeding did not show improvements in these traits in the present study. Further research with a larger number of animals and more locations may demonstrate a statistical advantage of

such progestin treatments, especially if the proportion of anestrous cows exceeds those observed in the present study. However, the cost of the CIDR treatment will need to be an important consideration in determining the benefits for producers.

### Literature Cited

- Anderson, L. H., and M. L. Day. 1994. Acute progesterone administration regresses persistent dominant follicles and improves fertility of cattle in which estrus was synchronized with melengesterol acetate. *J. Anim. Sci.* 72:2955-2961.
- Bellows, R. A., R.E. Short, and R. B. Staigmiller. 1979. Research areas in beef cattle reproduction. In: H. Hawk (Ed) *Proc. Beltsville Symp. on Animal Reproduction*, No. 3 p.3 Allanheld, Osmun, Montclair.
- Fike, K.E., M.L. Day, E.K. Inskeep, J.E. Kinder, P.E. Lewis, R.E. Short, and H.D. Hafs. 1997. Estrus and luteal function in suckled beef cows that were anestrous when treated with an intravaginal device containing progesterone with or without a subsequent injection of estradiol benzoate. *J. Anim. Sci.* 75:2009-2015.
- Lamb, G.C., J.S. Stevenson, D.J. Kesler, H.A. Garvericks, D.R. Brown, and B.E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin  $F_{2\alpha}$  for ovulation control in postpartum suckled beef cows. *J. Anim. Sci.* 79:2253-2259.
- Lucy, M.C., H.J. Billings, W.R. Butler, L.R. Ehnis, M.J. Fields, K.J. Kesler, J.E. Kinder, R.C. Mattos, R.E. Short, W.W. Thatcher, R.P. Wettemann, J.V. Yelich, and H.D. Hafs. 2001. Efficacy of an intravaginal progesterone insert and an injection of  $PGF_{2\alpha}$  for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. *J. Anim. Sci.* 79:982-995.
- Patterson, D.J., J.B. Hall, N.W. Bradley, K.K. Schillo, B.L. Woods, and J.M. Kearnan. 1995. Improved synchrony, conception rate, and fecundity in postpartum suckled beef cows fed melengesterol acetate prior to prostaglandin  $F_{2\alpha}$ . *J. Anim. Sci.* 73:954-959.
- Rhodes, F.M., S. McDougall, C.R. Burke, G. A. Verkerk, and K. L. Macmillan. 2003. Invited Review: Treatment of cows with an extended postpartum anestrous interval. *J. Dairy Sci.* 86:1876-1894.
- Roberts, A. J. and T. G. Jenkins. 2002. Effects of varying energy intake and sire breed on duration of postpartum anestrus, IGF-1 and GH in mature crossbred cows. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 53:454-456.
- Smith, V. G., J.R. Chenault, J. F. McAllister, and J. W. Lauderdale. 1987. Response of postpartum beef cows to exogenous progestogens and gonadotropin releasing hormone. *J. Anim. Sci.* 64:540.
- Stevenson, J.S., D.P. Hoffman, D.A. Nichols, R.M. McKee, and C.L. Krehbiel. 1997a. Fertility in estrus-cycling and noncycling virgin heifers and suckled beef cows after induced ovulation. *J. Anim. Sci.* 75:1343-1350.

Table 1. Distribution of cows by age and treatment at each location

Age	<u>Location 1</u>		<u>Location 2</u>	
	<u>CIDR</u>	<u>Control</u>	<u>CIDR</u>	<u>Control</u>
2	13	18	----	----
3	46	34	11	6
4	52	60	26	30

Table 2. The minimum, maximum, and standard error for body weight, days postpartum (PP), and BCS on d 0 of the study for cows within treatment groups at locations 1 and 2

	<u>BW (kg)</u>			<u>d PP</u>			<u>BCS</u>		
	Min	Max	SE	Min	Max	SE	Min	Max	SE
<b>Location 1(n =224)</b>									
<b>CIDR + PGF<sub>2α</sub></b>	864	1488	13.7	8.0	91	1.69	2.7	6.1	.06
<b>PGF<sub>2α</sub></b>	802	1472	15.0	4.0	92	1.74	3.1	5.2	.05
<b>Location 2 (n=73)</b>									
<b>CIDR + PGF<sub>2α</sub></b>	882	1376	20.97	23	69	1.55	3.8	6.2	.09
<b>PGF<sub>2α</sub></b>	834	1288	19.3	28	73	1.77	3.6	6.3	.09

Table 3. Least squares means ( $\pm$  SE), number of estrous cycles to conception (21 d periods), final number of estrous cycles for pregnancy (includes losses between first and second pregnancy diagnosis), days to conception, and days to final pregnancy at location 1 and 2 and for CIDR (CIDR + PGF<sub>2α</sub>) and Control (single i.m. injection PGF<sub>2α</sub>) treatments

	<b>Average estrous cycles to conception</b>	<b>Average estrous cycles to final pregnancy</b>	<b>Average d to conception</b>	<b>Average d to final pregnancy</b>
Location				
<b>Location 1</b>	1.43 $\pm$ .05 <sup>a</sup>	1.59 $\pm$ .07 <sup>a</sup>	20.6 $\pm$ 1.2 <sup>a</sup>	24.0 $\pm$ 1.5 <sup>a</sup>
<b>Location 2</b>	1.76 $\pm$ .11 <sup>a</sup>	1.79 $\pm$ .14 <sup>a</sup>	30.1 $\pm$ 2.7 <sup>a</sup>	30.6 $\pm$ 3.2 <sup>a</sup>
Treatment				
<b>CIDR + PGF<sub>2α</sub></b>	1.53 $\pm$ .07 <sup>b</sup>	1.73 $\pm$ .09	23.9 $\pm$ 1.7 <sup>b</sup>	25.9 $\pm$ 2.07
<b>PGF<sub>2α</sub></b>	1.66 $\pm$ .07 <sup>b</sup>	1.86 $\pm$ .09	26.9 $\pm$ 1.7 <sup>b</sup>	28.8 $\pm$ 2.0

<sup>a</sup> $P < 0.001$  for comparison between Location.<sup>b</sup> $P < 0.13$  for comparison between Treatments.

# GLUCOSE HALF-LIFE OF YOUNG POSTPARTUM LACTATING COWS WAS HALF THAT OF NON-LACTATING HERDMATES

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**ABSTRACT:** Lactation and diet quality have been implicated as regulators of nutrient partitioning by changing tissue sensitivity to insulin. Treatments were arranged as a 2 x 2 factorial to investigate the influence of lactation and season on serum glucose clearance. Glucose tolerance tests (GTT) were conducted on lactating (LACT, n = 4) and non-lactating (NLACT, n = 4) three-year-old crossbred beef cows grazing dormant native range in May (57 d postpartum) and July (135 d postpartum). In January, before calving, NLACT cows were heavier ( $P < 0.01$ ) than LACT cows ( $468 \pm 18$  vs  $414 \pm 9$  kg); all cows were body condition score (BCS)  $4.6 \pm 0.2$ . Calves from NLACT cows did not survive after parturition. NLACT cows gained condition ( $P = 0.08$ ) after calving (May BCS  $5.9 \pm 0.1$ ; July BCS  $6.5 \pm 0.3$ ) while LACT cows maintained condition (May BCS  $3.9 \pm 0.2$ ; July BCS  $4.4 \pm 0.1$ ). For each GTT, 50% dextrose solution was infused at 0.5 mL/kg BW via jugular catheter and serum was collected at 11 time intervals for 120 min. Serum glucose and insulin areas-under-the-curve (AUC) and glucose half-lives were calculated. Glucose and insulin AUC (glucose: 7299 vs 10599  $\pm$  1173 units; insulin: 185 vs 361  $\pm$  17 units) were smaller (glucose:  $P = 0.08$ ; insulin:  $P < 0.01$ ) for LACT cows than for NLACT cows. Glucose half-life was nearly 50% less ( $P = 0.03$ ) for LACT compared to NLACT cows (53 vs 100  $\pm$  12 min) and was longer ( $P = 0.08$ ) in July compared to May (94 vs 58  $\pm$  12 min). Diet quality as affected by season did influence glucose half-life. LACT cows were more responsive to insulin than NLACT cows since they cleared glucose in less time with less insulin, although it would be expected that NLACT cows would clear glucose in less time than LACT cows. Body condition may be as important as lactation in the regulation of glucose clearance.

Key Words: Glucose, Insulin, Lactation, Physiological state

## Introduction

Lactation and diet quality have been implicated as regulators of nutrient partitioning by changing tissue sensitivity to insulin (Bines and Hart, 1982; Tovar-Luna et al., 1995). Endecott et al. (2003) found that glucose half-life decreased in young postpartum cows from spring to summer. Unfortunately, the effect of season was confounded with stage of lactation, so it was not possible to determine whether the increased sensitivity to insulin was due to improved diet quality, progression of lactation, or an

additive effect of both. Cows were at different stages of lactation in spring and summer, and insulin sensitivity increases as lactation progresses (Bines and Hart, 1982). In order to further explore the influences of physiological state and seasonal diet quality changes on insulin sensitivity, we investigated tissue response to insulin and glucose clearance of lactating and non-lactating cows grazing dormant forage after 57 or 135 d postpartum.

## Materials and Methods

The New Mexico State University Institutional Animal Care and Use Committee approved all animal procedures. Glucose tolerance tests (GTT) were conducted on lactating (LACT, n = 4) and non-lactating (NLACT, n = 4) three-year-old crossbred beef cows grazing dormant native range at the Corona Range and Livestock Research Center. The first GTT (May) was in mid-May when cows were approximately 57 d postpartum, and the second GTT (July) took place in late July when cows were approximately 135 d postpartum. Both LACT and NLACT cows were diagnosed pregnant the previous fall and all had calves; however, the NLACT cows' calves died shortly after birth due to complications or unknown causes. Prior to calving, NLACT cows were heavier ( $P < 0.01$ ) than LACT cows ( $468 \pm 18$  vs  $414 \pm 9$  kg); all cows were body condition score (BCS)  $4.6 \pm 0.2$ . NLACT cows gained condition ( $P = 0.08$ ) after calving (May BCS  $5.9 \pm 0.1$ ; July BCS  $6.5 \pm 0.3$ ) while LACT cows maintained condition (May BCS  $3.9 \pm 0.2$ ; July BCS  $4.4 \pm 0.1$ ). All cows grazed the same pastures for the duration of the experiment. At the time of the May GTT, cows were individually fed a 30% CP supplement twice weekly at a rate of 1135 g•cow<sup>-1</sup>•d<sup>-1</sup>; supplementation had ceased at the time of the July GTT. The May GTT was conducted on a day after supplement had been fed.

Cows were gathered from the pasture at daylight the morning of each GTT; cows had access to water, but not feed after they had been gathered. For each GTT, 50% dextrose solution was infused at 0.5 mL/kg BW via indwelling jugular catheter. Catheters were inserted the morning of each GTT. A 12-gauge hypodermic needle (Ideal Instruments, Schiller Park, IL) was used to puncture the jugular vein. One-half of a 2.5-m length of sterile Tygon tubing (0.10 cm i.d., 0.18 cm o.d., Cole-Parmer Instrument Company, Vernon Hills, IL) was threaded through the needle and into the jugular vein. The remaining half of the tubing was taped to the neck and back of the

cow. Tubing near the puncture site was adhered to the cow's neck in a looped fashion so that it would remain stationary. A blunted 18-gauge needle (Becton-Dickinson, Franklin Lakes, NJ) was inserted in the end of the catheter and a 1-mL syringe (Air-Tite, Virginia Beach, VA) served as the tubing end cap. Serum was collected at -1, 0, 3, 6, 9, 12, 15, 20, 40, 60, 80, 100, and 120 min relative to infusion with the time-zero sample collected immediately after infusion. Catheters were flushed with 10 mL of saline solution immediately before and after each blood sample was collected and after glucose infusion. Blood samples (10 mL) collected at each sampling time were placed in Corvac serum separator tubes and centrifuged at  $2000 \times g$  at  $4^{\circ} \text{C}$  for 25 min for serum collection. Serum was stored in plastic vials at  $-20^{\circ} \text{C}$  for later analysis of glucose and insulin. Serum glucose concentrations were determined with a commercial kit ([Trinder] method, Sigma Diagnostics, St. Louis, MO), with volume modifications for a 96-well plate reader. Serum insulin was analyzed at the New Mexico State University Endocrinology Laboratory by solid-phase radioimmunoassay (DCP kit, Diagnostic Products Corp., Los Angeles, CA) as validated by Reimers et al. (1982). Intra-assay and inter-assay CV were 6.2% and 3.0%, respectively. Serum glucose and insulin areas under the curve (AUC) were calculated using trapezoidal summation. Glucose half-life was estimated by determining the time required for a 50% decrease in peak serum glucose concentration.

Data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) with cow as the experimental unit symmetry covariance structure. The model included effects of physiological state, season, and their interaction.

## Results and Discussion

The interaction of physiological state and season was not significant for glucose AUC ( $P = 0.21$ ), insulin AUC ( $P = 0.71$ ), or glucose half-life ( $P = 0.11$ ), suggesting that both physiological states responded to season similarly. Both glucose AUC (7299 vs  $10599 \pm 1173$  units) and insulin AUC ( $185$  vs  $361 \pm 17$  units) were smaller ( $P = 0.08$  and  $P < 0.01$ , respectively) for LACT cows than for NLACT cows. Glucose half-life was nearly 50% shorter ( $P = 0.03$ ) for LACT compared to NLACT cows ( $53$  vs  $100 \pm 12$  min). Both groups of cows exhibited extended glucose half-lives compared to the normal of 35 min (Kaneko, 1989); however it appears that LACT cows were more responsive to the effects of insulin than were NLACT cows. Because NLACT cows did not have the physiological changes occurring due to lactation, it would be expected that they would be more responsive to insulin than LACT cows (Bines and Hart, 1982). The negative effect of lactation on tissue responsiveness to insulin may be caused by growth hormone, which is elevated during this physiological state (Bines and Hart, 1982). Growth hormone apparently blocks the phosphorylation of glucose inside the cell after uptake (Ojeda and McCann, 2000); glucose freely diffuses back out of the cell and thus insulin has a limited effect. Weekes (1991) found that insulin

action was impaired in lactating ewes fed a restricted diet compared with non-lactating ewes and with lactating ewes fed ad libitum, which disagrees with the results from the present study. A potential explanation for the fact that LACT cows were more insulin responsive than NLACT cows may be differences in body condition, as the NLACT cows were in higher body condition than LACT cows for both GTT. Insulin AUC above baseline were smaller in lean compared to obese dairy heifers (McCann and Reimers, 1986), and lean dairy heifers were more responsive to insulin than were obese heifers after GTT (McCann and Reimers, 1985). Bergman et al. (1989) produced severe adult-onset obesity in ewes by overfeeding and found that obese sheep had decreased insulin sensitivity compared to lean controls, consistent with decreased insulin receptors in peripheral tissues. Serum glucose concentrations of the NLACT cows did not return to baseline in the 120 min after infusion at the July GTT, which agrees with the hypothesis that increased body condition has a negative effect on insulin sensitivity.

Diet quality, as affected by season, did not significantly influence glucose AUC ( $P = 0.14$ ) or insulin AUC ( $P = 0.42$ ), but did influence ( $P = 0.08$ ) glucose half-life, which was longer in July compared to May ( $94$  vs  $58 \pm 12$  min). Endecott et al. (2003) found that glucose half-life decreased from spring to summer, which appears to directly contradict the results of the present study. However, in the previous study, diet quality improved from spring to summer due to summer precipitation. In the present study, a lack of summer precipitation resulted in lower diet quality in the summer compared to the spring. Therefore, results from both the 2002 experiment (Endecott et al., 2003) and the present study support the hypothesis that diet quality does have an impact on glucose half-life. Additionally, the absence of an interaction between physiological state and seasonal diet quality further supports the interpretation of Endecott et al. (2003) that seasonal influences are independent of physiological state. If these were dependent, cows in different physiological states would have been expected to respond differently to seasonal diet quality changes.

## Implications

LACT cows were more responsive to insulin than NLACT cows as they cleared glucose in less time with less insulin, although it would be expected that NLACT cows would clear glucose in less time than LACT cows. Over-conditioned cows may be less efficient at nutrient uptake than thinner cows if they are insensitive to insulin. Body condition may be as important as lactation in the regulation of glucose clearance. Supplementation to improve energy balance of range cows is a common practice, particularly post-calving. Seasonal differences in insulin sensitivity may not allow the nutrients supplied by a supplement to be efficiently utilized for realimentation. Thus, supplementation when only dormant forage is available may not yield expected results if cows are insensitive to insulin at the time of supplementation.

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## **Literature Cited**

- Bergman, E. N., S. S. Reulein, and R. E. Corlett. 1989. Effects of obesity on insulin sensitivity and responsiveness in sheep. *Am. J. Physiol. Endocrinol. Metab.* 257:E772-E781.
- Bines, J. A., and I. C. Hart. 1982. Metabolic limits to milk production, especially roles of growth hormone and insulin. *J. Dairy Sci.* 65:1375-1389.
- Endecott, R. L., D. L. Dunlap, R. C. Waterman, A. C. Fitzgerald, V. A. Munn, K. L. Shirley, S. H. Cox, J. A. Hartung, C. A. Loest, and M. K. Petersen. 2003. Glucose half-life decreased in young postpartum cows from spring to summer. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 54:27-29.
- Kaneko, J. J. 1989. Carbohydrate metabolism and its diseases. In: J. J. Kaneko (ed.) *Clinical biochemistry of domestic animals*. Academic Press, San Diego, CA.
- McCann, J. P., and T. J. Reimers. 1985. Insulin response to glucose in estrous and diestrous obese and lean heifers. *J. Anim. Sci.* 61:619-623.
- McCann, J. P., and T. J. Reimers. 1986. Effects of obesity and glucose metabolism in cyclic heifers. *J. Anim. Sci.* 62:772-782.
- Ojeda, S. R., and S. M. McCann. 2000. The anterior pituitary and hypothalamus. In: J. E. Griffin and S. R. Ojeda (eds.) *Textbook of endocrine physiology*. p 128-162. Oxford University Press, Inc., New York, NY.
- Reimers, T. J., R. G. Cowan, J. P. McCann, and M. W. Ross. 1982. Validation of a rapid solid-phase radioimmunoassay for canine, bovine and equine insulin. *Am. J. Vet. Res.* 43:1274-1278.
- Tovar-Luna, I., J. S. Serrato-Corona, W. S. Ramsey, J. Bruemmer, and M. K. Petersen. 1995. Effect of frequency of escape protein supplementation on body weight, metabolic hormones, and blood metabolites in yearling heifers fed a roughage diet. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 46:9-12.
- Weekes, T. E. C. 1991. Hormonal control of glucose metabolism. In: T. Tsuda, Y. Sasaki and R. Sawashima (eds.) *Physiological aspects of digestion and metabolism in ruminants*. p 183-200. Academic Press, San Diego, CA.

# ENDOGENOUS PROSTAGLANDIN F<sub>2α</sub> CONCENTRATIONS IN BOVINE WHOLE SEMEN, SEMINAL PLASMA AND EXTENDED SEMEN

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**ABSTRACT:** Three experiments were conducted to quantify prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) in bovine semen, seminal plasma and extended semen by enzyme immunoassay. In experiment 1, PGF<sub>2α</sub> was measured in paired samples of whole and extended semen from beef (n = 6) and dairy bulls (n = 18). Levels of PGF<sub>2α</sub> did not differ between beef and dairy (273.8 ± 42.8 vs. 210.3 ± 18.5 pg/ml, respectively; P = 0.12), but tended to be greater for whole compared to extended semen (255.5 ± 29.8 vs. 194.5 ± 17.0 pg/ml, respectively; P = 0.08). In experiment 2, to elucidate why PGF<sub>2α</sub> levels in extended semen were comparable to whole semen, semen from dairy bulls (n = 7) was extended at eight dilutions (1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40 and 1:80). Semen was extended using a diluent consisting of two fractions: A (egg yolk based) and B (glycerol based). Samples collected after semen addition to fraction A and after each addition of fraction B resulted in four sub-samples. Prostaglandin F<sub>2α</sub> in sub-samples decreased at higher dilution rates and later steps of extension (P < 0.001). To quantify PGF<sub>2α</sub> synthesized during extension, amounts of PGF<sub>2α</sub> in semen and fractions A and B (52.8 and 87.7 pg/ml, respectively) were subtracted from each step. Higher dilution rates reduced the final amount of PGF<sub>2α</sub> synthesized (P < 0.001). In experiment 3, paired samples of whole semen and seminal plasma from dairy bulls (n = 7) were extended at three dilutions (1:15, 1:20 and 1:25). Initial PGF<sub>2α</sub> concentration was greater in whole semen compared to seminal plasma (430.0 ± 37.2 vs. 62.2 ± 15.0 pg/ml, respectively; P < 0.001). During extension, PGF<sub>2α</sub> synthesis resulted in less disparity than for original samples, but amount synthesized was greater for semen compared to seminal plasma (194.5 ± 15.8 vs. 150.5 ± 10.9 pg/ml, respectively; P = 0.03) and was not affected by dilution rate (P = 0.41). These data suggest that, although extension reduces the concentration of many seminal components, PGF<sub>2α</sub> synthesis during extension results in concentrations similar to whole semen.

Key Words: prostaglandin F<sub>2α</sub>, semen, bovine

## Introduction

Transport of sperm from the site of deposition, by natural service or artificial insemination (AI), is a critical

component of the reproductive process. A significant proportion of failed conceptions can be attributed to fertilization failure caused by impaired sperm transport. Forces involved in sperm transport from the site of semen deposition to the oviductal ampulla include smooth muscle contractions, ciliary beats and sperm flagellar motion.

Myometrial contractions appear to be an important component of sperm transport. Hawk and Echternkamp (1973) reported that ewes with inhibited sperm transport to the oviducts displayed a decreased number of uterine contractions moving toward the oviducts and an increased number moving toward the cervix compared to ewes with normal sperm transport. Prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) stimulates bovine myometrial contractions (Patil et al., 1980) and porcine myometrium exposed to 10<sup>-9</sup> to 10<sup>-5</sup> M PGF<sub>2α</sub> displayed a dose-dependent increase in contractility (Yu et al., 1993). In addition, Cheng et al. (2001) reported that PGF<sub>2α</sub> (1.76 X 10<sup>-9</sup> to 1.76 X 10<sup>-7</sup> M) added to extended boar semen or to extender alone increased *in vitro* myometrial contractility. In humans, intravenous administration of PGF<sub>2α</sub> and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), at 50 µg each resulted in uterine contractions, weak stimulation of tubal motility, and an increase in intraovarian pressure (Coutinho and Maia, 1971). Kelly (1978) reported that prostaglandin concentration in human semen seemed to be correlated with fertility when control patients were compared to patients in which infertility could not otherwise be explained, and prostaglandin concentration in this semen was not correlated with sperm count or motility.

Garner et al. (2001) observed improved post-thaw viability when bovine seminal plasma was added to extended bovine semen. These researchers also reported increased sperm metabolism and motility following addition of seminal plasma. There are numerous reports of improved fertility following administration of PGF<sub>2α</sub> near or at the time of breeding. Prostaglandin F<sub>2α</sub>, but not PGE<sub>1</sub> or PGE<sub>2</sub>, injected just before AI increased the number of embryos in rabbits (Spilman et al., 1973) and PGF<sub>2α</sub> injected at the time of AI improved farrowing rates of sows (Peña et al., 1998). Addition of PGF<sub>2α</sub> to semen has improved pregnancy rates in ewes (Gustafsson et al., 1975) and enhanced farrowing rates and litter sizes in sows (Peña et al., 2000). Improvement in fertility may be due in part to the observation of greater numbers of sperm recovered from each segment of the reproductive tract when ewes received a PGF<sub>2α</sub> injection before AI or were inseminated with PGF<sub>2α</sub>-supplemented semen (Edqvist et al., 1975).

Prostaglandin F<sub>2α</sub> has been reported to still elicit its affects after storage in extended fresh semen and in

<sup>1</sup>The authors wish to express gratitude to Select Sires Inc. for the generous donation of whole and extended semen, seminal plasma and diluent used in this study.

extended frozen semen. Addition of 5 mg PGF<sub>2α</sub> to extended boar semen remained bioactive, eliciting the same myometrial contractility, at 72 h post-addition when semen was stored at 17 ± 1 °C (Cheng et al., 2001). It has also been reported that addition of PGF<sub>2α</sub> to extended ram semen before freezing has no effect on post-thaw motility, morphology or survival of spermatozoa; moreover, it was demonstrated that this PGF<sub>2α</sub>-enhanced semen improved the pregnancy rate of ewes bred with this semen compared to ewes bred with semen lacking supplementary PGF<sub>2α</sub> (Gustafsson et al., 1975). In contrast, addition of increasing amounts PGF<sub>2α</sub> to bovine semen during the extension process resulted in a dose-dependent decrease in post-thaw motility (Abbitt et al., 1977).

Thin layer chromatography revealed that pooled bull semen contained 3.28 X 10<sup>-6</sup> M prostaglandin of the F series (Ledwozyw et al., 1986). This concentration is significantly greater than reported by Voglmayr (1973) when radioimmunoassay was utilized to measure PGF<sub>2α</sub> in genital tract secretions from dairy bulls and were reported to be 0.17 ng/ml. To our knowledge, PGF<sub>2α</sub> concentrations have not been previously determined in whole semen collected from beef bulls or in extended bovine semen.

Due to the equivocal PGF<sub>2α</sub> concentrations previously reported for bovine semen and lack of information pertaining to the concentration of PGF<sub>2α</sub> present in extended bovine semen, the quantity of PGF<sub>2α</sub> that could be added during the extension of bovine semen to enhance fertility is ambiguous. Therefore, the objective of the current study was to determine the endogenous concentrations of PGF<sub>2α</sub> whole and extended semen.

## Materials and Methods

### Experiment 1

Semen was collected from beef (n = 6) and dairy bulls (n = 18) at an AI stud farm using an artificial vagina. Following semen collection, a 1 ml aliquot of whole semen was harvested and immediately frozen. The remaining semen was extended following the normal protocol for this AI stud farm. Following shipment to the laboratory, samples of whole and extended semen were thawed, centrifuged at 15,000 x G, and the resulting supernatant was frozen at -20 °C until analysis for PGF<sub>2α</sub> concentration.

### Experiment 2

Semen was collected from dairy bulls (n = 7) at an AI stud farm using an artificial vagina. Following collection, a 1 ml aliquot of whole semen was harvested and immediately frozen. Following shipment to the laboratory, semen was thawed and extended at eight dilutions (1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, and 1:80). Semen was extended using a diluent consisting of two fractions: A (egg yolk based) and B (glycerol based) following an industry protocol. This protocol requires a four minute equilibration period after semen addition to fraction A and following each addition of fraction B. Samples collected after addition of semen to fraction A and

after each addition of fraction B resulted in four sub-samples. Sub-samples were centrifuged at 15,000 x G and the resulting supernatant was frozen at -20 °C until analysis for PGF<sub>2α</sub> concentration.

### Experiment 3

Semen was collected from dairy bulls (n = 7) at an AI stud farm using an artificial vagina. Following collection, a 1 ml aliquot of whole semen was harvested and immediately frozen. An additional 1.5 ml aliquot of whole semen was centrifuged at 15,000 x G and the resulting supernatant was harvested and immediately frozen. Following shipment to the laboratory, whole and seminal plasma samples were thawed and extended at three dilutions (1:15, 1:20, and 1:25) following the protocol described in Experiment 2. The resulting sub-samples were centrifuged at 15,000 x G, the supernatant was harvested and frozen at -20 °C until analysis for PGF<sub>2α</sub> concentration.

### Prostaglandin F<sub>2α</sub> Assay

Concentration of PGF<sub>2α</sub> in whole and extended semen and in sub-samples collected during the extension of whole semen and seminal plasma were determined by use of PGF<sub>2α</sub>-acetylcholinesterase (AChE) Competitive Enzyme Immunoassay (Cayman Chemical Company, Ann Arbor, MI). The sensitivity of the assay was 8 pg/ml, and the intra- and interassay coefficients of variation were 7.50% and 17.10%, respectively.

### Statistical Analysis

Prostaglandin F<sub>2α</sub> concentrations in whole and extended semen (Experiment 1) were analyzed by analysis of variance in SAS (SAS Inst. Inc., Cary, NC). Concentration of PGF<sub>2α</sub> in sub-samples collected during the extension of whole semen and seminal plasma (Experiments 2 and 3) were analyzed as repeated measures (step of extension process) by analysis of variance in SAS using the proc mixed statement. The statistical model consisted of treatment (whole semen vs. seminal plasma), dilution rate, step of extension process, and their interactions. To quantify amount of PGF<sub>2α</sub> synthesized during extension of whole semen and seminal plasma (Experiments 2 and 3), quantity of PGF<sub>2α</sub> present in semen, seminal plasma and fraction A and B of the diluent was subtracted from each respective dilution step before final analysis. Direct comparison of final PGF<sub>2α</sub> concentrations following extension of whole semen or seminal plasma were analyzed by analysis of variance and difference between means was determined with pair-wise t-tests.

## Results and Discussion

### Experiment 1

Quantity of PGF<sub>2α</sub> present in whole semen was slightly greater in samples collected from beef bulls (273.8 ± 29.8 pg/ml) compared to the concentration present in

samples collected from dairy bulls ( $210 \pm 18.5$  pg/ml); however this difference was not statistically different ( $P = 0.12$ ). These concentrations are significantly greater than was previously reported for bovine semen (170 pg/ml; Voglmayr 1973).

Comparison of  $\text{PGF}_{2\alpha}$  concentrations in whole and extended semen, collected from beef and dairy bulls, yielded a smaller difference than had been expected. Prostaglandin  $\text{F}_{2\alpha}$  concentration in whole semen did tend to be greater ( $P = 0.08$ ) than in extended semen ( $255.5 \pm 29.8$  vs.  $194.5 \pm 17.0$  pg/ml, respectively). However, following extension of bovine semen at the average industry dilution rate of 1:20, based on the average concentration of 255.5 pg/ml in whole semen observed in this study, a concentration of approximately 12.8 pg/ml would be expected to be present in extended semen. This augmentation of  $\text{PGF}_{2\alpha}$  concentration present in the extended semen could not be attributed to the diluent. Concentration of  $\text{PGF}_{2\alpha}$  was determined to be 52.8 pg/ml in fraction A and 87.7 pg/ml in fraction B.

In a previous study, when  $\text{PGF}_{2\alpha}$  was added to the glycerol fraction (fraction B) and final concentrations in extended bovine semen were calculated to be either 0  $\mu\text{g/ml}$ , 75  $\mu\text{g/ml}$ , 225  $\mu\text{g/ml}$ , or 675  $\mu\text{g/ml}$   $\text{PGF}_{2\alpha}$ , post-thaw motility of this semen following a 2 h incubation was 19.3, 17.8, 13.6, and 5.8%, respectively (Abbitt et al. 1974). If the observed phenomenon of  $\text{PGF}_{2\alpha}$  synthesis during semen extension also occurred during this previous study, final concentrations of  $\text{PGF}_{2\alpha}$  would have been nearly a million fold greater than endogenous levels observed in whole semen. This superfluous amount of  $\text{PGF}_{2\alpha}$  could have resulted in the disparaging effect on sperm motility. In contrast, 2500 ng/ml  $\text{PGF}_{2\alpha}$  added to sperm collected from subfertile men increased sperm motility; however a dose of 25,000 ng/ml (25  $\mu\text{g/ml}$ ), which exceeded physiological levels, reversed this effect and resulted in no net improvement in sperm motility (Grunberger et al., 1981). These data suggest that  $\text{PGF}_{2\alpha}$  may have paradoxical effects on sperm function, whereby addition of  $\text{PGF}_{2\alpha}$  at levels only slightly above endogenous levels may have positive effects on sperm motility, and addition of  $\text{PGF}_{2\alpha}$  at levels significantly greater than endogenous levels may have detrimental effects on sperm function.

### Experiment 2

Because the concentration of  $\text{PGF}_{2\alpha}$  in extended semen measured during Experiment 1 was greater than expected, semen was extended at eight dilution rates to elucidate if the quantity of semen initially added to the diluent would result in a dose-dependent response for quantity of  $\text{PGF}_{2\alpha}$  produced during the extension process. Prostaglandin  $\text{F}_{2\alpha}$  concentration in sub-samples decreased at higher dilution rates and later steps of extension ( $P < 0.001$ ; Figure 1), suggesting that a dose response does exist. To quantify  $\text{PGF}_{2\alpha}$  synthesized during extension, the amounts of  $\text{PGF}_{2\alpha}$  present in semen ( $\bar{X} = 255.5$  pg/ml) and fractions A and B (52.8 and 87.7 pg/ml, respectively) were subtracted from each sub-sample. Analysis of variance for

quantity of  $\text{PGF}_{2\alpha}$  present following semen extension revealed that higher dilution rates reduced the total amount of  $\text{PGF}_{2\alpha}$  synthesized during the extension process ( $P < 0.001$ ; Figure 2). However, there was no difference in quantity of  $\text{PGF}_{2\alpha}$  synthesized in extended semen diluted at 1:15, 1:20 or 1:25 which is inclusive of the range in which bovine semen is normally diluted. This suggests that sperm concentration, which determines semen dilution rate, will most likely not have an effect on the final  $\text{PGF}_{2\alpha}$  concentration present in extended semen unless the semen being extended is collected from a bull with abnormally low or high sperm concentration.

### Experiment 3

Initial  $\text{PGF}_{2\alpha}$  concentration was significantly greater ( $P < 0.001$ ) in whole semen compared to seminal plasma ( $430.0 \pm 37.2$  vs.  $62.2 \pm 15.0$  pg/ml, respectively). Following extension of these paired samples of whole semen and seminal plasma at 3 dilution rates (1:15, 1:20, and 1:25),  $\text{PGF}_{2\alpha}$  synthesis during the extension process resulted in less disparity than was observed in the original samples. As was observed in Experiment 2, after the quantity of  $\text{PGF}_{2\alpha}$  contributed from whole semen or seminal plasma and from fractions A and B of the diluent were subtracted, dilution rates of 1:10, 1:15 and 1:20 had no effect on the amount of  $\text{PGF}_{2\alpha}$  synthesized during the extension process, except for seminal plasma at the 1:20 dilution rate (Figure 3). However, when means for whole semen and seminal plasma were pooled for all dilution rates, the amount of  $\text{PGF}_{2\alpha}$  synthesized during extension was greater ( $P = 0.03$ ) for semen compared to seminal plasma ( $194.5 \pm 15.8$  vs.  $150.0 \pm 10.9$  pg/ml, respectively).

### Implications

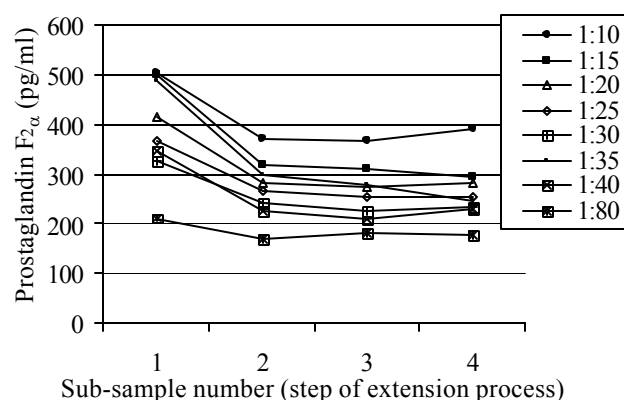
These data suggest that  $\text{PGF}_{2\alpha}$  is synthesized during extension of bovine semen, resulting in levels of  $\text{PGF}_{2\alpha}$  in extended semen near those observed in whole semen following collection with an artificial vagina. This documentation of the actual concentration of  $\text{PGF}_{2\alpha}$  present in whole and extended semen will assist in determining the quantity of  $\text{PGF}_{2\alpha}$  that could be added to extended bovine semen. Prostaglandin  $\text{F}_{2\alpha}$ -enhanced semen appears to have potential to improve conception rates following artificial insemination in cattle as has been observed following artificial insemination with  $\text{PGF}_{2\alpha}$ -enhanced semen in sows.

### Literature Cited

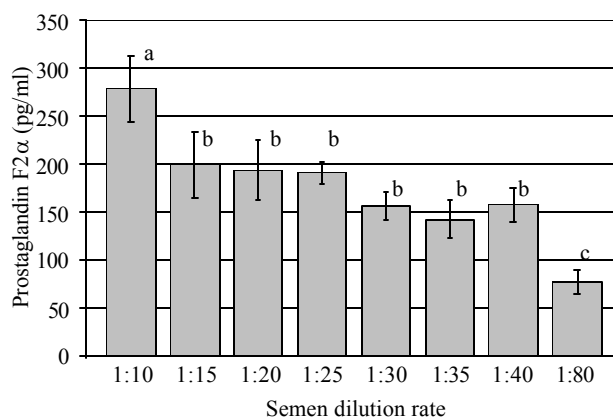
- Abbitt, B., G.E. Seidel, Jr., and W.E. Berndtson. 1977. Effect of tris (hydroxymethyl) aminomethane salt of prostaglandin  $\text{F}_{2\alpha}$  on post-thaw motility of bovine spermatozoa. *J. Dairy Sci.* 60:1991-1993.
- Cheng, H., G.C. Althouse, and W.H. Hsu. 2001. Prostaglandin  $\text{F}_{2\alpha}$  added to extended boar semen at processing elicits *in vitro* myometrial contractility 72 hours of storage. *Theriogenology*. 55:1901-1906.

- Coutinho, E.M., and H.S. Maia. 1971. The contractile response of the human uterus, fallopian tubes, and ovary to prostaglandins *in vivo*. Fertil. Steril. 22:539-543.
- Edqvist, S., S. Einarsson, and B. Gustafsson. 1975. Effect of prostaglandin  $F_{2\alpha}$  on sperm transport in the reproductive tract of the ewe. Acta Vet. Scand. 16:149-151.
- Garner, D.L., C.A. Thomas, C.G. Gravance, C.E. Marshall, J.M. DeJarnette, and C.H. Allen. 2001. Seminal plasma addition attenuates the dilution effect in bovine sperm. Theriogenology. 56:31-40.
- Gustafsson, B., S. Edqvist, and S. Einarsson. 1975. The fertility of deep-frozen ram semen supplemented with  $PGF_{2\alpha}$ . Acta Vet. Scand. 16:468-470.
- Hawk, H.W., and S.E. Echternkamp. 1973. Uterine contractions in the ewe during prostaglandin-regulated oestrus. J. Reprod. Fertil. 34:347-349.
- Kelly, R.W. 1978. Prostaglandins in semen: Their occurrence and possible physiological significance. International J. Andrology. 1:188-200.
- Ledwozyw, A., R. Pruszkowska, and A. Kadziolka. 1986. Isolation and identification of prostaglandins of the E and F series in testes and semen of lowland-black-white breed of bulls. Acta Physiologica Hungarica. 68:11-17.
- Patil, R.K., S.N. Sinha, S. Einarsson, and I. Settergren. 1980. The effect of prostaglandin  $F_{2\alpha}$  and oxytocin on bovine myometrium *in vitro*. Nord. Vet. Med. 32:474-479.
- Peña, F.J., J.C. Domínguez, B. Alegre, and J. Peláez. 1998. Effect of vulvomucosal injection of  $PGF_{2\alpha}$  at insemination on subsequent fertility and litter size in pigs under field conditions. Anim. Reprod. Sci. 52:63-69.
- Peña, F.J., J.C. Domínguez, J. Pelá, and B. Alegre. 2000. Intrauterine infusion of  $PGF_{2\alpha}$  at insemination enhances reproductive performance of sows during the low fertility season. The Vet. J. 159:259-261.
- Spilman, C.H., A.E. Finn, and J.F. Norland. 1973. Effect of prostaglandins on sperm transport and fertilization in the rabbit. Prostaglandins. 4:57-64.
- Voglmayr, J.K. 1973. Prostaglandin  $E_{\alpha}$  concentration in genital tract secretions of dairy bulls. Prostaglandins. 4:673-678.
- Yu, H., D.-H. Shin, and W.H. Hsu. 1993. Effects of oxytocin (OT), vasopressin (VP), and prostaglandin

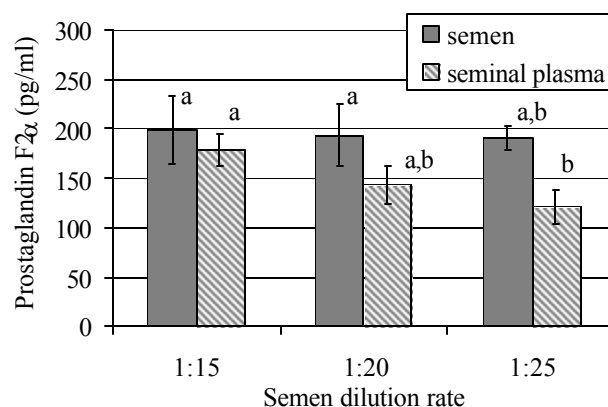
$F_{2\alpha}$  ( $PGF_{2\alpha}$ ) on porcine myometrial contractility *in vitro* and their dependence on  $Ca^{2+}$  influx. Biol. Reprod. 48(Suppl.):162. (Abstr.)



**Figure 1.** Prostaglandin  $F_{2\alpha}$  concentrations in sub-samples following each addition of diluent during extension of whole semen at eight dilution rates (1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, and 1:80). Dilution rate x dilution step interaction ( $P < 0.001$ ).



**Figure 2.** Mean  $PGF_{2\alpha}$  synthesis during extension of bovine semen at eight dilution rates. Bars lacking a common letter are different ( $P < 0.001$ ).



**Figure 3.** Mean  $PGF_{2\alpha}$  synthesis during extension of bovine whole semen or seminal plasma at three dilution rates. Bars lacking a common letter are different ( $P < 0.08$ ).

## RESPONSE OF GESTATING BEEF COWS TO LIMIT-FED DIETS CONTAINING ROLLED BARLEY

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**ABSTRACT:** In the Northern Great Plains, barley grain may be a more economical source of energy than hay. An experiment was conducted at South Dakota State University's Cottonwood Research Station to determine the efficacy of limit-fed, barley-based diets as an alternative to alfalfa hay for beef cows in late gestation. Ninety-six gestating, crossbred cows (age 3 to 11 years; average calving date of May 7) were stratified by age and weight and randomly assigned to one of 12 pens (8 cows/pen). Pens were randomly allotted to one of three winter feeding treatments (4 pens/treatment) from January 15 to April 10, 2003. Treatments were: 1) course-ground alfalfa hay (Hay; fed at approximately 1.6% of BW); 2) dry rolled barley replacing alfalfa hay at 29% of the diet (Low Barley; fed at approximately 1.4% of BW); 3) dry rolled barley replacing alfalfa hay at 67% of the diet (High Barley; fed at approximately 1.2% of BW). All diets were formulated using the 1996 NRC computer model to provide for maintenance of body condition score. A supplement (0.23 kg/d) supplied adequate protein, minerals, vitamins, and 200 mg/hd/d of Rumensin. Rations changed monthly to account for changing cow requirements during late gestation. All diets were consumed within a two-hour period each day. Treatment means were separated using orthogonal contrasts (Hay versus High and Low Barley; High Barley versus Low Barley). Cows fed barley gained more weight than Hay cows ( $P < 0.01$ ; weight change of 36, 59, and 60 kg for Hay, Low Barley, and High Barley, respectively). Cows fed barley also gained more body condition than Hay cows ( $P < 0.01$ ; body condition score change of -0.10, 0.24, and 0.38 for Hay, Low Barley, and High Barley, respectively). There were no differences ( $P > 0.10$ ) in weight or body condition score change between Low and High Barley treatments. There were no differences between treatments in subsequent pregnancy rates ( $P > 0.50$ ). Rolled barley can be used to replace alfalfa hay in diets for gestating beef cows.

Key Words: Limit-fed, Barley, Cows, Wintering, Gestation

### Introduction

Feed costs in many operations account for the largest proportion of operating costs. Limit feeding concentrate diets can lower feed costs while maintaining performance during gestation (Loerch, 1996). South Dakota can have harsh, severe winter conditions, which increase the maintenance requirements of beef cattle. It is not clear that limit feeding will work under such conditions.

The availability of barley is abundant in South Dakota with fifty-four percent (USDA) of United States' barley production coming from North Dakota, South Dakota, Minnesota, and Montana. Barley is a cheaper source of energy than hay in many situations. Most studies with barley have been with growing cattle, and most limit feeding studies with cows have used corn as a concentrate source. Loerch (1996) used limit-fed corn to maintain weight and lower feed cost on late gestating and early lactating cows. We hypothesize that barley can maintain cow body weight and body condition score during the winter months for cows in late gestation.

The objectives of this study were to evaluate body weight, body condition score, and reproduction on late gestating cows limit fed various levels of barley.

### Materials and Methods

This study was conducted from January 15 to April 10, 2003 at South Dakota State University's Cottonwood Range and Livestock Research Station, near Philip, SD. Ninety-six gestating, crossbred cows (age 3 – 11 yr; average calving date of May 7) were blocked by summer management, stratified by age, weight, body condition score, and randomly allotted to one of 12 pens (8 cows/pen). Pens were randomly allotted to one of three winter feeding treatments (4 pens/treatment): 1) alfalfa hay (**Hay**); 2) rolled barley replacing alfalfa hay at 29% of the diet (**Low Barley**); 3) rolled barley replacing alfalfa hay at 67% of the diet (**High Barley**).

Cows were housed in confinement pens and fed rations once daily in concrete bunks. All diets were formulated using the 1996 NRC computer model to result in maintenance of body condition score (Table 1). Rations changed monthly to account for changing cow requirements during late gestation. Alfalfa hay was course ground and analyzed 19.7% CP and 32.5% ADF (DM basis). Barley was dry rolled and analyzed 11.0% CP and 6.4% ADF (DM basis). A supplement (Table 2) was fed to all treatments at a rate of 0.23 kg/d throughout the trial and supplied 200 mg of Rumensin to each cow daily. All diets were adequate in degradable intake protein, undegradable intake protein, and minerals.

Cows were limit fed alfalfa hay for 5-d prior to initial weights. On d 1 of the trial (January 15), cows fed Low Barley and High Barley treatments were fed an adaptation diet of approximately 85% hay and 15% barley. On d 2, Low and High Barley cows were then placed on the Low Barley diet (Table 1). After four days on the Low Barley diet, High Barley cows were fed a third adaptation

diet consisting of 55% hay and 45% barley for an additional 6 d prior to being moved to their treatment diet (Table 1). Cows were limit fed the Hay diet for three days prior to the final weight measurements. The High Barley cows were fed the Low Barley Diet for two days prior to being placed on the final hay ration (adaptation to the hay). Cows were weighed on two consecutive days and a body condition score was assigned by two trained technicians at the beginning and end of the experiment. Pregnancy was determined by rectal ultrasonography in October of 2003.

Performance data were analyzed by ANOVA and means compared with orthogonal contrasts: Hay versus Barley (Low and High Barley); Low Barley versus High Barley. Due to management decisions unrelated to treatments, only 73 cows were available for pregnancy determination (26 cows in the Hay treatment, 25 cows in the Low Barley, and 22 cows in the High Barley treatment). Cows in this study were on one of two subsequent summer treatments (low versus high sulfate water). Pregnancy data were analyzed in Proc Genmod of SAS as a randomized complete block, with pen as the observation, animal as the trial within observation, and summer treatment as the block. (SAS Inst. Inc., Cary, NC)

### Results and Discussion

Daily feed was consumed within a 2-h period each day for all treatments. No digestive or health problems were observed. All cows gained weight over the course of the experiment (Table 3). Cows consuming Low Barley and High Barley had more weight gain ( $P < 0.01$ ) than those fed hay. In addition, the hay fed cows lost body condition during the experiment, whereas cows fed barley gained body condition ( $P < 0.01$ , Table 3). There were no differences ( $P > 0.10$ ) between the Low Barley and High Barley groups for the variables measured. There were no differences in pregnancy rates between treatments ( $P = 0.86$ , Table 3).

Loerch (1996) compared ad libitum hay and corn-based diets. The composition of hay was mainly orchardgrass with a small portion of alfalfa (approximately 75% NDF and 10.2% CP). Loerch found in yr 1 that there were no differences in cow weights but a higher BCS change for crossbred gestating cows limit-fed corn versus

cows consuming ad libitum hay. Tjardes et al. (1998) compared ad libitum hay, limit-fed whole corn with hay, and limit-fed cracked corn with hay for cows in early lactation. All treatments were supplied with ad libitum trace mineralized salt. Cows and calves experienced temperatures ranging from  $-22.8^{\circ}$  to  $22.8^{\circ}\text{C}$  with an average low of  $-2.4^{\circ}\text{C}$  and average high of  $6.7^{\circ}\text{C}$ . Tjardes et al. (1998) found no differences in cow weight change or body condition score when comparing ad libitum hay to either of the corn treatments. During the current study, cows experienced temperatures ranging from  $-5^{\circ}\text{C}$  to  $17^{\circ}\text{C}$  with an average high of  $5^{\circ}\text{C}$  and average low of  $-10^{\circ}\text{C}$  (weather data taken from a national weather station located on the research station). The low temperatures during this study were not as severe as some winter weather conditions in South Dakota. The Barley diets in our study resulted in better performance, but it is important to note that all diets were limit fed.

Barley, like corn, will work as an alternative to hay as a wintering program for late gestating cows.

### Implications

Barley can be used to replace alfalfa hay in limit-fed diets for gestating beef cows. The use of barley in limit feeding of gestating cows is an option during periods of low and/or expensive forage supply. There is the need for a comparison of limit fed barley diets to full-fed hay diets.

### Literature Cited

- Loerch, S.C. 1996. Limit-feeding corn as an alternative to hay for gestating beef cows. *J. Anim. Sci.* 74:1211-1216.
- NRC. 1996. *Nutrient Requirements of Beef Cattle*. 7<sup>th</sup> ed. National Academy Press, Washington, DC.
- Tjardes, K.E., D.B. Faulkner, D.D. Buskirk, D.F. Parrett, L.L. Berger, N.R. Merchen, and F.A. Ireland. 1998. The influence of processed corn and supplemental fat on digestion of limit-fed diets and performance of beef cows. *J. Anim. Sci.* 76:8-17.
- USDA. Major World Crop Areas and Climatic Profiles. [www.usda.gov](http://www.usda.gov).

**Table 1.** Daily feed offered to gestating cows across three treatments during three periods in late gestation. (DM basis)

Ingredient	Hay	Low Barley	High Barley
<i>January 15 – February 13</i>			
Hay, kg/d	9.2	5.7	2.2
Barley, kg/d	0.0	2.4	4.8
Supplement, kg/d	0.2	0.2	0.2
<i>February 14 – March 14</i>			
Hay, kg/d	9.9	6.2	2.4
Barley, kg/d	0.0	2.6	5.3
Supplement, kg/d	0.2	0.2	0.2
<i>March 15 – April 6<sup>a</sup></i>			
Hay, kg/d	10.7	6.3	2.6
Barley, kg/d	0.0	2.8	5.7
Supplement, kg/d	0.2	0.2	0.2

<sup>a</sup>All cows were fed the Hay diet on April 7, 8, 9, and 10. Final weights were taken April 10 and 11.

**Table 2.** Nutrient content of supplement feed to gestating cows across three treatments in late gestation.

Item	Amount (DM Basis)
Crude Protein, %	27.28
Crude Fat, %	3.15
NE <sub>M</sub> , Mcal/kg	0.25
NE <sub>G</sub> , Mcal/kg	0.16
Calcium, %	4.30
Phosphorus, %	3.64
Potassium, %	1.10
Sulfur, %	0.87
Zinc, mg/kg	1209
Iron, mg/kg	1431
Manganese, mg/kg	1714
Copper, mg/kg	631
Sodium, %	4.31
Magnesium, %	1.08
Rumensin, mg/kg	1,000

**Table 3.** Weight and body condition score (BCS) of cows program fed alfalfa hay (Hay), rolled barley replacing alfalfa hay at 29% of the diet (Low Barely), or rolled barley replacing alfalfa hay at 67% of the diet (High Barley) during the last trimester of gestation.

Item	Hay	Low Barley	High Barley
Initial wt, kg	643	648	633
Final wt, kg	679	705	693
Avg. Weight Change, kg <sup>a</sup>	36	57	60
Initial BCS	5.89	5.93	5.76
Final BCS	5.79	6.17	6.14
Avg. BCS Change <sup>a</sup>	-0.10	0.24	0.38
Pregnancy Rate, %	92.3	92.0	95.5

<sup>a</sup>Significant contrast: Hay versus Barley: P < 0.01.

# REPRODUCTIVE CHARACTERISTICS OF GRASS FED LHRH IMMUNOCASTRATED *BOS INDICUS* BULLS

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**ABSTRACT:** Two field trials were conducted in Brazil to evaluate LHRH immunocastration of *Bos indicus* bulls (d 0 = 2 yrs of age). In study I, 72 bulls were randomly assigned to one of three treatment groups: immunized, castrated and intact. Immunized animals (n = 25) received a primary and two booster injections of LHRH fusion proteins on d 0, 141, and 287. Twenty-three bulls were castrated on d 141, and 24 served as intact controls. All animals were slaughtered on d 385 at 3 yr of age. In study II, 216 bulls were randomly assigned to the same three treatments as in study I plus a fourth treatment in which immunized bulls received an additional booster on d 639 (late immunized), with all animals being slaughtered on d 741 (4 yrs of age). LHRH antibodies were not detectable in the castrate or intact animals in either study. In both studies, scrotal circumference decreased as LHRH antibody binding increased in immunized bulls. By d 287 serum testosterone concentrations for immunized bulls in both studies was decreased when compared to intact controls ( $P < 0.01$ ). In study II, testes and epididymides weight (g) at slaughter were higher ( $P < 0.01$ ) for intact ( $500 \pm 17$  and  $60 \pm 2$ , respectively) than immunized bulls ( $173 \pm 22$  and  $26 \pm 2$ , respectively) and late immunized bulls ( $78 \pm 23$  and  $20 \pm 2$ , respectively), the same significant effect was seen in study I. At the end of each study, BW was higher ( $P < 0.01$ ) in the intact bulls compared to the castrated and immunocastrated animals. In the two studies the efficacy of this LHRH fusion protein vaccine to induce castration effects at slaughter was considered 93%. These data support the concept that immunocastration of bulls at 2 yrs of age was successful and has practical application as a tool for managing grass fattened bulls in Brazil.

**Keywords:** LHRH, Immunocastration, *Bos indicus*, Bulls

## Introduction

Immunocastration has been suggested as an alternative to surgical castration in male cattle as a means to eliminate testicular function and reduce adverse sexual and

aggressive behaviors (Robertson et al., 1979; Price et al., 2003). Immunization against LHRH causes a reduction in the gonadotropins LH and FSH without affecting other pituitary hormones (Awoniyi et al., 1993). The decline or absence of serum LH and FSH lead to atrophy of the gonads and impairment of reproductive function (Robertson et al., 1982; Adams et al., 1996; Miller et al., 2000). In the male, immunization against LHRH induces azoospermia indirectly by suppressing the concentration LH and FSH followed by the decline of testosterone resulting in a castration effect. In areas of Brazil where bulls are fattened on grass, these animals are left intact to take advantage of the growth promotion effect of the testes, this is important because hormone implants are not allowed in Brazil. Two years of age would be the desired age to castrate the bulls to suppress aggressive behavior since they are grazed in groups of up to 500 bulls. However, surgical castration at this age is too traumatic while an immunocastration could accomplish the goals without the stress and estimated 1-2% death loss due to surgical castration at this age. The present two studies were designed to examine the effectiveness of immunocastration with LHRH fusion proteins on 2-yr old pasture fattened bulls raised in central Brazil.

## Materials and Methods

### Preparation of Antigen

Fusion proteins ovalbumin-LHRH-7 and thioredoxin-LHRH-7 were prepared as previously described (Zhang et al., 1999; Quesnell et al., 2000). Ovalbumin-LHRH-7 contains seven LHRH sequences inserted at four different positions in the ovalbumin gene fragment, while thioredoxin-LHRH-7 contains seven LHRH sequences inserted at three distinct positions of the thioredoxin gene. The *E. coli* strain BL21(DE3) was used to express the proteins. Each gene construct expressed a 6-histidine sequence (His-tag, Novagen, 1994) at the carboxyl terminus to facilitate purification of the LHRH fusions proteins via nickel affinity chromatography. Purified proteins were combined at an equal molar basis to yield a total of 1.5 mg of protein per injection. The LHRH fusion proteins were emulsified in modified Freund's complete adjuvant (CalBiochem, San Diego, CA), containing *Mycobacterium butyricum* for the primary immunization and incomplete Freund's adjuvant for booster injections. Shipment of the anti-LHRH vaccine was approved by the Brazilian Ministry of Agriculture under document numbers 01.044, 01.021 and 184.

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## Animals and Treatments

Nelore-cross bulls were maintained on two separate ranches, in Mato Grosso, Brazil. Bulls were approximately two yr of age at the initiation of the study (d 0) and grazed *Brachiaria brizantha* pastures. Seventy-two bulls from ranch I, and 216 bulls from ranch II were utilized in study I, and II respectively. Bulls were randomly assigned to one of three treatments. Intact controls received no treatment, a second group was castrated at 141-d after initiation of the study (d 0), and a third group of bulls was immunized against a cocktail of the two LHRH fusion proteins, receiving a primary injection and two booster injections on d 0, 141 and 287, respectively. Because of a very dry season, rancher II held his bulls for an extra year so consequently an additional booster was given to half of the immunized bulls during year two. In study II bulls received the same immunizations as previously described with the additional booster injection on d 639.

Blood samples (jugular venipuncture) collected on d 141, 287, 639 and at slaughter. These serum samples were treated with 0.2% citric acid to inactivate any potential Foot and Mouth virus, and shipped to Washington State University under APHIS permit number 50322. Serum testosterone, and LHRH antibody binding values were evaluated as an indication of vaccine efficacy. Serum T was quantified by using a commercial kit (DSL, Webster, TX). Percentage of  $^{125}\text{I}$  LHRH bound for each sample was quantified at a 1:1000 dilution with procedures described by Johnson et al. (1988). All serum samples and tubes were destroyed by incineration after assay analysis in fulfillment of APHIS regulations. Additionally, BW, and scrotal circumference were collected on d 0, 141, 287, 385, 639, and 741, and epididymides and testes weights were collected at the time of slaughter.

## Statistical Analysis

Percent LHRH antibody binding, testosterone, BW and scrotal circumference were analyzed using a Proc Mixed repeated measures of SAS (SAS Inst. Inc., Cary, NC). The model included treatment and was tested using animal within treatment as the error term. Day and treatment x day interaction was tested using the residual mean square. When a significant treatment x day interaction was detected, treatments were examined within day. Orthogonal polynomial contrasts were used to compare intact to the average of castrated and immunized animals for testosterone and BW. Comparisons for scrotal circumference were between immunized and intact bulls. Orthogonal contrast compared immunized to the average of intact and castrated animals for percent LHRH antibody binding. These comparisons were chosen prior to the study and compared the groups with the same expected biological response for specific traits. Epididymides and testes weights were subjected to a one-way completely random design. Least square means are reported.

## Results and Discussion

### Body Weight and Reproductive Measurements

A treatment x day interaction was detected for BW ( $P = 0.01$ ). On d 0, all groups weighed approximately 329 kg for study I, and 258 kg for study II. Likewise, BW were similar ( $P = 0.33$ ) 141-d after initiation of the study (Table 1). On d 287, BW for intact controls was greater than castrated and LHRH immunized animals. Intact bulls continued to differ from that of both castrate groups for the duration of each study ( $P < 0.01$ ). These data demonstrate the similar growth characteristics of immunized and castrated animals described by Cook et al. (2000) and Aïssat et al. (2002), suggesting the anabolic effects of testosterone are ablated in LHRH immunocastrated bulls.

**Table 1.** Body Weight Of Immunized, Castrated or Intact Bulls On Pasture In Brazil

	Day of Study <sup>a</sup>					
	0	141	287	385	639	741
Ranch I						
Intact	325	357	472*	523**		
Immunized	329	358	452	486		
Castrated	334	363	451	485		
SE <sup>b</sup>	7.6	7.8	7.8	7.6		
Ranch II						
Intact	260	255	367**	427**	513**	579**
Immunized <sup>c</sup>	256	247	341	393	455	513
Castrated	260	253	346	406	456	514
SE	5.9	6.0	6.1	6.1	6.8	6.8

<sup>a</sup>Bulls are approximately 2 yr of age at d 0.

<sup>b</sup>SE = largest standard error.

<sup>c</sup>Immunized and late immunized animals did not differ in BW therefore, mean BW were pooled.

\*Column values differ  $P < 0.05$ .

\*\*Column values differ  $P < 0.01$ .

Scrotal circumference was similar between treatments on d 0 ( $P = 0.74$ ), however by d 141 differences in SC were approaching significance ( $P = 0.06$ ) for intact and immunized groups (Figure 1 and 2). Intact bulls demonstrated a steady increase in scrotal circumference throughout the study. Conversely, scrotal circumference continued to decline for the immunized group on d 287, 385, 639 and 741 ( $P < 0.01$ ). Following the 639-d booster injection, intact bulls had scrotal circumference measurement of  $35 \pm 0.75$  cm compared with 23 and  $20 \pm 0.80$  cm for immunized and late immunized ( $P < 0.01$ ; Figure 2).

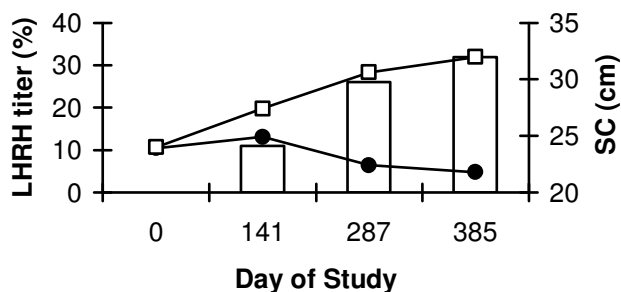


Figure 1. Line graph and right axis represents scrotal circumference of intact or LHRH immunized bulls during a 385-d study in which animals were intact (-□-), LHRH immunized (-●-). Bars indicate antibody titers as represented by % <sup>125</sup>I LHRH binding at 1:1000 serum dilution.

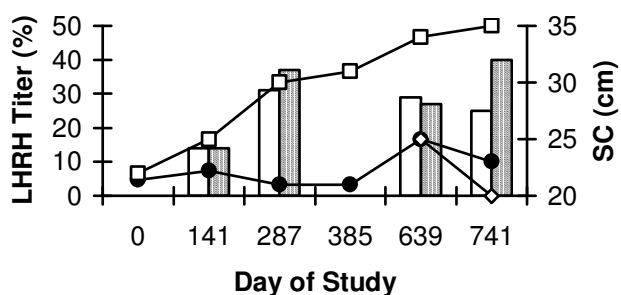


Figure 2. Line graph and right axis represents scrotal circumference of intact or LHRH immunized bulls on ranch II during a 741-d study in which animals were intact (-□-), LHRH immunized (-●-) or LHRH late immunized (-○-). Bar graph and left axis indicate antibody titers as represented by % <sup>125</sup>I LHRH binding at 1:1000 serum dilution of immunized animals (open bars) and late immunized (hatched bars) animals.

Paired testes and epididymides weights were measured on d 387 and 741 for ranch I and II, respectively (Table 3). Testes weights for the intact bulls were greater ( $P < 0.01$ ) than that of the LHRH immunized animals. Similarly, epididymides weights for intact bulls averaged  $47 \pm 1.1$  g compared to  $25 \pm 1.0$  g for the immunized group in ranch I. In ranch II epididymides weights were  $60 \pm 0.62$  g for intact bulls and 26 and 20 ( $\pm 0.87$ ) for immunized and late immunized animals. The decrease in testes and epididymides weights in LHRH immunized animals compared to the intact bulls are consistent with the scrotal circumference measurements, and agree with results of Huxsoll et al. (1998) and Cook et al. (2000). These data support the earlier findings that LHRH immunocastration does suppress reproductive function as demonstrated by atrophy of the gonads (Arimura et al., 1973).

#### LHRH Antibody Binding and Serum Testosterone

A treatment x day interaction ( $P < 0.01$ ) necessitated examination of LHRH immunization effects on

percent LHRH antibody bound by day. Antibodies to LHRH were detected by d 141 in immunized bulls ( $P < 0.01$ ; Figure 1 and 2). Antibody titer, as evaluated by <sup>125</sup>I LHRH, increased steadily and was elevated at all time points ( $P < 0.01$ ) for immunized animals compared with castrated and intact groups. As expected, castrate and intact animals had undetectable levels of LHRH antibody binding throughout the both studies. Immunized animals which received their final booster injection approximately 100 d prior to slaughter had LHRH antibody titers of  $31 \pm 2.6$  and  $39 \pm 1.6$  % for ranch I and II, respectively. When the final booster injection was given approximately 1 yr before slaughter, anti-LHRH binding had decreased to  $24 \pm 1.6$  %.

Repeated measures analysis revealed a treatment x day interaction ( $P < 0.01$ ); therefore castration method on testosterone was examined within day (Table 2). At the time of the first booster immunization (d 141), serum concentrations of testosterone were greater for intact bulls than immunized animals ( $P < 0.01$ ). Serum testosterone was markedly decreased in the group that was castrated after d 141 measurements were collected. Following the third immunization (d 287), mean testosterone concentrations were further reduced for immunized animals from ranch I and II, and did not differ from castrated animals ( $P = 0.51$ ). Immunized animals continued to demonstrate castrate levels of serum testosterone at the time of slaughter (d 387 and 741 for ranch I and II, respectively). Immunocastration using LHRH fusion proteins results in the production of anti-LHRH antibodies to levels that are effective in suppressing testicular growth and subsequently decrease circulating testosterone concentrations in treated animals.

**Table 2.** Serum Testosterone Concentrations Of Immunized, Castrated Or Intact Bulls During A 385 Or 741-D Study

	Day of study <sup>a</sup>				
	141	287	385	639	741
-----Testosterone concentration, ng/mL-----					
Ranch I, (n)					
Intact (24)	6.1	7.6**	3.2**		
Immunized (25)	2.5**	0.3	0.2		
Castrated (23)	8.6	0	0		
SE <sup>b</sup>	1.1	1.0	1.1		
Ranch II					
Intact (72)	1.4	2.5**	—	9.9**	2.2**
Immunized (72)	0.8	0.7	—	1.9	0.3
Castrated (72)	1.1	0	—	0	0
SE	0.8	0.8	—	0.8	0.8

<sup>a</sup>Serum was not collected on d 0 for either ranch or on d 385 for ranch II.

<sup>b</sup>SE = largest standard error.

\*Column values differ  $P < 0.01$ .

**Table 3.** Paired Testes And Epididymides Weights For Intact And Immunized Animals At Slaughter<sup>a</sup>

	Testes (g)	Epididymides (g)
Ranch I		
Intact	497**	47**
Immunized	172	25
SE <sup>b</sup>	11	1.1
Ranch II		
Intact	500 <sup>c</sup>	60 <sup>c</sup>
Immunized	174 <sup>d</sup>	26 <sup>df</sup>
Late		
Immunized	77 <sup>c</sup>	20 <sup>eg</sup>
SE	22	2.1

<sup>a</sup>Slaughter = d 387 for ranch I and d 741 for ranch II.

<sup>b</sup>SE = largest standard error.

\*\*Columns differ  $P < 0.01$ .

<sup>cde</sup>Column values differ  $P < 0.01$ .

<sup>fg</sup>Column values differ  $P < 0.05$ .

### Implications

Starting immunization against LHRH fusion proteins in two-year-old *Bos indicus* bulls is effective in producing growth and reproductive hormone profiles similar to that demonstrated by surgically castrated two-year-old bulls. Inducing a castrate like effect in immunized animals at two years of age allows producers to take advantage of the endogenous testosterone anabolic effects followed by desired steer characteristics without the associated trauma and possible death of surgical castration. The LHRH vaccine appears to be an effective alternative to traditional castration methods of 2-year-old grazing bulls in Brazil.

### Literature Cited

- Adams, T.E., C.A. Daley, D.M. Adams, and H. Sakurai. 1996. Testes function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: Effect of age at immunization. *J. Anim. Sci.* 74: 950-954.
- Aïssat, D., J.M. Sosa, D.M. de Avila, K.P. Bertrand, and J.J. Reeves. 2002. Endocrine, growth, and carcass characteristics of bulls immunized against luteinizing hormone-releasing hormone fusion proteins. *J. Anim. Sci.* 80:2209-2213.
- Arimura, A., H. Sato, T.Kumasaka, R.B. Worobec, L. Debeljuk, J.Dunn, and A.V. Schally. 1973. Production of antiserum to LH-releasing hormone (LH-RH) associated with gonadal atrophy in rabbits: Development of radioimmunoassays for LH-RH. *Endocrinology* 93(3):1092-1103.
- Awoniyi, C.A., M.S. Reece, B.S. Hurst, K.A. Faber, V. Chandrashekar, and W.D. Schlaff. 1993. Maintenance of sexual function with testosterone in the gonadotropin-releasing hormone-immunized hypogonadotropic infertile male rat. *Biol. Reprod.* 49:1170-1176.

- Cook, R.B. J.B. Popp, J.P. Kastelic, S. Robbins, and R. Harland. 2000. The effects of active immunization against GnRH on testicular development, feedlot performance, and carcass characteristics of beef bulls. *J. Anim. Sci.* 78:2778-2783.
- Huxsoll, C.C., E.O. Price, and T.E. Adams. 1998. Testis function, carcass traits, and aggressive behavior of beef bulls actively immunized against gonadotropin-releasing hormone. *J. Anim. Sci.* 76:1760-1766.
- Johnson, H.E., D.M. de Avila, C.F. Chang, and J.J. Reeves. 1988. Active immunization of heifers against luteinizing hormone releasing hormone, human chorionic gonadotropin, and bovine luteinizing hormone. *J. Anim. Sci.* 66:719-726.
- Miller, L.A., B.E. Johns, and G.J. Killian. 2000. Immunocontraception of white-tailed deer with GnRH vaccine. *American Journal of Reproductive Immunology* 44:266-274.
- Price, E.O., T.E. Adams, C.C. Huxsoll, and R.E. Borgwardt. 2003. Aggressive behavior is reduced in bulls actively immunized against gonadotropin-releasing hormone. *J. Anim. Sci.* 81:411-415.
- Robertson, I.S., J.C. Wilson, and H.M. Fraser. 1979. Immunological castration in male cattle. *Vet. Rec.* 105:556-557.
- Robertson, I.S., H.M. Fraser, G.M. Innes, and A.S. Jones. 1982. Effect of immunological castration on sexual and production characteristics of male cattle. *Vet. Rec.* 111:529.
- Quesnell, M.M., Y. Zhang, D.M. de Avila, K.P. Bertrand, and J.J. Reeves. 2000. Immunization of male mice with luteinizing hormone-releasing hormone fusion proteins reduces testicular and accessory sex gland function. *Biol. Reprod.* 63:347-353.
- Zhang, Y., T.G. Rozell, D.M. de Avila, K.P. Bertrand, and J.J. Reeves. 1999. Development of recombinant ovalbumin-luteinizing hormone releasing hormone as a potential sterilization vaccine. *Vaccine* 17:2185-2191.

# EFFECTS OF SUPPLEMENTAL HIGH-LINOLEATE OR HIGH-OLEATE SAFFLOWER SEEDS ON ADIPOSE TISSUE FATTY ACIDS, APPARENT MOBILIZATION, AND POTENTIAL UPTAKE AND STORAGE IN POSTPARTUM COWS<sup>1</sup>

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**ABSTRACT:** Three-year-old Angus × Gelbvieh beef cows nutritionally managed to achieve a BCS of  $4 \pm 0.07$  (BW =  $479.3 \pm 36.3$  kg) or  $6 \pm 0.07$  (BW  $579.6 \pm 53.1$  kg) at parturition were used in a 2-yr experiment ( $n = 36/\text{yr}$ ) to determine the effects of dietary lipid supplementation on cow adipose tissue fatty acid (FA) profile, apparent mobilization, and potential uptake and storage. Beginning 3 d postpartum, cows within each BCS were randomly assigned to be fed hay and a low-fat control supplement or supplements with either high-linoleate cracked safflower seeds or high-oleate cracked safflower seeds until d-60 of lactation. Rations were formulated to be isonitrogenous and isocaloric, and safflower seed supplements provided 5% DMI as fat. Cow adipose tissue biopsies were collected near the tail-head region on d-30 and 60 of lactation. Postpartum dietary treatment did not affect cow BW ( $P = 0.27$ ) or BCS change ( $P = 0.94$ ), adipose FA profile ( $P \geq 0.11$ ), plasma NEFA ( $P = 0.93$ ), adipose tissue lipoprotein lipase (LPL) activity ( $P = 0.86$ ), or rate of palmitate esterification into total acylglycerols ( $P = 0.92$ ). A BCS × day interaction ( $P = 0.02$ ) was noted for BCS change because BCS 4 cows increased condition while BCS 6 cows decreased BCS at d 60. Adipose tissue of cows in BCS 4 had greater proportions of 18:0 ( $P = 0.003$ ) and tended to have greater proportions of 18:2 ( $P = 0.07$ ) than BCS 6 cows. Cows in BCS 4 had increased rate of palmitate esterification into acylglycerols ( $P = 0.05$ ) and tended to have increased LPL activity ( $P = 0.08$ ) and decreased plasma NEFA ( $P = 0.08$ ), which is consistent with the increased condition observed at d-60 of lactation for these cows. Additionally, lack of dietary treatment response on adipose tissue FA profile and metabolism suggests that the majority of dietary FA were utilized to support mammary gland function through d-60 of lactation.

**Keywords:** Beef cattle, Lipid supplementation, Lipid metabolism

## Introduction

During early to peak lactation the nutrient demands of the mammary gland exceed those of the rest of the body, resulting in increased lipid mobilization for body reserves (Barber et al., 1997). A divergence of lipogenic activity towards milk fat synthesis in the mammary gland and away from lipid deposition in adipose tissue occurs, which results in lower body condition of the animal (Smith and Walsh, 1988). As a result of fatty acid mobilization, plasma NEFA concentrations have significantly increased after parturition (Bauman and

Currie, 1980; Pike and Roberts, 1980). This change in physiological state due to lactation alters the metabolism of the adipocytes to help support mammary function. Regulation of lipid synthesis and mobilization is a reciprocal process such that factors that increase one process tend to decrease the other process (Bauman and Currie, 1980). Cows in negative energy balance mobilize lipid stores from adipocytes, while cows in positive energy balance store dietary energy in the form of triacylglycerols in adipocytes. In the lactating animal, decreased adipose tissue lipoprotein lipase activity (Bauman and Currie, 1980; McNamara et al., 1987), coupled with increased demands of milk production (Bauman and Currie, 1980) may dictate the partitioning of nutrients towards mammary function and milk production, resulting in little if any exogenous lipid reaching the adipocyte for storage. Although the inherent homeostatic regulation involved with lactation makes repartitioning of nutrients away from the mammary gland difficult, provision of certain dietary fatty acids has been associated with repartitioning of nutrients to support specific productive functions (McNamara et al., 1995; Bottger et al., 2002). For example, Bottger et al. (2002) attributed maintenance of greater body condition in lactating beef cattle to supplementation of linoleic acid, whereas dietary oleic acid increased milk fat synthesis.

Increasing the condition of thin lactating beef cows to a more optimal condition by repartitioning nutrients toward adipose tissue reserves rather than milk fat synthesis could lead to reproductive improvement (Houghton et al., 1990a) and decreased maintenance requirement (Wagner et al., 1988).

Our hypothesis was that dietary supplementation of specific fatty acids during early lactation would influence metabolic signals that potentially mediate responses of adipose tissue storage and mobilization in thin and above average condition beef cows. Therefore, our objective was to evaluate the interaction of BCS at parturition and supplementation of high-linoleic or high-oleic acid safflower seeds on cow performance and adipose tissue fatty acid profile, apparent uptake, and mobilization.

## Materials and Methods

### General

The University of Wyoming Institutional Animal Care and Use Committee approved all procedures for the following study. Three-year-old Angus × Gelbvieh beef cows nutritionally managed to achieve a BCS (1 =

<sup>1</sup>Research was supported by the USDA-NRI Competitive Grants Program (USDA-NRI #2001-03314)

emaciated, 9 = obese, Wagner et al., 1988) of  $4 \pm 0.07$  (BW =  $479.3 \pm 36.3$  kg) or  $6 \pm 0.07$  (BW  $579.6 \pm 53.1$  kg) at parturition were used in a 2-yr experiment ( $n = 36/\text{yr}$ ). Cows were randomly assigned within BCS to dietary treatment as they calved; average calving interval was not greater than 7 d across treatments for both years. Beginning 3 d postpartum, cows were fed twice daily in individual feeding stanchions. Previous research (Bottger et al., 2002) at the University of Wyoming reported that cows of similar genetics produced 9 kg of milk during peak lactation. Therefore, our diets (Table 1) were formulated to meet the energy requirements of a 544 kg beef cow producing 9 kg of milk at peak lactation. Diets were formulated to provide equal quantities of N and TDN within each year. Dietary CP was higher in yr 2, due to differences in hay used during year 1 (Brome grass hay; CP % = 8.5) and yr 2 (Fox-tail Millet hay; CP % = 10.6). Dietary TDN was equal between years, and lipid supplemented diets were formulated to be isolipidic between years, providing 5% DMI as fat.

#### *Sampling and Laboratory Analyses*

Cows were allowed 2 d postpartum to become acclimated with their calves and surroundings before being placed on the experiment. Initial cow BW was determined from an average of weights taken on two consecutive days immediately before the beginning of the experiment, and additional 2 d weights were recorded at d 30 to 31 and d 60 to 61 of lactation. Cow BCS was determined at the beginning of the experiment 3 d after parturition and again at d 30 and d 60 of lactation by two independent evaluators. Milk production was measured on d 30 and d 60 of lactation using a modified weigh-suckle-weigh technique. Each cow was administered 20 USP units of oxytocin (Vedco, Inc., St. Joseph, MO. 64509) after removal of the calf, and then milked using a mechanical milking device; remaining milk was hand-stripped. Cows were then allowed 2 h to consume their rations, after which they were released from their individual feeding stanchions and given free access to water for an additional 2 h. After the 4 h period, cows were given a second injection of oxytocin (20 USP units) and milked as previously described. Each cow's 4 h milk production was extrapolated to 24 h milk production. A sub-sample (approximately 20 mL) of milk was sent to a commercial laboratory (Rocky Mountain DHIA, Logan, UT) and analyzed for total crude fat.

On d 30 and again on d 60, each cow was given approximately 400 mg of lidocain hydrochloride (Vedco, Inc. St. Joseph, MO. 64509) subcutaneously as a local anesthetic to desensitize a 10-cm<sup>2</sup> area adjacent to the tail head region. Adipose tissue biopsies (5 g) were removed (Rule and Beitz, 1986) and immediately placed in sterile Krebs Ringer Bicarbonate buffer (pH 7.4, 37 °C). Half of the adipose tissue sample was snap-frozen in liquid N and stored at -80°C for later analysis of activity of lipoprotein lipase (Andersen et al., 1996) and acetyl-CoA carboxylase (Vernon and Taylor, 1986) using tissue homogenates. For rate of palmitate esterification in vitro, approximately 100 mg of minced adipose tissue was incubated for 90 min in 3.0 mL of Krebs Ringer Bicarbonate buffer supplemented

with 10 mM glucose, 1000 µU/mL insulin, and 0.75 mM palmitate. Thin layer chromatography was used to quantify palmitate esterification into acylglycerols. A sub-sample (100 mg tissue) of adipose tissue was used for analysis of fatty acid composition (Murrieta et al., 2003).

Blood samples were taken immediately before feeding, 2 h from the onset of feeding, and 4 h after feeding on d 31 and 61 of lactation and analyzed using an enzymatic colorimetric assay for the quantification of NEFA concentrations in plasma (NEFA C kit ACS-ACOD method, WAKO Chemicals USA, inc. Richmond VA.)

#### *Statistical Analyses*

Data were analyzed using the MIXED procedures of SAS (SAS Institute, Cary, NC). The model included the effects of BCS at parturition, dietary treatment, day of sampling, and all possible interactions. The effects of BCS at parturition and dietary treatment were tested using cow within BCS at parturition  $\times$  dietary treatment as the RANDOM statement. Time course data for NEFA concentration was analyzed using PROC MIXED with effects of BCS at parturition, dietary treatment, day of sampling, time of sampling, and all possible interactions. The effect of BCS at parturition and dietary treatment were the main plot tested using cow within BCS at parturition  $\times$  dietary treatment  $\times$  day of sampling as the RANDOM statement. Comparisons of main effects were determined using least square means.

### **Results and Discussion**

#### *Cow Production*

*Effects of body condition score.* The only interaction detected for cow production traits was a BCS at parturition  $\times$  day of lactation ( $P = 0.04$ ) for change in BW. Cows with a BCS of 4 lost more BW from d 0 to 30 compared to d 30 to 60 (-6.3 vs -5.5 kg), while cows with a BCS of 6 at parturition lost more weight from d 30 to 60 compared to d 0 to 30 (-15.4 vs -5.3 kg). Cow BCS at parturition did not affect ( $P \geq 0.54$ ) 24 h milk production or milk fat (Table 2). Houghton et al. (1990b) suggested that cows fed a low-energy diet prepartum had a lower maintenance requirement per unit of metabolic BW. Results from the current study were consistent with those of Houghton et al (1990b) where cows at a lower BCS at parturition were able to increase BCS through d 60 of lactation compared to cows with a higher BCS at parturition. Although Houghton et al. (1990a) reported that thinner cows had a longer period of postpartum anestrus, they also reported that cows in sub-optimal condition at parturition that were increasing BCS had increased first service conception rate compared to fleshy cows with decreasing BCS. In the current study, however, percentage of cows pregnant at the end of the breeding season was lower ( $P = 0.01$ ) for cows with BCS 4 (63.9%) than with BCS 6 (88.9%), suggesting that the increase in BCS for BCS 4 cows was not sufficient to elicit an increase in reproductive performance.

*Effects of dietary treatment.* Dietary treatment did not affect BCS change ( $P = 0.94$ ), BW change ( $P = 0.27$ ), milk fat percentage ( $P = 0.41$ ), or milk yield ( $P = 0.47$ ). These findings support our previous research (Lake et al., 2003) and others showing no differences in milk fat percentage (Eastridge et al., 1988; Komaragiri et al., 1998), or total milk yield (Komaragiri et al., 1998; Bottger et al., 2002) due to dietary lipid supplementation. Through homeorhetic regulatory functions, the endocrine system may supersede dietary influences on nutrient partitioning to support specific functions (Komaragiri et al., 1998). Therefore, the nutrient demands of lactation may have superseded any partitioning effect due to specific fatty acids. Bottger et al. (2002) suggested that partitioning of nutrients was influenced by type of fatty acid supplementation; however, the lack of production response due to lipid supplementation in the present study would be expected because all diets provided equal quantities of N and TDN.

*Effects of day of sampling.* Twenty-four-hour milk yield was not affected ( $P = 0.55$ ) by day of sampling. Change in cow BW tended to be greater ( $P = 0.08$ ) at d 60 than d 30 of lactation, and milk fat percentage was decreased ( $P = 0.02$ ) at d 60 compared to d 30. Average peak milk production in beef cows occurs at about 60 d of lactation (NRC, 1996). Although milk production was not affected by day of sampling, it is possible that cows in the current study lost more weight at d 60 due to the increased nutrient demands required to support mammary function as they approached peak lactation. Furthermore, milk fat percentage was expected to decrease with the concomitant increase in milk production as cows reached peak lactation (McDonald et al., 2002).

#### *Adipose tissue fatty acid profile*

*Effects of body condition score.* A BCS at parturition  $\times$  day of lactation interaction was noted for 18:2 ( $P = 0.04$ ) and CLA ( $P < 0.001$ ), with both fatty acids increasing in BCS 4 cows from d 30 to d 60, while a decrease was observed from d 30 to d 60 in BCS 6 cows, suggesting a greater propensity to store dietary lipid at d 60 for cows in BCS 4. Cows in BCS 6 at parturition tended to have greater 14:0 ( $P = 0.07$ ) and had greater proportions of 16:0 ( $P = 0.003$ ), while BCS 4 cows had greater proportions of 18:0 ( $P = 0.003$ ). The products of de novo fatty acid synthesis included short to medium chain fatty acids to palmitic acid (16:0). Greater 14:0 and 16:0 in adipose tissue of cows in BCS 6 may indicate incorporation of fatty acids synthesized de novo, while greater 18:0, 18:2, and CLA in adipose tissue of cows in BCS 4 may be indicative of the uptake and storage of exogenous fatty acid.

*Effects of dietary treatment.* No differences ( $P \geq 0.11$ ) were noted in adipose tissue fatty acid profile due to dietary treatment. The lack of differences suggests that the nutrient demands of lactation most likely dictated the utilization of dietary fatty acids for milk fat synthesis and mammary function, regardless of treatment. For instance, dietary lipid supplementation increased incorporation of

exogenous lipid in the milk (DePeters et al., 2001; Lake et al., 2004); this change in milk fatty acid content along with the lack of change in adipose tissue fatty acid profile in the current study suggests that the majority of dietary fatty acids were utilized for milk fat synthesis.

*Effects of day of sampling.* Day of sampling had no effect ( $P = 0.14$  to  $0.56$ ) on adipose tissue fatty acid proportions of 14:0, 18:1*trans*-9, 18:1*trans*-11, 18:1*cis*-9, 18:2, and 18:3. The lack of differences for these fatty acids in adipose tissue was likely due to the utilization of dietary lipids for milk fat synthesis during early to peak lactation. Cow adipose tissue on d 30 had greater ( $P \leq 0.01$ ) proportions of 16:0 and CLA, while d-60 adipose tissue had greater proportions of 18:0.

#### *Apparent fatty acid uptake and mobilization.*

*Effects of body condition score.* In vitro lipoprotein lipase activity and rate of palmitate esterification into acylglycerols was determined to more fully investigate the potential for uptake of fatty acids for storage as influenced by BCS and dietary treatment. Concentrations of NEFA in plasma were determined as an estimate of lipolysis. Cows in BCS 4 tended to have greater ( $P = 0.08$ ) lipoprotein lipase activity and had a greater ( $P = 0.05$ ) rate of palmitate incorporation whereas cows in BCS 6 tended to have greater ( $P = 0.08$ ) plasma NEFA concentrations (Table 2). In agreement with the current study, we (Lake et al., 2003) previously noted greater lipoprotein lipase activity of cows in sub-optimal condition. Adipose lipoprotein lipase catalyzes hydrolysis of fatty acids from circulating lipoprotein-triacylglycerols, after which the fatty acids are transported into the adipocyte and then incorporated into triacylglycerol for storage. Therefore, greater lipoprotein lipase activity with BCS 4 cows would infer that if circulating triacylglycerol containing chylomicrons increased at the adipocytes there would be a greater supply of fatty acids presented to the adipocyte surface. Increased rate of palmitate esterification in vitro for cows with BCS 4 indicates that adipocytes of BCS 4 cows could incorporate and store more fatty acids in the cell as triacylglycerols if dietary lipid was presented at the cell surface. In addition, the tendency for less NEFA concentration in cows with a BCS of 4 along with the greater apparent uptake of fatty acid and storage could potentially lead to an increase in body condition. This conclusion is supported by the increase in dietary fatty acids stored in the adipocytes along with the increased body condition of cows in BCS 4 in the current study.

*Effects of dietary treatment.* The addition of dietary fat during early to peak lactation is commonly thought to improve energy balance resulting in decreased body tissue mobilization (Komaragiri et al., 1998). In the current study, however, provision of supplemental lipid did not influence lipoprotein lipase activity ( $P = 0.86$ ), rate of fatty acid esterification ( $P = 0.92$ ), or plasma NEFA concentration ( $P = 0.94$ ). A nutrient partitioning effect was expected between the linoleate and oleate fed cows, as was suggested by Bottger et al. (2002). In the current

study, lack of dietary treatment effects on lipid uptake and mobilization corresponds to the lack of production responses and adipose tissue fatty acid profile changes due to supplementation with specific fatty acids. The nutrient demands during early to peak lactation appear to have dictated nutrient partitioning regardless of dietary supplementation, which may have been attributed to endocrine control over nutrient partitioning during early lactation (Komaragiri et al., 1998).

*Effects of day of sampling.* In dairy cattle, as peak lactation approaches, activity of adipose tissue lipogenic enzymes continually decreases (Barber et al., 1998). The NRC (1996) suggests that average peak milk production occurs at about d 60 of lactation for beef cows. In the current study lipoprotein lipase activity ( $P = 0.01$ ), rate of palmitate esterification into acylglycerols ( $P = 0.002$ ), and mobilization of NEFA was greater ( $P = 0.05$ ) at d 30 than d 60. This decrease in apparent uptake of fatty acids in adipose tissue agrees with Lake et al. (2003) who also reported a decrease in lipoprotein lipase activity and rate of palmitate incorporation in acylglycerols at peak lactation. Acetyl-CoA carboxylase catalyzes the rate-limiting step in fatty acid biosynthesis. Research with beef cows (Lake et al., 2003) and dairy cows (Barber et al., 1998) was consistent with the current study in which the adipose tissue activity of acetyl-CoA carboxylase decreased as peak lactation approached. The decrease in acetyl-coA carboxylase activity observed from d 30 to d 60 of lactation was consistent with the decreased in adipose tissue 16:0. Once more, the inability of adipose tissue to incorporate dietary fatty acids suggests that nutrient demands of lactation dictated nutrient utilization, irrespective of day of sampling.

*Effects of time of sampling.* A BCS at parturition  $\times$  time of sampling ( $P = 0.04$ ) and a BCS at parturition  $\times$  day of sampling  $\times$  time of sampling ( $P = 0.02$ ; data not shown) interaction was noted for plasma NEFA concentrations. Concentrations of plasma NEFA were higher ( $P < 0.001$ ) in samples taken preprandially and immediately after feeding than 4 h post feeding. This change in NEFA concentration over time was expected because cows in the preprandial state mobilize energy stores from adipocytes in order to meet their energy requirements, whereas cows in the postprandial state can rely on by-products of fermentation as their primary source of energy (Blum et al., 2000; Nielsen et al., 2003).

In conclusion, dietary lipid supplementation during early to peak lactation did not appear to influence adipocyte uptake, incorporation, storage, or mobilization of fatty acids. The onset of lactation alters the metabolism of the mammary gland in such a way as to partition nutrients towards milk production (Vernon et al., 1981; McNamara et al., 1995) through mobilization of fatty acids from adipocytes, resulting in a decrease in body condition of the animal (Smith and Walsh, 1988). The inability of the dietary treatment to alter adipose tissue fatty acid profile reflects the utilization of dietary lipids for milk fat synthesis. However, cows in sub-optimal condition appear to have the proclivity to

incorporate and store more dietary fatty acids as acylglycerols in adipocytes, as evidenced by the greater deposition of exogenous fatty acids. Moreover, increased lipoprotein lipase activity along with increased rate of palmitate incorporation into acylglycerols was consistent with increased BCS of cows with BCS 4 compared to cows with BCS 6.

## Implications

The provision of high-linoleate and high-oleate safflower seeds during early to peak lactation did not appear to partition nutrients away from milk production in beef cows. Further research should be directed at investigating the impact of body condition at parturition on metabolic signals associated with lipid storage and mobilization in order to develop feeding strategies to improve the condition of cows in sub-optimal condition as the breeding season approaches.

## Literature Cited

- Andersen, M. K., J. W. Bailey, C. Wilken, and D. C. Rule. 1996. Lipoprotein lipase and glycerophosphate acyltransferase in ovine tissues are influenced by growth and energy intake regimen. *J. Nutr. Biochem.* 7:610-616.
- Barber, C. B., R. A. Clegg, M. T. Travens, and R. G. Vernon. 1997. Lipid metabolism in the lactating mammary gland. *Biochim. Biophys. Acta.* 1347:101-126.
- Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* 63:1514-1529.
- Blum, J. W., R. M. Bruckmaier, P. Y. Vacher, A. Munger, and F. Jans. 2000. Twenty-four-hour patterns of hormones and metabolites in week 9 and 19 of lactation in high-yielding dairy cows fed triglycerides and free fatty acids. *J. Vet. Med.* A47:43-60.
- Bottger, J. D., B. W. Hess, B. M. Alexander, D. L. Hixon, L. F. Woodard, R. N. Funston, D. M. Hallford, and G. E. Moss. 2002. Effects of supplementation with high linoleic or oleic cracked safflower seeds on postpartum reproduction and calf performance of primiparous beef heifers. *J. Anim. Sci.* 80:2023-2030.
- DePeters, E. J., J. B. German, S. J. Taylor, S. T. Essex, and H. Perez-Monti. 2001. Fatty acid and triglyceride composition of milk fat from lactating Holstein cows in response to supplemental canola oil. *J. Dairy Sci.* 84:929-936.
- Eastridge, M. L., M. D. Cunningham, and J. A. Patterson. 1988. Effect of dietary energy source and concentration on performance of dairy cows during early lactation. *J. Dairy Sci.* 71:2959-2966.
- Houghton, P. L., R. P. Lemenager, L. A. Horstman, K. S. Hendrix, and G. E. Moss. 1990a. Effects of body composition, pre- and postpartum energy level and

- early weaning on reproductive performance of beef cows and preweaning calf gain. *J. Anim. Sci.* 68:1438-1446.
- Houghton, P. L., R. P. Lemenager, K. S. Hendrix, G. E. Moss, and T. S. Stewart. 1990b. Effects of body composition, pre- and postpartum energy intake and stage of production on energy utilization by beef cows. *J. Anim. Sci.* 68:1447-1456.
- Komaragiri, M. V., D. P. Casper, and R. A. Erdman. 1998. Factors affecting body tissue mobilization in early lactation dairy cows. 2. Effect of dietary fat on mobilization of body fat and protein. *J. Dairy Sci.* 81:169-175.
- Lake, S. L., B. W. Hess, D. C. Rule, C. M. Murrieta, E. J. Scholljegerdes, V. Nayigihugu, and R. L. Atkinson. 2003. Effects of supplemental high-linoleate or high-oleate safflower seeds on production and lipogenesis by adipose tissue of postpartum cows. *Proc. West. Sect. Amer. Soc. Anim. Sci.* 54:40-46.
- Lake, S. L., B. W. Hess, E. J. Scholljegerdes, R. L. Atkinson, and D. C. Rule. 2004. Milk and calf adipose tissue fatty acid changes in response to maternal supplementation with high-linoleate or high-oleate safflower seeds. *Midwestern Section ASAS and Midwest Branch ADSA 2004 Meeting, Des Moines, IA Abstr.* 307.
- McDonald, P., R. A. Edwards, J. F. D. Greenhalgh, and C. A. Morgan. 2002. *Animal Nutrition*. 6<sup>th</sup> ed. Ashford Colour Press Ltd., Gosport, UK.
- McNamara, J. P., D. C. McFarland, and S. Bai. 1987. Regulation of bovine adipose tissue metabolism during lactation. 3. Adaptations of hormone sensitive and lipoprotein lipases. *J. Dairy Sci.* 70:1377-1384.
- McNamara, J. P., J. H. Harrison, R. L. Kincaid, and S. S. Waltner. 1995. Lipid metabolism in adipose tissue of cows fed high fat diets during lactation. *J. Dairy Sci.* 78:2782-2796.
- Murrieta, C. M., B. W. Hess, and D. C. Rule. 2003. Comparison of acidic and alkaline catalysts for preparation of fatty acid methyl esters from ovine muscle with emphasis on conjugated linoleic acid. *Meat Sci.* 65:523-529.
- NRC. 1996. *Nutrient Requirements of Beef Cattle*. 7<sup>th</sup> rev. ed. Natl. Acad. Press, Washington, DC.
- Nielsen, N. I., K. L. Ingvarsen, and T. Larsen. 2003. Diurnal variation and effect of feed restriction on plasma and milk metabolites in TMR-fed dairy cows. *J. Vet. Med.* A50:88-97.
- Pike, B.V., and C. J. Roberts. 1980. The metabolic activity of bovine adipocytes before and after parturition. *Res. Vet. Sci.* 29:108-110.
- Rule, D. C., and D. C. Beitz. 1986. Fatty acids of adipose tissue, plasma, muscle and duodenal ingesta of steers fed extruded soybeans. *J. Am. Oil Chem. Soc.* 63:1429-1435.
- Smith, R. W., and A. Walsh. 1988. Effects of pregnancy and lactation on the metabolism of bovine adipose tissue. *Res. Vet. Sci.* 44:349-353.
- Vernon, R. G., R. A. Clegg, and D. J. Flint. 1981. Metabolism of sheep adipose tissue during pregnancy and lactation. *Biochem. J.* 200:307-314.
- Vernon, R. G., R. A. Clegg, and D. J. Flint. 1981. Metabolism of sheep adipose tissue during pregnancy and lactation. *Biochem. J.* 200:307-314.
- Wagner, J. J., K. S. Lusby, J. W. Oltjen, J. Rakestraw, R. P. Wettemann, and L. E. Walters. 1988. Carcass composition in mature Hereford cows: Estimation and effect on daily metabolizable energy during winter. *J. Anim. Sci.* 66:603-612.

Table 1. Ingredient and chemical composition of diets consumed by lactating beef cows<sup>a</sup>

Item	Diet year 1			Diet year 2		
	Control	High-linoleate	High-oleate	Control	High-linoleate	High-oleate
-----Ingredients, %-----						
Hay <sup>b</sup>	79.3	85.4	85.3	87.2	89.7	89.6
High-linoleate safflower seed	-	11.8	-	-	8.1	-
High-oleate safflower seed	-	-	9.6	-	-	7.6
Soybean meal	2.8	-	2.1	0.7	-	0.7
Molasses	0.8	0.8	0.8	0.6	0.6	0.6
Beet pulp	15.0	-	-	10.0	-	-
Minerals	2.8	2.1	2.1	1.6	1.6	1.6
-----Chemical composition, % DM-----						
CP	10.4	10.2	10.4	11.2	11.4	11.4
TDN	70.6	71.1	72.0	69.7	70.1	70.1
Crude fat	1.2	5.0	5.0	2.2	5.0	5.0
-----Fatty acid, weight %-----						
16:0	28.7	9.9	7.8	19.8	10.0	8.0
18:0	5.6	3.2	0.3	2.7	3.2	0.2
18:1	10.1	10.2	73.2	10.4	10.3	71.3
18:2	20.3	69.7	10.4	22.4	68.1	10.9
18:3	6.6	0.6	0.1	1.7	0.4	0.6

<sup>a</sup>Diets were formulated to be isocaloric and isonitrogenous and to meet the energy requirements of a 544 kg beef cow producing 9 kg of milk during peak lactation. Lipid supplemented diets were formulated to provide 5% DMI as fat.

<sup>b</sup>Brome grass hay (CP = 8.5%) was fed in year 1, Foxtail Millet hay (CP = 10.8%) was fed in year 2.

Table 2. Main effects of BCS at parturition, dietary treatment, and day of sampling on cow production, adipose tissue uptake, mobilization and fatty acid profile in lactating beef cows<sup>a</sup>

Item	BCS		Treatment			Day		SE	P Value		
	4	6	C	L	O	30	60		BCS	TRT	DAY
----- Cow production -----											
Weight change, kg	-5.90	-10.36	-5.26	-10.28	-8.85	-5.80	-10.47	2.26	0.09	0.27	0.08
BCS change	0.07	-0.11	-0.02	-0.01	-0.04	-0.12	0.07	0.06	0.02	0.94	0.01
24 h milk yield, kg	8.60	8.76	9.04	8.62	8.38	8.57	8.79	0.38	0.71	0.47	0.55
Milk fat, %	3.39	3.49	3.57	3.31	3.42	3.57	3.30	0.14	0.54	0.41	0.02
----- Adipose tissue fatty acid, weight % -----											
14:0	2.84	3.06	3.05	2.89	2.90	3.01	2.89	0.11	0.07	0.47	0.14
16:0	26.01	28.04	27.38	27.10	26.73	27.61	26.53	0.57	0.003	0.70	0.01
18:0	12.95	11.08	12.29	12.64	11.13	11.59	12.44	0.54	0.003	0.11	0.01
18:1 <i>trans</i> -9	0.08	0.08	0.09	0.07	0.08	0.07	0.09	0.01	0.80	0.44	0.18
18:1 <i>trans</i> -11	1.24	1.40	1.58	1.21	1.17	1.21	1.43	0.24	0.56	0.39	0.42
18:1 <i>cis</i> -9	36.77	37.35	37.23	37.00	36.95	36.93	37.19	0.65	0.43	0.94	0.56
18:2	1.32	0.96	1.00	1.11	1.31	1.10	1.17	0.17	0.07	0.39	0.17
18:3	0.43	0.43	0.44	0.42	0.43	0.42	0.44	0.03	0.93	0.85	0.27
CLA <sup>b</sup>	0.38	0.47	0.42	0.40	0.46	0.47	0.38	0.05	0.10	0.65	0.001
----- Apparent fatty acid uptake and mobilization -----											
LPL activity <sup>c</sup>	32.25	27.14	28.78	30.76	29.4	33.72	25.67	2.54	0.08	0.86	0.01
ACC activity <sup>d</sup>	10.73	11.66	9.68	10.47	13.43	15.60	6.72	2.94	0.79	0.64	0.001
Palmitate incorporation <sup>e</sup>	63.38	42.98	53.62	50.45	55.46	69.43	36.92	8.73	0.05	0.92	0.002
Plasma NEFA concentration <sup>f</sup>	0.37	0.42	0.39	0.40	0.39	0.42	0.37	0.02	0.08	0.94	0.05

<sup>a</sup>Diets were formulated to be isocaloric and isonitrogenous and to meet the energy requirements of a 544 kg beef cow producing 9 kg of milk during peak lactation. Lipid supplemented diets were formulated to provide 5% DMI as fat.

<sup>b</sup>Conjugated linoleic acid, 18:2 *cis*-9, *trans*-11.

<sup>c</sup>Adipose tissue lipoprotein lipase activity,  $\text{neq} \cdot \text{min}^{-1} \cdot \text{g}$  adipose tissue<sup>-1</sup>.

<sup>d</sup>Adipose tissue acetyl-CoA carboxylase activity,  $\text{nmol} \cdot \text{min}^{-1} \cdot \text{g}$  adipose tissue<sup>-1</sup>.

<sup>e</sup>Palmitate incorporation into acylglycerol in adipose tissue,  $\text{nmol} \cdot \text{min}^{-1} \cdot \text{g}$  lipid<sup>-1</sup>.

<sup>f</sup>NEFA concentration,  $\text{mEq} \cdot \text{L}^{-1}$ .

**Genetic Parameter Estimates for Yearling Scrotal Circumference and Semen Traits of Line 1 Hereford Bulls**

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**ABSTRACT:** Objectives of this research were to estimate heritabilities of scrotal circumference and semen traits, and genetic correlations between these traits and birth weight. Line 1 Hereford bulls (n = 841), born in 1963 or from 1967 to 2000, were selected either for use by USDA-ARS at Miles City, Montana or for sale. Semen was collected by electro-ejaculation when the bulls were approximately one year of age (mean = 446d) and all samples were evaluated by one person. Traits analyzed were scrotal circumference, volume, concentration, motility, and percents normal, live, and primary and secondary abnormalities. Primary abnormalities were abnormal heads, abnormal mid-pieces, and proximal droplets. Secondary abnormalities were bent tails, coiled tails, and distal droplets. Data were analyzed using MTDF-REML. The model included fixed effects for contemporary group and age of dam, covariates for age of bull at evaluation and inbreeding of the bull and his dam, and random animal and residual effects. Random maternal effects were also included for birth weight. Heritability estimates for birth weight, scrotal circumference, volume, concentration, motility, and percents normal, live, and primary and secondary abnormalities were 0.46, 0.57, 0.09, 0.16, 0.22, 0.34, 0.23, 0.09, and 0.13, respectively. Estimates of genetic correlations between birth weight and scrotal circumference, volume, concentration, motility, and percents normal, live, and primary and secondary abnormalities were 0.36, 0.07, 0.58, 0.21, 0.20, 0.34, -0.25, and 0.05, respectively. The moderate estimates of heritability for many of the traits indicate potential for favorable selection response. Positive genetic correlations between birth weight and majority of the traits suggest selection to reduce birth weight may compromise semen traits. However, for most traits the expected correlated responses are small.

**Key Words:** Semen Characters, Cattle, Genetic Parameters

**Introduction**

Bull fertility can only be directly measured by the production of a calf crop. However, this measurement is impractical because establishing fertility before the breeding season as opposed to after the breeding season is required to insure maximal reproductive success. Therefore, methods to predict fertility have been developed. Breeding soundness evaluations are the most commonly used method to predict bull fertility.

According to the Society for Theriogenology (Chenoweth et al., 1992), three classifications are possible for bulls based on the results of a breeding soundness evaluation. These classifications are satisfactory breeder, unsatisfactory breeder, or deferred for a later test. A satisfactory breeder must reach a minimum scrotal circumference for their age, have at least 70% morphologically normal spermatozoa, and at least 30% motile spermatozoa (Chenoweth et al., 1992). Kennedy et al. (2000) reported 76.2% of bulls tested were classified as satisfactory breeders. Percentages of motile and morphologically normal spermatozoa and scrotal circumference are emphasized by the breeding soundness evaluation and have been shown to influence fertility (Coulter and Kozub, 1989; Farrell et al., 1998; Hulroyd et al., 2002).

Estimates of heritability have been reported for some of the traits evaluated in a breeding soundness evaluation. However, there are still several semen traits that influence fertility for which heritability estimates have not been reported. A void in the literature also exists for correlations between semen traits and birth weight. Therefore, the objectives of this study were to estimate heritability of scrotal circumference and semen traits, and genetic correlations between these traits and birth weight.

**Materials and Methods**

Semen evaluations and scrotal circumference measurements were collected by one person from 841 yearling bulls during the years 1963 and 1967 to 2000. Bulls were from the Line 1 Hereford population maintained by the USDA-ARS Fort Keogh Livestock and Range Research Laboratory, Miles City, MT. This population serves as an excellent resource for animal breeders because pedigree information and management practices are available for the population from its establishment. The initial mating of two half-sib bulls, Advanced Domino 20<sup>th</sup> and Advanced Domino 54<sup>th</sup>, to 50 registered Hereford females occurred in 1934. Inbreeding accumulated quickly in the first few generations, but in later generations the rate of inbreeding declined due to the use of planned matings (Knapp et al., 1951). The present pedigree inbreeding level is approximately 30% with a 2% increase with every generation (MacNeil et al., 1992). From the late 1950's to present all bull calves remained intact and were placed on feed at weaning and fed for 140, 168, or 196d (MacNeil et al., 1992).

Measurements made during the semen evaluations were volume, concentration, motility, and percents normal,

live, and primary and secondary abnormalities. Primary abnormalities were percents abnormal heads, abnormal mid-pieces, and proximal cytoplasmic droplets. Secondary abnormalities were percents bent tails, coiled tails, and distal cytoplasmic droplets. For each bull, the semen collection nearest to one year of age was used for analysis. For most bulls it was the first collection and took place either in the spring prior to breeding or the fall for a pre-sale examination. Average age of the bulls, at the time of evaluation, was 446 d.

Semen samples were collected by electroejaculation. During collection the semen tube was maintained at 36°-40° C using an insulated water jacket. A water bath and slide warmer were used to maintain the sample at 37° C throughout the evaluation. Total volume was recorded within 5 minutes of collection. An undiluted drop of neat semen was placed on a microscope slide and given a score for swirl (0-5; 0 = none, 1 = very weak, 2 = weak, 3 = intermediate, 4 = strong and 5 = very strong) at 100x magnification. Semen concentration and motility were likewise scored (0-5) by viewing a drop of neat semen placed on a warmed glass slide under a cover slip at 400x magnification. Percents of live, normal, primary abnormalities and secondary abnormalities were estimated by staining a drop of semen with morphology stain (Lane Manufacturing, Inc., Denver, CO) and counting 100 spermatozoa at 400x magnification. Scrotal circumference was measured with a flexible measuring tape at the greatest horizontal distance around the scrotum after pulling the testicles into the bottom of the scrotum.

A linear model with the fixed effects of contemporary group and age of dam and covariates for age of bull at evaluation, inbreeding of the bull, and inbreeding of his dam was used to obtain residuals. Contemporary group is defined as year of birth, year of evaluation, and season of evaluation (spring, summer, and fall). The distributions of residuals were then tested for normality using the univariate procedures of SAS (SAS Institute., Inc., Cary, NC, 2004). Birth weight and scrotal circumference were the only traits with normally distributed residuals. Standard transformations were used on the data and the residuals were tested again for normality. The transformations did not cause the residuals to become normally distributed; therefore the original data were used for the analysis.

Multiple-trait derivative-free restricted maximum likelihood (MTDFREML) program was used to estimate (co)variances from which estimates of heritability and genetic correlation were calculated. Maximization of the likelihood function (?) is the same as minimizing  $-2 \log ?$  or the variance function values. When the variance function values were less than  $1 \times 10^{-9}$ , iterations were stopped. Another analysis for the same trait was restarted with values different from the first run. Additional runs were carried out until the variance function values changed less than 0.001. Once the variance function values did not change between analyses by more than 0.001, global convergence was assumed.

Two-trait analyses allowed for the estimation of correlations among the semen characteristics, scrotal circumference, and birth weight. Birth weight was

included in the model because it was measured on every animal and helped account for any nonrandom selection of bulls. The model included the same fixed effects and covariates as the linear model described above in addition to the random animal and residual effects. Random maternal genetic effects were also included for birth weight. Predicted correlated response to 1 SD of selection intensity applied to birth weight was calculated as the product of the square root of the heritability of birth weight, the estimated genetic correlation between birth weight and the trait of interest, and the genetic standard deviation of the trait of interest.

## Results and Discussion

It is noteworthy that data collection for this research was initiated before the establishment of standardized methods for breeding soundness evaluation of bulls. As a consequence, some comparisons with current literature may be complicated by differing definitions of the various phenotypes. All of the semen samples were evaluated by one person and the semen evaluation protocols used did not change throughout the course of the study, thus providing physical control of some potential sources of environmental variation.

### *Heritability*

Heritability estimates were obtained for all traits (Table 1). Heritability estimates were also obtained for the transformed traits to determine if any differences existed between the estimates for the original data and the transformed data. No differences were found between the two estimates supporting the decision to use the untransformed data for the remaining analyses.

Scrotal circumference was estimated to have a heritability of 0.57, which falls within the range of 0.32 to 0.71 estimated in previous studies. Bourdon and Brinks (1986) and Kriese et al. (1991) estimated the heritability of scrotal circumference to be 0.53, in close agreement with the present results. The average estimate from the literature is only slightly lower, 0.47 (Koots et al., 1994). Both the results from this study and the average heritability estimate from the literature indicate scrotal circumference is highly heritable, which is unusual since heritability estimates for reproductive traits are generally low (Koots et al., 1994). Coulter and Kozub (1989) further suggested that fertility can be improved when bulls with large scrotal circumference are selected for use as herd sires.

Heritability of ejaculate concentration was estimated to be 0.16. Knights et al. (1984) and Rege et al. (2000) reported values of 0.13 and 0.16 in close agreement with the estimate from this study. Heritability of ejaculate volume was estimated to be 0.09. Rege et al. (2000) reported an estimate for semen volume in 12 month rams to be 0.11. The low estimates of heritability for volume of the ejaculate and ejaculate concentration may result from increased environmental variation introduced by using an electro-ejaculator for semen collection. During

the course of this research, several different people were responsible for operating the electro-ejaculator.

Percentages of motile and morphologically normal spermatozoa were positively related to number of calves sired (Hulroyd et al., 2002). In these data, heritability of motility was estimated to be 0.22. Values in the literature range from 0.08 to 0.27 for males at 12 months of age. Knights et al. (1984) and Smith et al. (1989) reported lower estimates than reported in this study. However, Rege et al. (2000) reported a moderate estimate of heritability (0.27) for rams at 12 months of age. Percentage of motile spermatozoa explained 34% of the variation observed in fertility (Farrell et al., 1998). Heritability of percent normal spermatozoa was estimated to be 0.34. Smith et al. (1989) reported a lower estimate of heritability (0.07). Heritability estimates for percentages of primary and secondary abnormalities were 0.09 and 0.13, respectively. Smith et al. (1989) reported values of 0.31 and 0.02 for primary and secondary abnormalities, respectively. The low value reported by Smith et al. (1989) for secondary abnormalities agrees with that reported here. The difference between values for primary abnormalities may result from various differences between the two studies. Three breeds, Hereford, Angus, and Red Angus, totaling 549 yearling bulls were used by Smith et al. (1989). The data were also analyzed differently. Smith et al. (1989) used least squares as opposed to maximum likelihood methods. Both data sets are small and sampling may also contribute to the difference in estimates.

Zemjanis (1974) reported that primary abnormalities result from disturbances of spermatogenesis and secondary abnormalities are influenced by factors occurring after spermatogenesis. Thus, the difference in results with respect to the heritability of percent primary abnormalities, between the present study and that of Smith et al. (1989) may have potentially important implications. The moderate heritability estimate reported by Smith et al. (1989) is indicative of a significant genetic basis for the failure of spermatogenesis that results in an elevated level of primary abnormalities. In contrast, the present results indicate little genetic basis of the origin of primary abnormalities. However, the present low estimate for percent secondary abnormalities is in agreement with the expectation of a trait that is mostly influenced by the environment.

Estimated heritability for percent live spermatozoa was 0.23. Rege et al. (2000) previously reported an estimate for percent dead of 0.01.

#### *Genetic correlations*

Table 1 lists genetic correlations between birth weight ( $h^2 = 0.46$ ) and the traits recorded as part of the breeding soundness examination of the bulls. Birth weight was positively correlated with scrotal circumference, volume, concentration, motility, percent normal, percent secondary abnormalities, and percent live. Primary abnormalities were negatively correlated with birth weight. Thus, attempts to reduce calving difficulty and increase calf survival through selection for reduced birth weight may

adversely impact yearling breeding soundness evaluations. Conversely, selection of bulls based on results of yearling breeding soundness evaluations may tend to increase birth weight as a correlated response.

Werre and Brinks (1986) reported a negative genetic correlation between birth weight and age of puberty in females, -0.16. Assuming the correlation between birth weight and age at puberty is the same between males and females, bulls with greater birth weights will tend to reach puberty at younger ages than bulls weighing less at birth. Lunstra and Echternkamp (1982) reported a rapid increase in progressive motility with post-pubertal age in yearling bulls. Percent normal spermatozoa and concentration also increased as age increased (Hultnäs, 1959; Madrid et al., 1987; Rege et al., 2000). The positive relationship between chronological age and semen characteristics, along with the negative correlation between birth weight and age of puberty, leads to the inference that low birth weight could be antagonistically related to semen characteristics associated with the attainment of puberty. Madrid et al. (1987) reported a negative relationship between proximal droplets, a primary abnormality, and age.

#### **Implications**

The moderate heritability estimates for scrotal circumference and most of the semen traits evaluated, imply improvement in these traits can be achieved through genetic selection. The genetic correlations between birth weight and semen characteristics indicate that there may be some negative impacts on bull fertility if selection for low birth weight is practiced.

Table 1. Phenotypic standard deviations (SD) and heritability estimates ( $h^2$ ) for scrotal circumference and semen traits, genetic correlations ( $r_g$ ) between these traits and birth weight, and predicted correlated responses (CR) in scrotal circumference and semen traits to selection on birth weight.

Trait	SD	$h^2$	$r_g$	CR
Scrotal circumference	1.86	0.57	0.36	0.34
Volume	1.70	0.09	0.07	0.02
Concentration	0.96	0.16	0.58	0.15
Motility	1.14	0.22	0.21	0.08
% normal	12.54	0.34	0.20	0.99
% live	11.63	0.23	0.34	1.27
% 1° abnormalities	28.16	0.09	-0.25	-1.40
% 2° abnormalities	28.15	0.13	0.05	0.34

#### **Literature Cited**

- Bourdon, R. M. and J. S. Brinks. 1986. Scrotal circumference in yearling Hereford bulls: adjustments factors, heritabilities and genetic, environmental and phenotypic relationships with growth traits. *J. Anim. Sci.* 62:958-967.

- Chenoweth, P. J., J. C. Spitzer, and F. M. Hopkins. 1992. A new breeding soundness evaluation form. Proc. Soc. For Theriogenology. Annual Mtg. San Antonio, Tx.
- Coulter, G. H. and G. C. Kozub. 1989. Efficacy of methods used to test fertility of beef bulls used for multiple-sire breeding under range conditions. J. Anim. Sci. 67:1757-1766.
- Farrell, P. B., G. A. Presicce, C. C. Brockett, and R. H. Foote. 1998. Quantification of bull sperm characteristics measured by computer-assisted sperm analysis (CASA) and the relationship to fertility. Theriogenology. 49:871-879.
- Higdon, H. L., J. C. Spitzer, F. M. Hopkins, and W. C. Bridges Jr. 2000. Outcomes of breeding soundness evaluations of 2898 yearling bulls subjected to different classification systems. Theriogenology. 53:1321-1332.
- Hulroyd, R. G., V. J. Doogan, J. De Faveri, G. Fordyce, M. R. McGowan, J. D. Bertram, D. M. Vanka, L. A. Fitzpatrick, G. Jayawardhana, and R. G. Miller. 2002. Bull selection and use in northern Australia. 4. Calf output and predictors of fertility of bulls in multiple-sire herds. Anim. Reprod. Sci. 71:67-79.
- Hultnäs, C. A. 1959. Studies on variation in mating behavior and semen picture in young bulls of the Swedish Red-and White breed and on causes of this variation. Acta. Agric. Scand. Suppl. 6:1-82.
- Kennedy, S. P., J. C. Spitzer, F. M. Hopkins, H. L. Higdon, and W. C. Bridges Jr. 2002. Breeding soundness evaluations of 3,648 yearling beef bulls using the 1993 Society for Theriogenology guidelines. Theriogenology. 58:947-961.
- Knapp, B., R. C. Church and A. E. Flower. 1951. Genetic history of Line 1 Hereford cattle at the United States Range Livestock Experiment Station. Miles City, Montana. Bull. 479. Montana State College, Agric. Exp. Sta., Bozeman.
- Knight, S. A., R. L. Baker, D. Gianóla and J. B. Gibb. 1984. Estimates of heritabilities and of genetic and phenotypic correlations among growth and reproductive traits in yearling Angus bulls. J. Anim. Sci. 58:887-893.
- Koots, K. R., J. P. Gibson, C. Smith and J. W. Wilton. 1994. Analyses of published genetic parameter estimates for beef production traits. 1. Heritability. Anim. Breeding Abstracts. 62:309-338.
- Kriese, L. A., J. K. Bertand and L. L. Benyshek. 1991. Age adjustment factors, heritabilities and genetic correlations for scrotal circumference and related growth traits in Hereford and Brangus bulls. J. Anim. Sci. 69:478-489.
- Luntstra, D. D. and S. E. Echternkamp. 1982. Puberty in beef bulls: Acrosome morphology and semen quality in bulls of different breeds. J. Anim. Sci. 55:638-648.
- Mackinnon, M. J., J. F. Taylor, and D. J. S. Hetzel. 1990. Genetic variation and covariation in beef cow and bull fertility. J. Anim. Sci. 68:1208-1214.
- MacNeil, M. D., J. J. Urick, and G. Deocoudu. 2000. Characteristics of Line 1 Hereford females resulting from selection by independent culling level for below-average birth weight and high yearling weight or by mass selection for high yearling weight. J. Anim. Sci. 78:2292-2298.
- MacNeil, M. D., J. J. Urick, S. Newman and B. W. Knapp. 1992. Selection for postweaning growth in inbred Hereford cattle: the Fort Keogh Montana Line 1 example. J. Anim. Sci. 70:723-733.
- Madrid, R. S. Ott, D. N. Rao Veeramachaneni, D.F. Parrett and C. L. Willms. 1988. Scrotal circumference, seminal characteristics and testicular lesions of yearling Angus bulls. Am. Vet. Res. 49:579-585.
- Martinez-Velazquez, G., K. E. Gregory, G. L. Bennett and L. D. Van Vleck. 2003. Genetic relationships between scrotal circumference and female reproductive traits. J. Ani. Sci. 81:395-401.
- Rege, J. E. O., F. Toe, E. Mukasa-Muerwa, S. Tembely, D. Anindo, R. L. Baker and A. Lahlou-Kassi. 2000. Reproductive characteristics of Ethiopian highland sheep II. genetic parameters of semen characteristics and their relationships with testicular measurements in ram lambs. Sm. Rum. Res. 37: 173-187.
- Smith, B. A., J. S. Brinks and G. V. Richardson. 1989. Estimation of genetic parameters among breeding soundness examination components and growth traits in yearling bulls. J. Anim. Sci. 67:2892-2896.
- Werre, J. F. and J. S. Brinks. 1986. Relationships of age at puberty with growth and subsequent productivity in beef heifers. Proc. West. Soc. Am. Soc. Anim. Sci. 37:300-306.
- Zemjanis, R. 1974. Diagnostic and Therapeutic Techniques in Animal Reproduction, 2nd ed. The Williams and Wilkins Co., Baltimore, MD.

# EFFECTS OF SHORT-TERM PROPYLTHIOURACIL AND MELATONIN TREATMENT DURING GESTATION ON SERUM THYROXINE, POSTPARTUM REPRODUCTION, AND LAMB PERFORMANCE IN FINE-WOOL EWES

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**ABSTRACT:** Thyroid hormones play a permissive role in onset of anestrus in ewes, and thyroid hormone inhibition before onset of anestrus extends the breeding season. In this study, 11 pregnant Rambouillet ewes were used to examine effects of short-term administration of propylthiouracil (PTU) and melatonin supplementation on prepartum serum thyroxine (T4) concentrations, BW, and postpartum reproduction along with lamb performance. After the fall breeding season, ewes were maintained in a single pen and fed alfalfa hay (1.8 kg/d). Beginning on d 0 (January 2, 76.8  $\pm$  4.7 d of gestation), ewes received treatments (gavage) consisting of either 0 (n = 3) or 40 (n = 8) mg PTU/kg BW/d for 15 d. After 15 d, the 40 mg dosage of PTU was decreased to 20 mg/kg BW for an additional 20 d, and melatonin was given (i.m. injections at 5 mg/d) for 30 d. Blood samples were collected daily for 48 d. Lamb BW and blood samples were collected at parturition, 14, and 28 d after parturition. Postpartum ewe blood collections were at parturition and alternating days for 30 d; serum progesterone (P4) was analyzed to determine cyclicity. Ewe BW were similar (P > 0.90) throughout the treatment and postpartum period (77.4 and 73.9  $\pm$  3.0 kg in control and treated ewes, respectively, 3 d after treatment ended). Propylthiouracil decreased (P  $\leq$  0.05) serum T4 on d 23 through 41 of the treatment period. Unexpectedly, the decrease was not as pronounced as in previous studies. Three ewes appeared resistant to PTU and marginally responded. Postpartum P4 was below 1 ng/mL for all ewes on all days indicating acyclicity. Lamb BW (13.2 and 13.9  $\pm$  1.6 kg at 28 d of age) and serum IGF-I (391.2 and 309.2  $\pm$  72.5 ng/mL at 28 d of age) were similar (P > 0.60) for lambs born to control and PTU-treated ewes, respectively. Results indicate that 40/20 mg PTU with melatonin supplementation does not induce postpartum cyclicity in ewes and has little effect on lamb growth for 28 d after parturition.

Key words: Sheep, Thyroid, Melatonin, Reproduction

## Introduction

Ewes exhibit seasonal ovarian cyclicity characterized by shifts in response to the negative feedback of estradiol on GnRH secretion (Karsch et al., 1993). Photoperiod appears to synchronize the circannual rhythm (Turek and Campbell, 1979) and is mediated by the pineal hormone melatonin thus providing an endocrine signal for day length (Bittman et al., 1985). Graham et al. (2000) reported that summer melatonin patterns provide an effective entraining signal, and short-day signals are required for maintaining a full duration breeding season

(Malpaux and Karsch, 1990). Specifically, the premammillary hypothalamic area (PMH) exhibits high melatonin receptor binding, and melatonin microimplants within the PMH stimulate LH secretion (Malpaux et al., 1998).

Thyroidectomy during the breeding season prevents occurrence of anestrus (Karsch et al., 1995). Furthermore, thyroid hormones affect GnRH secretion only during the last 60 to 90 d of the breeding season (Thrun et al. 1997). Indeed, chemical thyroid inhibition during this period extends the breeding season in ewes (Hernandez et al., 2003). The mechanism by which thyroid hormones seasonally decrease GnRH and LH pulse frequency is unclear; however, Anderson et al. (2003) suggested that thyroid hormones act within the ventromedial preoptic area and the PMH of the hypothalamus to allow seasonal transition. Therefore, thyroid hormones and melatonin may work in concert to initiate anestrus within the PMH. Watanabe et al. (2004) showed that melatonin injections reduced expression of type 2 iodothyronine deiodinase (Dio2) in the cell-clear zone overlying the tuberoinfundibular sulcus of Djungarian hamsters and speculated that expression of Dio2 may be regulated in a similar manner in the PMH of ewes.

The gestation length in a ewe is 150 d which would allow two lamb crops each year if cyclicity could be induced early postpartum. Therefore, we hypothesized that chemical thyroid inhibition with melatonin supplementation during mid gestation would induce early postpartum cyclicity in ewes; our objective was to determine effects of these treatments on serum thyroid hormones, postpartum reproduction, and lamb performance.

## Materials and Methods

Originally, 20 pregnant Rambouillet ewes were assigned to either control (n = 5) or treatment (n = 15) groups. However, two control and four treatment ewes aborted (toxoplasmosis) and were excluded from the experiment. Therefore, 11 pregnant Rambouillet ewes (BW = 70.1  $\pm$  1.2 kg; 76.8  $\pm$  4.7 d of gestation on d 0 of treatment) were utilized in this experiment. Animals were maintained in a single pen (12 x 4 m) and had ad libitum access to shade, water, and salt, and alfalfa hay was fed at 1.8 kg/animal/d throughout the study. Ewes were weighed 3 d before treatment began, stratified by BW, and assigned to one of two treatment groups. Body weight measurements were then taken every 2 wk until the end of the treatment period. Previous data from our laboratory showed that 40 mg 6-N-propyl-2-thiouracil (PTU)

decreased thyroxine (T4) concentrations below 20 ng/mL when administered to pregnant ewes in late gestation (Duffey et al., 2002). Therefore, 40 mg of PTU (Sigma, St. Louis, MO)/kg BW was administered (gavage in gelatin capsules) to the treatment group (n = 8) for 15 d. After 15 d, the 40 mg dosage was decreased to 20 mg/kg BW for an additional 20 d (35 d of PTU treatment), and melatonin (5 mg suspended in 2 mL safflower oil) was administered (i.m. injections at alternating sites at 1600) for 30 d. Previous data from our laboratory showed that 5 mg melatonin suspended in safflower oil and administered via i.m. injections was sufficient to elevate serum melatonin concentrations to > 1 ng/mL 3 h after treatment before returning to pretreatment concentrations 6 h later (Perez-Eguia and Hallford, 1994). The control group (n = 3) received no treatment but was administered (i.m. injections at alternating sites at 1600) 2 mL safflower oil on all days of melatonin treatment.

Beginning on d 0 (January 2, first day of treatment), blood was collected (jugular venipuncture) daily for the duration of the treatment period and for 4 d after melatonin treatment ended (14 d after termination of PTU treatment) to measure serum T4 and 3,5,3'-triiodothyronine (T3) concentrations in response to PTU treatment. Additional samples were collected from ewes at parturition and on alternating days for 30 d thereafter to measure serum progesterone (P4) as an indicator of postpartum cyclicity. The time interval between breeding date and birth date was used as gestation length. Blood samples were collected from lambs at parturition, 14, and 28 d after parturition to measure serum T4, T3, and IGF-I to assess thyroid status and growth. Lamb thyroid glands were palpated on these days and were assigned a thyroid score of 1 to 5 (1 = normal, 5 = large goiter). Lambs were weighed at birth and both ewes and lambs were weighed 14 and 28 d after parturition. Serum was harvested by centrifugation at 1500 x g for 15 min at 4°C and was stored at -20°C until analyzed. Serum T4 (Richards et al., 1999), T3 (Wells et al., 2003), and serum P4 (Schneider and Hallford, 1996) were quantified by RIA utilizing components of commercial kits from Diagnostic Products Corp. (Los Angeles, CA). The procedures described by Berrie et al. (1995) were used to quantify IGF-I concentrations. The within and between assay coefficients of variation for all hormone RIA were less than 15%. All animal procedures were approved by the Institutional Animal Care and Use Committee.

Effects of PTU and melatonin on ewe BW, ewe T4, ewe T3, lamb T4, lamb T3, lamb IGF-I, and lamb BW were examined as repeated measures using Proc Mixed of SAS (SAS Inst. Inc., Cary, NC). When significant treatment x day interactions were detected, effects of PTU and melatonin were examined within day. All analyses were computed using the Mixed procedure of SAS.

## Results and Discussion

### *Prepartum*

A treatment x day interaction was not detected ( $P > 0.90$ ) for ewe BW; therefore, treatment effects were examined across weigh periods and no difference ( $P = 0.57$ )

was observed between treatments. However, control ewes gained 6.5 kg while PTU-treated ewes only gained 1.5 kg throughout the treatment period. Likewise, Duffey et al. (2002) and Hernandez et al. (2003) noted reduced BW gains in pregnant ewes treated with PTU in late gestation and non-pregnant ewes receiving 40 mg PTU, respectively. Collectively, these data indicate that short-term PTU-induced hypothyroidism in late gestation may decrease BW gains.

Serum T4 and T3 during the 35-d treatment period are shown in Figure 1. A PTU x day interaction for T4 and T3 was detected ( $P < 0.001$ ) which necessitated evaluation of PTU effects within day. On d 0, PTU-treated ewes tended ( $P = 0.08$ ) to have higher serum T4 ( $115 \pm 11$  ng/mL) than control ewes ( $88 \pm 11$  ng/mL). Values were similar ( $P > 0.10$ ) between treated and control ewes through d 22; but on d 23, serum T4 in controls was 128 compared with  $55 (\pm 28)$  ng/mL ( $P = 0.05$ ) for PTU-treated females. Serum T4 continued to decrease and remained lower ( $P < 0.05$ ) in PTU-treated ewes through d 35 when treatment ended, and T4 was 139 and  $17 (\pm 11)$  ng/mL for control and treated ewes, respectively. Interestingly, the decrease in serum T4 in response to PTU was not as rapid or pronounced as previous results using the same 40/20 mg/kg BW PTU treatment regimen (Duffey et al., 2002; Hernandez et al., 2003). Specifically, Duffey et al. (2002) found decreases in serum T4 by d 5 and T4 values of 15.2 ng/mL in PTU-treated pregnant ewes on d 9, which is below the 20 ng/mL nadir suggested by Dahl et al. (1995) to be necessary to influence seasonal cyclicity in sheep. Duffey et al. (2002) and Hernandez et al. (2003) found a continued decrease in serum T4 to a nadir ( $< 1$  ng/mL) by approximately d 15 (day when 40 mg PTU dosage was reduced to 20 mg/kg BW), and the 20 mg/kg BW PTU dosage was sufficient to maintain the T4 nadir. In this study, serum T4 in treated ewes was not lower ( $P > 0.10$ ) than controls until d 23, which is well past d 15 when the PTU dosage was decreased. Serum T4 declined throughout the treatment period in PTU-treated females to the lowest value of  $17 \pm 11$  ng/mL on d 35 (last day of treatment), and this was the only day in which T4 was below 20 ng/mL for PTU-treated ewes. Much of this discrepancy can be accounted for by the inter-animal variation in response to PTU treatment as some ewes in the treatment group only marginally responded while others reached a serum T4 nadir similar to those reported by Duffey et al. (2002). Also, Duffey et al. (2002) utilized nulliparous ewes without melatonin treatment. This study utilized a mix of nulliparous and multiparous ewes as well as melatonin supplementation. Because some PTU-treated ewes exhibited almost no T4 production and evidence by Brammer et al. (1979) found no interaction between melatonin and T4 secretion in rats, it is unlikely that melatonin supplementation negated the PTU effect on T4 production. In addition, Wells et al. (2003) noted the decline in serum T4 of ewe lambs treated with PTU was more rapid than that of mature ewes given PTU in the study by Hernandez et al. (2003). It is possible that, as ewes age, they become more resistant to PTU in terms of T4 production; further studies are needed to verify this possibility.

Aside from inhibiting T4 production, PTU inhibits Dio2 thereby decreasing conversion of T4 to the active form T3. On d 0, both treated and control ewes had  $1.5 (\pm 0.11)$  ng/mL T3. However, by d 8, PTU decreased ( $P = 0.03$ ) serum T3 in treated ewes ( $0.97 \pm 0.10$  ng/mL) compared with controls ( $1.36 \pm 0.10$  ng/mL). Treated ewes remained lower ( $P \leq 0.05$ ) throughout the treatment period, and the 20 mg/kg BW dosage was sufficient to keep serum T3 depressed with values of 2.73 and  $0.72 (\pm 0.26)$  ng/mL in control and treated ewes, respectively, on d 34 ( $P < 0.001$ ). By d 37 (3 d after PTU treatment ended), serum T3 in the treated group rebounded above that of controls with values of 2.14 and  $3.85 (\pm 0.65)$  ng/mL for control and treated ewes, respectively ( $P = 0.05$ ), and tended ( $P \leq 0.08$ ) to be higher through d 40 with a peak on d 38 ( $3.13$  and  $5.66 \pm 0.98$  ng/mL in control and treated ewes, respectively;  $P = 0.06$ ). Wells et al. (2003) observed a similar decrease in serum T3 in ewe lambs treated with PTU and showed a rebound of serum T3 above control values when PTU treatment ended. Interestingly, the T3 pattern in our study is similar to that observed by Wells et al. (2003), but these workers also showed a marked depression in serum T4. These data imply that ewes in the present study were hypothyroid as characterized by serum T3 status.

#### *Postpartum*

Ewes treated with PTU and melatonin had a gestation length of 155 compared with  $150 (\pm 0.8)$  d for controls ( $P = 0.0006$ ) indicating that gestation may be lengthened by PTU and melatonin treatment. A treatment x day interaction was not detected ( $P = 0.90$ ) for ewe BW after parturition. Analysis of treatment effects examined across postpartum weigh periods showed no difference ( $P = 0.80$ ) between treatments. Serum P4 was below 1 ng/mL for all ewes on all days indicating that PTU and melatonin treatment in late gestation did not induce early postpartum cyclicity.

Because a treatment x day interaction was not detected for lamb weights ( $P > 0.60$ ) or IGF-I concentrations, treatment effects were examined across sampling days. Lamb weights were similar ( $P = 0.66$ ) for control lambs and those born to treated ewes. Likewise, IGF-I concentrations did not differ ( $P = 0.45$ ) with values of 311 and 277 ( $\pm 36$ ) ng/mL for offspring of control and treated ewes, respectively. These data indicate that PTU treatment with melatonin supplementation in late gestation has little effect on subsequent lamb performance. Conversely, Duffey et al. (2002) reported decreased birth weights for offspring of PTU-treated ewes. This finding is particularly interesting as ewes in the current experiment exhibited similar decreased gains during the treatment period as those reported by Duffey et al. (2002) indicating that both groups were exposed to similar conditions in terms of nutritional status. It is unlikely that melatonin would account for the higher birth weights observed in our study; therefore, the difference may again be due to differences in nulliparous versus multiparous ewes.

In addition, a treatment x day interaction was not detected for lamb serum T4 ( $P > 0.70$ ) or T3; therefore, treatment effects were examined across sampling periods. Lamb serum T4 values did not differ ( $P = 0.24$ ) between

treatments and were 172 and  $188 (\pm 11)$  ng/mL for lambs born to control and treated ewes, respectively. Similarly, T3 was not different ( $P = 0.46$ ) between treatments with values of 4.58 and  $4.99 (\pm 0.45)$  ng/mL for offspring of control and treated ewes, respectively. Likewise, Duffey et al. (2002) reported no differences in serum T4 concentrations between lambs born to treated and control ewes. However, in our study, 56 % of lambs born to treated ewes had enlarged thyroid glands compared with none of the controls indicating that a hypothyroid condition existed in response to maternal PTU treatment. Likely, lamb thyroid status was able to recover during the 1.5 mo period between when maternal PTU treatment ended and parturition resulting in normal thyroid hormone concentrations in offspring at birth.

Even though serum T4 was not as depressed as reported in other studies (Duffey et al., 2002; Hernandez et al., 2003), it appears that 40/20 mg PTU/kg BW with melatonin still induced a hypothyroid condition at the tissue level. Ewes exposed to PTU and melatonin gained less, and had depressed T3 values indicating that PTU was effective in inhibiting Dio2. Also, lambs born to treated females exhibited enlarged thyroid glands at birth.

#### **Implications**

A hypothyroid-like condition was induced by propylthiouracil and melatonin treatment in this study, but treatments were unable to induce early postpartum cyclicity. Several factors could account for lack of cyclicity including duration of treatment. Propylthiouracil treatment could be extended; however, detrimental effects on offspring would likely occur. Further studies are needed to determine methods of inducing early postpartum cyclicity in ewes.

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#### **Literature Cited**

- Anderson, G. M., S. L. Hardy, M. Valent, H. J. Billings, J. M. Connors, and R. L. Goodman. 2003. Evidence that thyroid hormones act in the ventromedial preoptic area and the premammillary region of the brain to allow the termination of the breeding season in the ewe. *Endocrinology* 144:2892-2901.
- Barrell, G. K., L. A. Thrun, M. E. Brown, C. Viguie, and F. J. Karsch. 2000. Importance of photoperiodic signal quality to entrainment of the circannual reproductive rhythm of the ewe. *Biol. Reprod.* 63:769-774.

- Berrie, R. A., D. M. Hallford, and M. L. Galyean. 1995. Effects of zinc source and level on performance, carcass characteristics, and metabolic hormone concentrations of growing and finishing lambs. *Prof. Anim. Sci.* 11:149-156.
- Brammer, G. L., J. E. Morley, E. Geller, A. Yuwiler, and J. M. Hershman. 1979. Hypothalamus-pituitary-thyroid axis interactions with pineal gland in the rat. *Am J Physiol Gastrointes. Liver Physiol.* 236: G416-G420.
- Dahl, G. E., N. P. Evans, L. A. Thrun, and F. J. Karsch. 1995. Thyroxine is permissive to seasonal transition in reproductive neuroendocrine activity in the ewe. *Biol. Reprod.* 52:690-696.
- Duffey, J.L., D. M. Hallford, C. A. Gifford, and R. L. Rosencrans. 2002. Effects of thyroid inhibition during gestation on serum thyroxine and weight responses of fine-wool ewes. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 53:164-166.
- Hernandez, J. A., D. M. Hallford, and N. H. Wells. 2003. Ovarian cyclicity in thyroid-suppressed ewes treated with propylthiouracil immediately before onset of seasonal anestrus. *J. Anim. Sci.* 81:29-34.
- Karsch, F. J., G. E. Dahl, N. P. Evans, J. M. Manning, K. P. Mayfield, S. M. Moenter, and D. L. Foster. 1993. Seasonal changes in gonadotropin-releasing hormone secretion in the ewe: alteration in response to the negative feedback action of estradiol. *Biol. Reprod.* 49:1377-1383.
- Karsch, F. J., G. E. Dahl, T. M. Hachigian, and C. A. Thrun. 1995. Involvement of thyroid hormones in seasonal reproduction. *J. Reprod. Fertil. (Suppl. 49):* 409-422.
- Malpaux, B. A. Daveau, F. Maurice-Mandon, G. Duarte, and P. Chemineau. 1998. Evidence that melatonin acts in the premammillary hypothalamic area to control reproduction in the ewe: presence of binding sites and stimulation of luteinizing hormone secretion by in situ microimplant delivery. *Endocrinology* 139:1508-1516.
- Malpaux, B. and F. J. Karsch. 1990. A role for short days in sustaining seasonal reproductive activity in the ewe. *J. Reprod. Fertil.* 90:555-562.
- Perez-Eguia, E., and D. M. Hallford. 1994. Reproductive and endocrine characteristics of ewe lambs after short- or long-term administration of melatonin. *Sheep and Goat Res. J.* 10:194-200.
- Richards, J. B., D. M. Hallford, and G. C. Duff. 1999. Serum luteinizing hormone, testosterone, and thyroxine and growth responses of ram lambs fed locoweed (*Oxytropis sericea*) and treated with vitamin E/selenium. *Theriogenology* 52:1055-1066.
- Schneider, F. A., and D. M. Hallford. 1996. Use of a rapid progesterone radioimmunoassay to predict pregnancy and fetal numbers in ewes. *Sheep and Goat Res. J.* 12:33-38.
- Thrun, L. A., G. E. Dahl, N. P. Evans, and F. J. Karsch. 1997. A critical period for thyroid hormone action on seasonal changes in reproductive neuroendocrine function in the ewe. *Endocrinology* 138:3402-3409.
- Turek, F. W. and C. S. Campbell. 1979. Photoperiodic regulation of neuroendocrine-gonadal activity. *Biol. Reprod.* 20:32-50.
- Watanabe, M., S. Yasuo, T. Watanabe, T. Yamamura, N. Nakao, S. Ebihara, and T. Yoshimura. 2004. Photoperiodic regulation of type 2 deiodinase gene in djungarian hamster: possible homologies between avian and mammalian photoperiodic regulation of reproduction. *Endocrinology* 145:1546-1549.
- Wells, N. H., D. M. Hallford, and J. A. Hernandez. 2003. Serum thyroid hormones and reproductive characteristics of Rambouillet ewe lambs treated with propylthiouracil before puberty. *Theriogenology* 59:1403-1413.

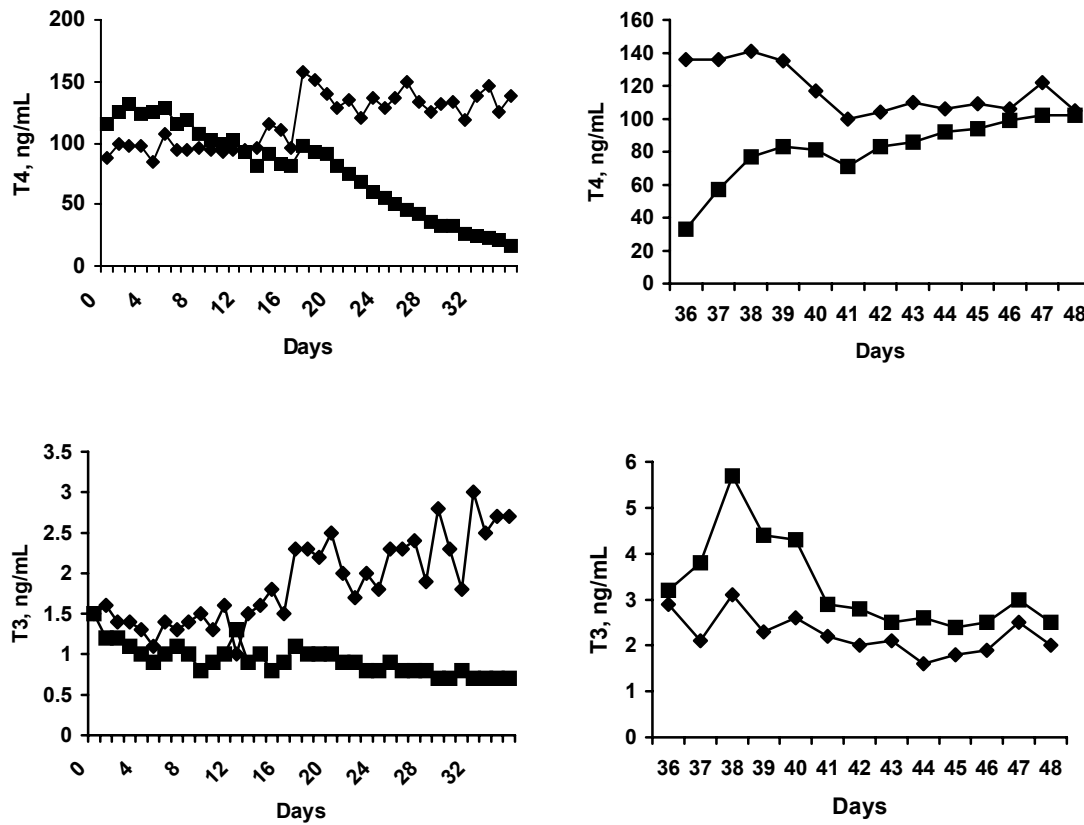


Figure 1. Serum thyroxine (T4) (top row) and 3,5,3'-triiodothyronine (T3) (bottom row) in pregnant Rambouillet ewes during (left column) and after (right column) a 35-d treatment period (d 0 = first day of treatment) in which ewes received 0 (---♦---) or 40 (---■---) mg of propylthiouracil (PTU)/kg body weight (BW)/day beginning when all ewes were  $76.8 \pm 4.7$  d of gestation (Jan 2). After 14 d, the dosage of PTU was decreased to 20 mg/kg BW for 20 d, and treatment with 5 mg/d of melatonin was initiated and administered for 30 d.

**SUPPLEMENTAL FAT IN LIMIT-FED, HIGH GRAIN PREPARTUM DIETS OF BEEF COWS:  
EFFECTS ON COW WEIGHT GAIN, REPRODUCTION, AND CALF HEALTH, IMMUNITY,  
AND PERFORMANCE**

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**ABSTRACT:** Multiparous Angus  $\times$  Gelbvieh ( $n = 155$ ) beef cows (initial BW =  $604.5 \pm 4.2$  kg) were used to determine the effect of prepartum fat supplementation on cow BW, BCS, reproduction, and calf birth weight, health, immunity, plasma fatty acid (FA) concentrations, and performance. Starting approximately 80 d prepartum, cows were adapted to a limit-fed (60% rolled corn:40% millet hay) diet. At  $60 \pm 1.3$  d prior to calving, cows were allotted to one of two isocaloric, isonitrogenous diets containing either 2.69% (CON;  $n = 79$ ) or 4.63% (FSUP;  $n = 76$ ) dietary fat. Following parturition, all cows received CON diet plus ad libitum grass hay until breeding. Cows were synchronized for estrus on d  $61 \pm 1.32$  postpartum and calves were weaned at  $101 \pm 1.1$  d of age received a s.q. antigen injection at  $116 \pm 1.08$  d of age. Prepartum diet did not affect ( $P \geq 0.35$ ) cow BW or BCS change. Calf birth weight, vigor score, and rectal temperature were not influenced ( $P \geq 0.52$ ) by cow diet; however, FSUP calves had higher plasma IgG levels ( $P = 0.05$ ;  $15.44$  vs  $11.00 \pm 1.63$  mg/ml) at  $14 \pm 0.5$  h after birth. Calves from FSUP dams had higher plasma FA concentrations of 18:1 *trans*-11 ( $P < 0.01$ ;  $0.89$  vs  $0.42 \pm 0.07$  %), and CLA ( $P < 0.01$ ;  $0.22$  vs  $0.13 \pm 0.01$  %), than CON calves. Cows detected in estrus ( $P = 0.56$ ) and first service conception rates ( $P = 0.87$ ) were not different due to diet. Calf weaning weight ( $P = 0.26$ ) and BW gain ( $P \geq 0.39$ ) were not affected by dams prepartum diet. Calf response to antigen injection was not different ( $P = 0.90$ ) between treatments. Although cow and calf performance was not influenced by prepartum fat supplementation, calf IgG levels and plasma FA associated with immune function were increased in calves from FSUP dams. Thus, prepartum supplemental fat may have positive effects on calf immune status and overall health.

Key Words: Beef cows, Calf health, Fat supplementation

### Introduction

Previous studies have established the importance of prepartum nutrition on subsequent reproduction in beef cattle (Randel, 1990; Short et al., 1990; Dunn and Moss,

1992). It is difficult to reverse the negative reproductive impacts of inadequate prepartum nutrition during the postpartum interval due to the dramatic increase in nutrient demands during lactation, (Lalman et al., 2000). Feeding supplemental fat, specifically diets high in linoleic acid, to beef cows during late gestation has been evaluated as method to minimize the negative impacts of nutritional inadequacy on reproductive performance (Bellows et al., 2001; Grings et al., 2001; Alexander et al., 2002). Additionally, the influence of diet on the fatty acid (FA) profile of phospholipid pools may lead to residual effects (Staples et al., 1998) which could influence subsequent reproduction of cows provided with supplemental fat during late gestation (Hess et al., 2003). The apparent carry-over effect associated with feeding fat prepartum may also serve as an important functional link with calf survivability (Hess et al., 2003). Lammoglia et al. (1999a) indicated an increase in cold tolerance of neonatal calves from dams receiving supplemental fat for 8 wk prior to calving. In addition, Dietz et al. (2003) observed increased IgG levels in calves born when temperatures were below 6°C from dams supplemented fat prepartum. However, effects on calf vigor, and birth weight have been less conclusive (Bellows et al., 2001; Alexander et al., 2002; Dietz et al., 2003). Concerning the effects of supplemental fat on calf weights and performance, Espinoza et al. (1995) reported increased calf weight gains when dams were fed fat pre- and postpartum. However, Alexander et al. (2002) indicated no increase in weight gain by calves from dams fed fat prepartum. Therefore the objective of this study was to evaluate the effects of prepartum fat supplementation on cow body condition score and reproductive performance, as well as calf immunity and weight gain.

### Materials and Methods

#### *Cow performance and early calf health*

One hundred fifty five Angus  $\times$  Gelbvieh, multiparous cows ( $604.5 \pm 4.2$  kg,  $6.2 \pm 0.2$  yrs) from the University of Wyoming beef herd, were initially weighed and body condition scored (Wagner et al., 1988) by two independent technicians approximately 80 d prior to calving and adapted

to a limit-fed, corn and millet hay based diet over a 21 d period. At the end of the adaptation period, cows were weighed on two consecutive days, body condition scored, and allotted to treatment based on BW, BCS, estimated calving date, cow age, and service sire. Treatments consisted of a limit-fed control ration (CON;  $n = 79$ ) or an added fat ration (FSUP;  $n = 76$ ; table 1). Rations were formulated to be isocaloric and isonitrogenous and were delivered once daily for an average of  $61 \pm 1.3$  d prepartum. At approximately  $14 \text{ h} \pm 0.5 \text{ h}$  postpartum, calf birth weight, rectal temperature, and vigor score (1 = nursed immediately, 4 = died shortly after birth) were recorded, and three 10 ml jugular blood samples were collected into EDTA, sodium heparin, and non-anticoagulant vacutainers. Blood samples collected into EDTA tubes were used to determine total white blood cell counts (cells/ml) and white blood cell percentages (neutrophils, lymphocytes, monocytes, and eosinophils). Samples in non-anticoagulant tubes were utilized to determine total IgG concentrations as determined by quantitative ELISA (Bethyl laboratories, Montgomery, TX). Serum IgG concentrations were classified according to Perino et al. (1995) as adequate ( $> 16.00 \text{ mg/ml}$ ), marginal ( $8.00 - 16.00 \text{ mg/ml}$ ), or inadequate ( $< 8.00 \text{ mg/ml}$ ). Blood samples collected in sodium heparin tubes were used to determine calf plasma FA profile (Murrieta et al., 2003). Following calving, cows were commingled and managed similarly receiving the CON diet plus ad libitum grass hay (13.5% CP) until breeding. Cow BW and BCS were taken  $19 \pm 0.7$  d postpartum. On d  $61 \pm 1.3$  postpartum, cow BW and BCS were recorded and cows were synchronized for estrus. Intravaginal progesterone inserts (CIDR; EAZI-BREED™ CIDR®, Pfizer, Inc., New York, NY) were inserted on d 1 of synchronization. On d 7 CIDRs were removed, and 5 ml of prostaglandin  $F_{2\alpha}$  ( $\text{PGF}_{2\alpha}$ ; Lutalyse, Pharmacia, Corp., Kalamazoo, MI) was administered intramuscularly, following Beef Quality Assurance guidelines. Cows were then monitored for estrus for 7 d following  $\text{PGF}_{2\alpha}$  injection and bred via artificial insemination 12 h following estrus detection. Response to synchronization was determined as the number of cows synchronized divided by the number of cows detected in estrus. Following initial estrus detection period, cows were hauled 56 km to summer pasture and then monitored for estrus for an additional 21 d heat cycle and artificially inseminated upon return to estrus. Bulls were then turned in following the second heat detection period for 30 d. First service conception was determined at 38 d following estrus synchronization (99 d postpartum) via transrectal ultrasoundography. Final conception was determined by rectal palpation following removal of bulls.

#### *Calf health and performance*

At  $101 \pm 1$  d of age 129 calves were weaned, dry-lotted 7 h, and hauled 56 km to the University of Wyoming livestock center. Calves were weighed on two consecutive days, vaccinated for respiratory and clostridial diseases (Bovashield 4, Ultravac-7, Pfizer, Inc., New York, NY), and allotted to one of four pens based on dam's prepartum nutrition (CON or FSUP). Calves were provided 30.5 cm per head of bunk space and fed once daily at 0700. Calves

were initially offered access to grass hay (13.5% CP) and 0.91 kg of commercial supplement (RT31, 14.5% CP or RT32, 18.5% CP; ADM Alliance Nutrition, Inc., Quincy, IL) per head. Hay was increased or decreased based upon the previous day's consumption and supplement was increased at a rate of 0.23 kg/d until supplement consumption reached 2.27 kg/hd/d. Calves were maintained at this supplement level for the remainder of the 35 d receiving trial. An ovalbumin (OVA) antigen injection was administered on d 15 of the receiving trial to determine calf immune response. Prior to OVA injection (d 0), calves were bled via jugular veinapuncture into 10 ml, non-anticoagulant vacutainers and then administered 1 ml OVA s.q.. Blood samples were then collected on d 7, 14, and 21 following injection. Response to antigen was measured by total OVA specific antibody, as determined by indirect ELISA. Calves were monitored for morbidity daily and treated according to symptoms. Final weights were determined by the average weights on d 34 and 35.

#### *Statistical Analysis*

Effects of prepartum supplement on cow BW, BCS, reproductive performance, and calf birth weight and weaning weight were analyzed as a completely randomized design using the General Linear Model (GLM) procedures of SAS (SAS Inst. Inc., Cary, NC) with cow or calf as the experimental unit. Calf IgG levels, FA levels, morbidity, and mortality were analyzed using the GLM procedure with cow treatment in the model statement. Chi-Square was used to evaluate the distribution of IgG intervals within treatment. Calf post-weaning performance and response to antigen injection was analyzed as a two by two factorial with cow treatment and calf supplement in the model statement.

### **Results and Discussion**

#### *Cow/Calf Weight Gain*

Diet had no effect on 19 d postpartum cow BW ( $P = 0.69$ ) or BCS ( $P = 0.95$ ) or, 61 d BW ( $P = 0.84$ ) and BCS ( $P = 0.63$ ) (Table 2). During the feeding period, cow BCS was not affected ( $P \geq 0.35$ ) by diet with CON and FSUP gaining  $0.27 \pm 0.1$  and  $0.35 \pm 0.1$  BCS respectively, from the initiation of the trial to estrus synchronization (d 61). Similarly, Tjardes et al. (1998) observed no difference in performance between postpartum cows consuming limit-fed diets containing cracked corn and hay with either 4.0 or 0.0 % supplemental fat. Calf birth weight was not affected ( $P = 0.52$ ) by diet, which is agreement with results from Dietz et al. (2003). This is in contrast to Lammoglia et al. (1999b) who reported increased calf birth weights from cows supplemented fat 8 wk prepartum. Calf weaning weights ( $P = 0.52$ ) and 101 d weight gain ( $P = 0.16$ ) were similar between treatments (Table 5). These results are similar to those of Alexander et al. (2002) who noted no differences in weight gain from birth to 90 d of age in calves from heifers supplemented fat 62 d prepartum. The lack of significant findings in cow weight and BCS changes is partially expected due to the isocaloric nature of the diets. This combined with the similarity in weight gain by the calves may suggest that there is similar milk production

between dietary treatments, which is in concurrence with Alexander et al. (2002) where no difference in milk production between fat supplemented and non fat supplemented cows was noted.

### *Cow Reproduction*

In agreement with Alexander et al. (2000) the addition of supplemental fat to the limit-fed prepartum diet did not increase the number of cows exhibiting estrus 60 d postpartum or improve artificial insemination first service conception rates or final conception rate (Table 2). Bellows et al. (2001) observed varying results in conception rates across study years in cows supplemented fat prepartum. In the present study, prepartum plane of nutrition and adequate body condition of the cows may contribute to the lack of treatment responses. Wiltbank et al. (1962) found conception rates of 95% in cows that were on a high plane of nutrition both before and after parturition. Likewise, Hughton et al. (1990) indicated that cows in moderate body condition at parturition that maintained their condition until breeding had higher pregnancy rates than cows that were thin at calving or excessively conditioned at breeding. Cows in the present trial were in adequate condition at breeding (BCS =  $5.37 \pm 0.1$ ), increasing 0.31 BCS from 60 d prepartum until breeding. This would indicate that cows may have already been at peak reproductive performance, thus the potential beneficial reproductive effects of fat supplementation were unnoticed.

### *Calf Health and Immunity*

Calf vigor score and rectal temperature at birth were not affected ( $P = 0.98$  and  $P = 0.98$  respectively; Table 3). Similarly, Dietz et al. (2003), Alexander, et al (2002), and Bellows et al. (2001) indicated no difference in calf vigor score from dams receiving supplemental fat prepartum. Lammoglia et al. (1999a) and Dietz et al. (2003) reported that calf rectal temperature was also not affected by fat supplementation. This is in contrast to Lammoglia et al. (1999b) who observed increased rectal temperatures in calves with prolonged cold exposure from dams fed fat prepartum. In the present study, weather conditions were mild for the majority of the calving season, and may not have elicited a cold tolerance response (Hess et al., 2003). Total white blood cell counts and concentrations were not different ( $P = 0.95$  and  $P = 0.94$  respectively) between treatments, with similar percentages of lymphocytes, neutrophils, monocytes, and eosinophils ( $P \geq 0.11$ ; Table 3). Meek et al. (2004) reported similar results in Holstein calves fed colostrum containing high levels of CLA. Calves from FSUP dams had higher ( $P = 0.05$ ) mean levels of total IgG, as well as a greater ( $P = 0.04$ ) percentage of calves with adequate ( $> 16.0$  mg/ml) IgG levels and fewer calves with marginal (8.0 – 16.0 mg/ml) or inadequate ( $< 8.0$  mg/ml) levels of IgG (Table 3). This may indicate improved passive transfer in calves from fat supplemented dams. Dietz et al. (2003) observed no differences in cow colostrum IgG concentrations or calf serum IgG levels at 36 h. However, Dietz et al. did note greater serum IgG concentrations in calves from fat supplemented dams born at an ambient temperature below 6°C. In the present study,

pre-weaning calf morbidity and mortality was not affected ( $P = 0.25$  and  $P = 0.78$  respectively). The addition of supplemental fat prepartum altered calf plasma FA profile (Table 4). Fatty acid concentrations were higher for 18:0 ( $P = 0.02$ ), 18:1 *trans* 11 ( $P < 0.001$ ), CLA ( $P < 0.001$ ), 20:3 ( $P = 0.006$ ) and tended ( $P = 0.08$ ) to be higher for 18:3 in calves from FSUP dams. Lake et al. (2004a) observed similar alterations in adipose tissue fatty acid profiles from calves suckling dams receiving high-oleate or high-linoleate diets for 60 d postpartum. Several of these FA have been shown to play a role in immune function or act as intermediates to FA associated with immune function (Hwang, 2000; Suchner et al., 2000; Calder, 2001). Therefore, it may be possible that the increases in FA associated with immune function could alter early calf immune status.

### *Early Weaned Calf Weight Gain and Immunity*

There was no cow treatment by receiving ration interaction for calf weight gain ( $P \geq 0.24$ ), morbidity ( $P \geq 0.56$ ), or response to immune challenge ( $P \geq 0.41$ ), so only main effects of cow prepartum fat supplementation were reported. During the 35 d weaning period there was no difference ( $P = 0.39$ ) in weight gain due to dams prepartum dietary fat. Morbidity was similar ( $P = 0.39$ ), with no mortality during the 35 d period for either treatment (Table 5). Due to the decrease in failure of passive transfer of immunity observed in calves from FSUP dams, one would have expected FSUP calves to have a lower morbidity rate. Wittum and Perino (1995) noted that calves not acquiring adequate levels of IgG had over  $3 \times$  greater chance of becoming ill, however, in the present study this increase in number of morbid calves was not observed. Results from the OVA antigen injection are reported in Table 6. Fat supplemented calves had a slower ( $P = 0.007$ ) response to the antigen from d 0 to 7. Antibody response from d 14, and 21 were not different ( $P = 0.58$  and  $P = 0.29$  respectively) between treatments. As well, total response to antigen was not different ( $P = 0.90$ ) between treatments over the 21 d period (Figure 1). Lake et al. (2004b) noted a trend for a decrease in humoral response to antigen injection and no difference in cell mediated response for calves suckling dams supplemented fat postpartum. In the present study fat supplementation was stopped at parturition, and while calf plasma fatty acid profiles were similar to the adipose tissue fatty acid profiles observed by Lake et al. (2004b) at birth, these differences may not have been apparent at the time of antigen injection. This may warrant further investigation into the possibility of alterations in immune function within the first few weeks following birth of calves from dams supplemented fat prepartum.

### **Implications**

The addition of supplemental fat to prepartum diets did not affect cow reproductive performance, weight gain, or calf performance. However, the addition of fat may decrease failure of passive immune transfer and possibly lead to improved neonatal calf health.

## Literature Cited

- Alexander, B. M., B. W. Hess, D. L. Hixon, B. L. Garrett, D. C. Rule, M. McFarland, J. D. Bottger, D. D. Simms, and G. E. Moss. 2002. Influence of prepartum fat supplementation on subsequent beef cow reproduction and calf performance. *Prof. Anim. Sci.* 18:351-357.
- Bellows, R. A., E. E. Grings, D. D. Simms, T. W. Geary, and J. W. Bergman. 2001. Effects of feeding supplemental fat during gestation to first-calf beef heifers. *Prof. Anim. Sci.* 17:81-89.
- Calder, P. C. and E. A. Miles. 2000. Fatty acids and atopic disease. *Pediatr. Allergy Immunol. Suppl.* 13:29-36.
- Dietz, R. E., J. B. Hall, W. D. Whittier, F. Elvinger, and D. E. Eversole. 2003. Effects of feeding supplemental fat to beef cows on cold tolerance in newborn calves. *J. Anim. Sci.* 81:885-894.
- Dunn, T. G. and G. E. Moss. 1992. Effects of nutrient deficiencies and excesses on reproductive efficiency of livestock. *J. Anim. Sci.* 70:1580-1593.
- Espinoza, J. L., J. A. Ramirez-Godinez, J. A. Jimenez, and A. Flores. 1995. Effects of calcium soaps of fatty acids on postpartum reproductive activity in beef cows and growth of calves. *J. Anim. Sci.* 73:2888-2892.
- Grings, E. E., R. E. Short, M. Blummel, M. D. MacNeil, and R. A. Bellows. 2001. Prepartum supplementation with protein or fat and protein for grazing cows in three seasons of calving. *Proc. West. Sect. Am. Soc. Anim. Sci.* 52.
- Hess, B. W., D. C. Rule, and G. E. Moss. 2003. High fat supplements for reproducing beef cows: Have we discovered the magic bullet? *Proc. Pacific Northwest Nurt. Conf.*
- Houghton, P. L., R. P. Lemenager, L. A. Horstman, K. S. Hendrix, and G. E. Moss. 1990. Effects of body composition, pre- and postpartum energy level and early weaning on reproductive performance of beef cows and preweaning calf gain. *J. Anim. Sci.* 68:1438-1446.
- Hwang, D. 2000. Fatty acids and immune responses-A new perspective in searching for clues to mechanism. *Annu. Rev. Nutr.* 20:431-456.
- Lake, S. L., B. W. Hess, E. J. Scholljegerdes, R. L. Atkinson, and D. C. Rule. 2004a. Milk and calf adipose tissue fatty acid changes in response to maternal supplementation with high-linoleate or high-oleate safflower seeds. *Proc. Midwest Sect. Am. Soc. Anim. Sci.* 74 (abstr.)
- Lake, S. L., E. J. Scholljegerdes, E. L. Belden, R. L. Atkinson, D. C. Rule, and B. W. Hess. 2004b. Calf adipose tissue fatty acid profile, immune function and performance while nursing beef cows consuming high-linoleate or high-oleate safflower seed supplements. *Nat. Am. Soc. Anim. Sci.* (Excepted)
- Lalman, D. L., J. E. Williams, B. W. Hess, M. G. Thomas, and D. H. Keisler. 2000. Effect of dietary energy on milk production and metabolic hormones in thin, primiparous beef heifers. *J. Anim. Sci.* 78:530-538.
- Lammoglia, M. A., R. A. Bellows, E. E. Grings, J. W. Bergman, R. E. Short, and M. D. MacNeil. 1999a. Effects of feeding beef females supplemental fat during gestation on cold tolerance in newborn calves. *J. Anim. Sci.* 77:824-834.
- Lammoglia, M. A., R. A. Bellows, E. E. Grings, and J. W. Bergman. 1999b. Effects of prepartum supplementary fat and muscle hypertrophy genotype on cold tolerance in newborn calves. *J. Anim. Sci.* 77:2227-2233.
- Meek, K. I., S. T. Franklin, L. J. Driedger, J. A. Jackson, M. W. Schilling, and M. T. Sands. 2004. Dietary conjugated linoleic acid (CLA) affects the immune system of neonatal Holstein calves. *Proc. Midwest Sect. Am. Soc. Anim. Sci.* 18 (abstr.)
- Murrieta, C.M., B.W. Hess, and D.C. Rule. 2003. Comparison of acidic and alkaline catalysts for preparation of fatty acid methyl esters from ovine muscle with emphasis on conjugated linoleic acid. *Meat Sci.* 65:523-529.
- Perino, L. J., T. E. Wittum, and G. S. Ross. 1995. Effects of various risk factors on plasma protein and serum immunoglobulin concentrations of calves at postpartum hours 10 and 24. *Am. J. Vet. Res.* 56:1144-1148.
- Randel, R. D. 1989. Nutrition and postpartum rebreeding in cattle. *J. Anim. Sci.* 68:853-862.
- Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. *J. Anim. Sci.* 68:799-816.
- Staples, C. R., J. M. Burke, and W. W. Thatcher. 1998. Optimizing energy nutrition for reproducing dairy cows. *J. Dairy Sci.* 81:856-871.
- Suchner, U., K. S. Kuhn, and P. Furst. 2000. The scientific basis of immunonutrition. *Proc. Nutr. Soc.* 59:553-563.
- Tjardes, K. E., D. B. Faulkner, D. D. Buskirk, D. F. Parrett, L. L. Berger, N. R. Merchen, and F. A. Ireland. 1998. The influence of processed corn and supplemental fat on digestion of limit-fed diets and performance of beef cows. *J. Anim. Sci.* 76:8-17.
- Wagner, J. J., K. S. Lusby, J. W. Oltjen, J. Rakestraw, R. P. Wettemann, and L. E. Walters. 1988. Carcass composition in mature Hereford cows: Estimation and effect on daily metabolizable energy during the winter. *J. Anim. Sci.* 66: 603-612.
- Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, K. E. Gregory, and R. M. Koch. 1964. Effect of energy level on reproductive phenomena of mature Hereford cows. *J. Anim. Sci.* 21: 219.

**Table 1.** Diet composition

Item	Diet <sup>a</sup>	
	Control	Fat supplement
Chemical composition, % DM basis		
DM, %	87.73	88.46
CP	10.28	10.15
TDN	75.36	75.15
Fat	2.69	4.63
ADF	16.64	19.87
NDF	43.15	44.59
OM	92.89	92.37
DMI, Kg/hd <sup>b</sup>	9.98	9.97
TDN, Kg/hd	7.52	7.49
Fatty acid analysis		
FA, mg/g <sup>c</sup>		
16:0	28.18	64.25
18:0	4.19	9.74
18:1 <i>cis</i> 9	27.97	70.04
18:2	69.27	148.41
18:3	18.33	24.20
Total	172.73	340.11
FA, % <sup>d</sup>		
16:0	15.77	17.10
18:0	2.36	2.56
18:1 <i>cis</i> 9	17.36	17.51
18:2	42.81	42.01
18:3	10.01	10.29
other	11.70	10.53

<sup>a</sup> Total mixed rations formulated to be isocaloric and isonitrogenous.

<sup>b</sup> Dry matter intake limited to amount reported.

<sup>c</sup> Expressed as mg of fatty acid per gram of feed.

<sup>d</sup> Fatty acid percentage of mg of total fatty acid.

**Table 2.** Effect of prepartum diet on cow performance and reproduction

Item	Treatment		SE	P-Value
	Control	Fat supplement		
Initial wt., kg	604	604	6.01	0.99
Initial BCS	5.08	5.05	0.06	0.73
Postpartum wt., kg <sup>a</sup>	604	600	6.71	0.69
Postpartum BCS <sup>a</sup>	5.49	5.50	0.08	0.95
Final wt., kg <sup>b</sup>	578	576	6.21	0.84
Final BCS <sup>b</sup>	5.34	5.39	0.07	0.63
Calf BW, kg	41.44	42.12	0.76	0.52
Estrus detection, % <sup>c</sup>	92.31	94.67	2.87	0.56
First service conception, % <sup>de</sup>	67.95	66.67	5.45	0.87
Final conception, % <sup>c</sup>	97.47	97.37	1.83	0.97

<sup>a</sup> Taken at 19 d postpartum.

<sup>b</sup> Taken at 61 d postpartum, time of estrus synchronization.

<sup>c</sup> Cows were synchronized on d 61 postpartum. Calculated as number of cows synchronized divided by number of cows detected in estrus.

<sup>d</sup> Verified d 99 postpartum.

<sup>e</sup> Calculated as number pregnant divided by number of cow synchronized.

**Table 3.** Effect of prepartum fat supplementation on early calf health

Item	Treatment		SE	P-Value
	Control	Fat supplement		
Calf vigor <sup>a</sup>	1.13	1.21	0.02	0.97
Rectal temperature, °C <sup>b</sup>	38.70	38.70	0.05	0.71
Morbidity, % <sup>c</sup>	29.11	21.05	4.99	0.25
Mortality, % <sup>d</sup>	6.33	5.26	2.70	0.78
IgG conc., mg/ml <sup>b</sup>	11.00	15.44	1.63	0.05
Passive transfer level <sup>ef</sup>				
% Adequate	21.52	39.47	-	0.04
% Marginal	31.65	21.05	-	0.04
% Inadequate	46.84	39.47	-	0.04
White blood cell counts				
Neutrophils, % <sup>g</sup>	65.77	66.32	1.61	0.81
Lymphocytes, % <sup>g</sup>	30.16	29.85	1.54	0.89
Monocytes, % <sup>g</sup>	3.34	3.13	0.25	0.55
Eosinophils, % <sup>g</sup>	0.33	0.61	0.13	0.11
Total white blood cells	214.45	215.28	10.31	0.95
White blood cell conc. <sup>h</sup>	10.71	10.77	0.52	0.94

<sup>a</sup> 1 = normal, stood quickly and nursed, 2 = slow to stand and nurse but did so unassisted, 3 = required assistance to nurse, 4 = died shortly after birth.

<sup>b</sup> Taken approximately 14 h after birth.

<sup>c</sup> % of calves treated for illness pre-weaning.

<sup>d</sup> Calf death loss % pre-weaning.

<sup>e</sup> Expressed as mg/ml with Adequate = > 16.00 mg/ml, Marginal = 8.00 – 16.00 mg/ml, and Inadequate = < 8.00 mg/ml.

<sup>f</sup> Percentage of calves within passive transfer level differs,  $\chi^2(2, n = 155) = 6.25, P = 0.04$

<sup>g</sup> Percent of total white blood cells.

<sup>h</sup> Cells/ml expressed as  $10^6$ .

**Table 4.** Effect of prepartum fat supplementation on calf plasma fatty acid profile

Fatty acid	Treatment		SE	P value
	Control	Fat supplement		
% of total FA <sup>a</sup>				
10:0	0.05	0.033	0.006	0.10
12:0	0.415	0.254	0.039	0.004
14:0	4.269	2.959	0.273	0.0009
14:1	0.549	0.532	0.020	0.55
15:0	0.266	0.266	0.013	0.99
16:0	29.498	27.502	0.591	0.02
16:1	4.077	3.675	0.141	0.04
17:0	0.752	0.764	0.030	0.78
17:1	0.546	0.535	0.022	0.72
18:0	11.930	12.865	0.279	0.02
18:1 <i>trans</i> 10	0.264	0.280	0.023	0.62
18:1 <i>trans</i> 11	0.416	0.887	0.067	<.0001
18:1 <i>cis</i> 9	26.616	27.923	0.566	0.10
18:2	8.024	8.945	0.513	0.20
18:3	0.471	0.392	0.319	0.08
CLA	0.129	0.222	0.014	<.0001
20:3	1.519	1.775	0.066	0.006
20:4	2.957	3.077	0.115	0.45
20:5	0.492	0.476	0.034	0.73
22:5	0.545	0.564	0.026	0.60
22:6	0.511	0.539	0.033	0.53
28:0	0.110	0.120	0.015	0.62
Unknown	5.599	5.415	0.243	0.58

<sup>a</sup> Expressed as % of total amount of fatty acid.

**Table 5.** Effect of prepartum cow diet on calf performance

Item	Treatment		SE	P-Value
	Control	Fat supplement		
Pre-weaning gain, kg <sup>a</sup>	105.22	99.64	2.77	0.16
Pre-weaning ADG, kg/d <sup>b</sup>	1.03	0.99	0.02	0.18
Weaning age, d	102.25	100.31	1.54	0.37
Weaning wt., kg	146.58	141.81	3.00	0.26
35 d wt., kg <sup>c</sup>	181.55	176.62	3.43	0.31
35 d gain, kg <sup>d</sup>	33.78	34.87	0.90	0.39
35 d ADG, kg/d <sup>e</sup>	1.02	1.05	0.03	0.49
Morbidity, % <sup>f</sup>	9.58	9.66	3.79	0.99

<sup>a</sup> Calf weight gain from birth to weaning.

<sup>b</sup> Calf average daily gain from birth to weaning.

<sup>c</sup> Calf weight at end of 35 d weaning period.

<sup>d</sup> Calf weight gain during 35 d weaning period.

<sup>e</sup> Calf average daily gain during 35 d weaning period.

<sup>f</sup> % of calves treated for illness during 35 d weaning period.

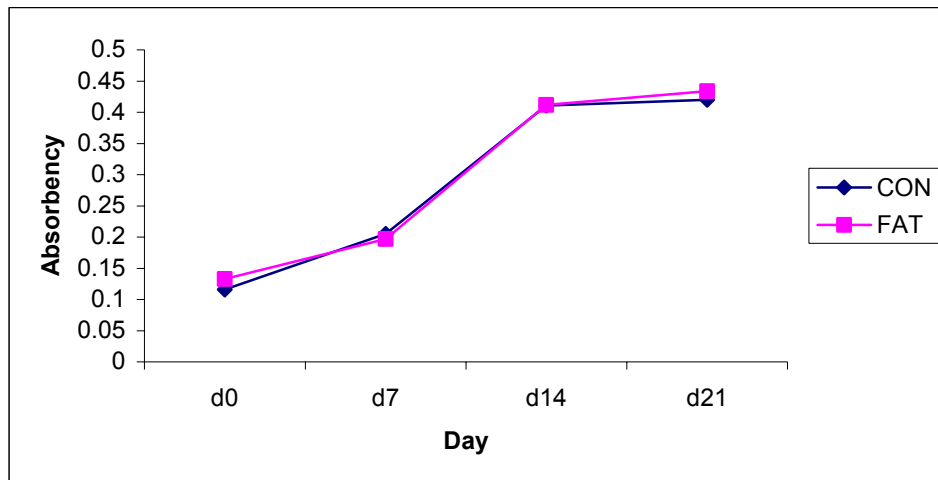
**Table 6.** Effects of prepartum fat supplementation on calf response to OVA antigen injection

Item	Treatment		SE	P-Value
	Control	Fat supplement		
Absorbency <sup>a</sup>				
d 0 <sup>b</sup>	0.116	0.133	0.01	0.08
d 7	0.205	0.197	0.01	0.55
d 14	0.411	0.412	0.01	0.97
d 21	0.420	0.434	0.01	0.26
Response change <sup>c</sup>				
d0-7	0.089	0.064	0.01	0.01
d7-14	0.207	0.214	0.01	0.58
d14-21	0.008	0.022	0.01	0.29
d0-21	0.303	0.301	0.01	0.90

<sup>a</sup> Measurement of total OVA specific antibody as determined by indirect ELISA.

<sup>b</sup> Taken prior to administration of antigen.

<sup>c</sup> Change in absorbency between blood collections.

**Figure 1.** Calf response to OVA antigen injection

# EFFECTS OF BARLEY CULTIVAR AND GROWING ENVIRONMENT ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF FINISHING BEEF CATTLE

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**ABSTRACT:** Thirty-two crossbreed beef heifers (initial weight  $349 \text{ kg} \pm 2.21 \text{ kg}$ ) were individually fed finishing diets for 84 d in a 2 X 2 factorial experiment examining the effects of barley cultivar (Harrington vs. Valier) and growing environment (irrigated vs. dryland) on animal performance, carcass characteristics, and nutrient digestibility. No differences in ADG ( $P = 0.46$ ; average  $1.78 \text{ kg/d}$ ) or final weight ( $P = 0.23$ ; average  $498 \text{ kg}$ ) were detected due to cultivar. Cultivar did not affect DMI ( $P = 0.80$ ; average  $9.8 \text{ kg/d}$ ), or feed efficiency ( $P = 0.63$ ; average  $18.3 \text{ kg gain/100 kg of feed}$ ). Growing environment did not affect ADG ( $P = 0.17$ ; average  $1.77 \text{ kg/d}$ ), or final weight ( $P = 0.20$ ; average  $498 \text{ kg}$ ). Heifers fed diets containing irrigated barley had lower ( $P = 0.009$ ) DMI than heifers fed diets containing dryland barley ( $9.3 \text{ vs. } 10.3 \text{ kg/d}$ , respectively). Feed efficiency was higher ( $P = 0.001$ ) for heifers fed diets containing irrigated barley than for those fed dryland barley ( $19.8 \text{ vs. } 16.8 \text{ kg gain/100 kg of feed}$ ). Barley  $\text{NE}_m$  ( $P = 0.63$ ; average  $2.41 \text{ Mcal/kg}$ ) and  $\text{NE}_g$  ( $P = 0.56$ ; average  $1.64 \text{ Mcal/kg}$ ) were not affected by cultivar. Irrigated barley  $\text{NE}_m$  and  $\text{NE}_g$  ( $2.58$  and  $1.79 \text{ Mcal/kg}$ , respectively) contents were higher ( $P = 0.001$ ) than dryland barley  $\text{NE}_m$  and  $\text{NE}_g$  ( $2.24$  and  $1.49 \text{ Mcal/kg}$ , respectively). Cultivar, growing environment or their interaction did not affect ( $P > 0.06$ ) carcass characteristics. Dry matter digestibility was higher ( $P = 0.02$ ) for diets containing Valier than for diets containing Harrington ( $77.6 \text{ vs. } 74.9 \%$ , respectively). Starch digestibility was not affected ( $P = 0.13$ ) by cultivar. Growing environment did not affect ( $P > 0.06$ ) nutrient digestibility. The dryland growing environment increased barley ADF content  $47\%$  and decreased starch content  $12\%$ , resulting in lower NE relative to irrigated barley. The lower starch content of dryland barley may have caused heifers to increase DMI to meet their energy requirements, thus making irrigated barley a more efficient feed source.

Barley, growing environment, finishing diet

## Introduction

Barley (*Hordeum vulgare* L.) is commonly used as the principle energy source in feedlot diets throughout the Northern U.S., where its' adaptability to diverse growing environments gives it advantages over other cereal grains (Nolan and Ullrich, 1993). Typically  $40\%$  of U.S. barley acreage is seeded with feed cultivars, while the remaining acreage is seeded with malting cultivars (U.S. Grains Council, 2004). However, variation in barley nutrient content, caused by cultivar and growing

environment, results in about  $35\%$  of malt barley failing to meet commercial malting criteria, subsequently entering the feed market (Davis, 2002). Research by Molina-Cano et al. (1997) showed a positive correlation between most malting and feeding characteristics. Boss and Bowman (1996) reported that steers fed finishing diets containing Harrington, a two-rowed malting cultivar, had greater animal performance than steers fed diets containing Medallion, a six-rowed feed barley, supporting this idea. Similar trials found no difference in animal performance of beef steers fed finishing diets containing two-rowed malting cultivars compared to those fed diets containing six-rowed feed cultivars (Hinman, 1979; Bradshaw et al., 1996). Growing environment has been shown to be equally important in creating variability in barley nutrient content (Reynolds et al., 1992; Surber et al., 1999). However, while numerous studies have evaluated environmental influences on barley composition (Åman and Newman, 1986; Tester, 1997; Berthodsson, 1998), few dealt directly with how it relates to beef cattle performance.

The objectives of this trial were to compare the animal performance of finishing beef cattle fed Harrington, a two-rowed malting cultivar, or Valier, a two-rowed feed cultivar, grown under two growing environments (irrigated vs. dryland). Influences of barley cultivar, growing environment (irrigated vs. dryland) and cultivar x growing environment interactions were investigated.

## Materials and Methods

Thirty-two crossbred heifers (average initial wt  $348.6 \pm 2.21 \text{ kg}$ ) were assigned to one of four dietary treatments using a randomized complete block design with a 2 X 2 factorial arrangement of treatments. The heifers were placed into a Calan Gates feeding system (American Calan, Inc; Northwood, NH) located in Bozeman, Montana for a feedlot trial lasting 84 d. Heifers were assigned to one of eight pens, based on equal pen weight. Within a pen, heifers were randomly assigned to one of four gates. Gates within a pen were then randomly assigned to one of four dietary treatments. A 3-wk adaptation and training period was allowed to ensure that heifers adapted to the environment and diet. Initial and final weights were determined by taking the average of weights measured on two consecutive days. Interim weights were taken every 28-d. Heifers were fed daily at 0930 and allowed ad libitum access to feed and water. Diet samples were obtained when diets were mixed to ensure a representative sample. Orts were weighed and sampled daily. Fecal grab

samples were obtained every 28 d. Diet, ort and fecal samples were composited by animal and period. Fecal samples were dried in a forced air oven at 60° C for 48 h. Diet, ort, and fecal samples were ground to pass a 1-mm screen in a Wiley mill and analyzed for DM, OM, N, starch (AOAC, 2000), ADF (Van Soest et al. 1991) and acid insoluble ash (Van Keulen and Young, 1977). Acid-insoluble ash was used as an internal marker to estimate fecal output for the calculation of DM, starch and CP apparent total tract digestibility for every 28-d period. Using NRC (1996) equations,  $NE_m$  and  $NE_g$  for each of the four barleys were calculated from heifer average weight, DMI and ADG. All procedures were conducted following a protocol approved by the Montana State University Institutional Animal Care and Use Committee.

Harrington and Valier barleys grown under irrigated and dryland environments were the basal grains in the dietary treatments. Irrigated barley was grown in Southwestern MT near the town of Bozeman. Dryland barley was grown at the Northern Agricultural Research Center in Havre, MT. As shown in Table 1, barleys were evaluated for DM, OM, N, starch (AOAC, 2000) and ADF (Van Soest et al., 1991) content.

Dietary treatments consisted of four finishing diets, which were; 1) Harrington irrigated; 2) Harrington dryland; 3) Valier irrigated; and 4) Valier dryland. Diet and nutrient composition of the four dietary treatments are shown in Table 2. Supplements were formulated specifically for individual treatment barleys ensuring that diets were isonitrogenous (2.24% N) and isocaloric (2.01 Mcal/kg  $NE_m$  and 1.35 Mcal/kg  $NE_g$ ). All barleys were cracked prior to feeding.

Heifers were visually assessed and slaughtered when 70% were estimated to grade choice. Hot carcass weights were taken the day of slaughter. Carcass data was obtained following a 24-h chill period and included: 1) longissimus muscle area, measured by tracing the longissimus muscle at the 12<sup>th</sup> rib, and the area determined by a planimeter; 2) subcutaneous fat thickness over the longissimus muscle at the 12<sup>th</sup> rib, measured at  $\frac{3}{4}$  the lateral length from the chine bone; 3) kidney, pelvic and heart fat as a percentage of carcass weight; 4) marbling score; and 5) quality and yield grades, as assigned by a USDA grader.

A randomized complete block design with a 2 X 2 factorial arrangement of treatments was employed, testing the effects of cultivar (Harrington vs. Valier), growing environment (irrigated vs. dryland) and cultivar x growing environment interactions. The GLM procedure of SAS (SAS Inst. Inc., Cary, NC) was used to analyze the data. Means with F-values found to be significant were separated using the LSD method.

## Results and Discussion

No cultivar by growing environment interactions ( $P > 0.18$ ) were detected for animal performance or carcass characteristics, so the main effect means are presented

(Table 3). No differences in ADG ( $P = 0.46$ ; average 1.78 kg/d) or final weight ( $P = 0.23$ ; average 498 kg) were detected due to cultivar (Table 3). As shown in Table 3, barley cultivar did not affect DMI ( $P = 0.80$ ; average 9.8 kg/d), or feed efficiency ( $P = 0.63$ ; average 18.3 kg gain/100 kg of feed). The lack of a cultivar effect on animal performance parameters measured agrees with the work of Hinman (1979) and Bradshaw et al. (1996). Conversely, Boss and Bowman (1996) found that steers fed diets containing Harrington, a two-rowed malting cultivar, exhibited greater animal performance than steers fed diets containing Medallion, a six-rowed feed barley or Gunhilde, a two-rowed feed cultivar. However, steers fed diets containing Gunhilde exhibited similar animal performance as steers fed diets containing Medallion (Boss and Bowman, 1996).

Growing environment did not influence ADG ( $P = 0.17$ ; average 1.77 kg/d), or final weight ( $P = 0.20$ ; average 498 kg; Table 3). Heifers fed diets containing irrigated barley had lower ( $P = 0.009$ ) DMI than heifers fed diets containing dryland barley (9.3 vs. 10.3 kg/d, respectively). Feed efficiency was higher ( $P = 0.001$ ) for heifers fed diets containing irrigated barley than for those fed dryland barley (19.8 vs. 16.8 kg gain/100 kg of feed). The higher DMI of heifers consuming dryland cultivars was not accompanied by similar increases in animal performance. As shown in table 3, irrigated barley  $NE_m$  and  $NE_g$  (2.58 and 1.79 Mcal/kg, respectively) content was higher ( $P = 0.001$ ) than dryland barley  $NE_m$  and  $NE_g$  (2.24 and 1.49 Mcal/kg, respectively) content. The lower energy content seen in the dryland barley may have caused heifers to consume more to meet their energy requirements. Additionally, dryland barley had a higher ADF and lower starch content than irrigated barley (Table 1). High ADF content has been shown to adversely affect starch intake ultimately hindering ADG (Surber et al., 2000), which could explain the inferior feed efficiencies of heifers fed diets containing dryland barley. Barley  $NE_m$  ( $P = 0.63$ ; average 2.41 Mcal/kg) or  $NE_g$  ( $P = 0.56$ ; average 1.64 Mcal/kg) was not affected by cultivar.

As shown in Table 3, no differences ( $P > 0.06$ ) in carcass characteristics were detected due to barley cultivar, growing environment or their interaction. These findings agree with the work of Bradshaw et al. (1996) who attributed no differences in carcass characteristics to Klages, a two-rowed malting cultivar, or Steptoe, a six-rowed feed cultivar. Hinman (1979) attributed no differences in carcass characteristics to cultivar (Klages vs. Steptoe) or growing environment (irrigated vs. dryland). Conversely, Boss and Bowman (1996) found that the carcass characteristics of steers that had consumed diets containing Harrington had higher hot carcass weights, marbling scores, and tended to have higher yield and quality grades than steers consuming Gunhilde or Medallion.

Diet nutrient digestibility is shown in Table 4. Dry matter digestibility was higher ( $P = 0.02$ ) for diets containing Valier barley than for diets containing

Harrington barley (77.6 vs. 74.9 %, respectively), contradicting findings by Boss and Bowman (1996) who reported greater DMD for diets containing malting cultivars. Starch, ADF, and N digestibility were not affected ( $P = 0.13$ ) by cultivar. Growing environment did not affect ( $P > 0.06$ ) nutrient digestibility. A cultivar x growing environment interaction ( $P = 0.005$ ) was detected for ADF digestibility. Digestibility of ADF was lowest ( $P = 0.005$ ) for diets containing irrigated Harrington (14.1%), intermediate for diets containing dryland Valier (22.9%), and greatest for diets containing irrigated Harrington and dryland Valier (average 30.6%).

### Implications

The results of this study imply that no differences in animal performance can be attributed to cultivar when feeding diets containing Harrington or Valier barley to finishing beef heifers. The lower  $NE_m$  and  $NE_g$  content of dryland barley may have caused heifers fed diets containing dryland barley to increase DMI to meet their energy requirements. The lower ADF content and higher starch content of barleys grown in irrigated growing environments made diets containing irrigated barley the more efficient feed source.

### Literature cited

- Åman, p. and C.W. Newman. 1986. Chemical composition of some different types of barley grown in Montana, U.S.A.. *J. Cer. Sci.* 4 :133-141
- AOAC. 2000. Official Methods of Analysis of AOAC International (17<sup>th</sup> Ed.) AOAC International, Gaithersburg, MD.
- Bertholdsson, N.O.. 1999. Characterization of malting barley cultivars with more or less stable grain protein content under varying environmental conditions. *European J. Agron.* 10: 1-8.
- Boss, D.L. and J.G.P. Bowman. 1996. Barley variety for finishing steers: I. Feedlot performance, in vivo diet digestion, and carcass characteristics. *J. Anim. Sci.* 74:1967-1972.
- Bradshaw, W.L., D.D. Hinman, R.C. Bull, D.O. Everson, and S.J. Sorensen. 1996. Effects of barley variety and rocessing method on feedlot steer performance and carcass characteristics. *J. Anim. Sci.* 74: 18-24.
- Davis, Mike. 2002. Personal communication. American Malt Barley Association, [mpdavis@mail.execpc.com](mailto:mpdavis@mail.execpc.com), November, 13, 2002.
- Hinman, D.D.. 1979. A comparison of malting vs. feed barley varieties on beef cattle performance. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 30:49.
- Molina-Cano, J. L., M. Franceshh, A.M. Perez-Vebdrell, J. Voltas and J. Brufau. 1997. Genetic and environmental variation in malting and feed quality of barley. *J. Cer. Sci.* 25 : 37-47.
- Nilan, R.A. and S.E. Ullrich. 1993. Barley: Taxonomy, Origin, Distribution, Production, Genetics and Breeding.

- In. Alexander W. MacGregor and Rattan S. Bhatti (Ed.) Barley Chemistry and Technology. P. 1-5.
- NRC. 1996. Nutrient Requirements of Beef Cattle. National Academy Press, Washington, D.C.
- Surber , L.M.M., J.G.P. Bowman, T.K. Blake, D.D. Hinman, D.L. Boss and T.C. Blackhurst. 2000. Prediction of barley feed quality for beef cattle from laboratory analyses. *Proc. West. Am. Soc. Anim. Sci.* 51: 454-457.
- Tester, Richard F.. 1997. Influence of growth conditions on barley starch properties. *J. Biological Macromoloecules.* 21:37-45.
- U.S. Grain Council. 2002. U.S. Grain Council Home Pages. Available at: <http://www.grains.org/grains/barley.html> Accessed October 8, 2002
- Van Keulen, J. and B.A. Lewis. 1997. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* 70:2866.
- Van Soest, P.J., J.B. Robertson and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 44:3583-3597.

Table 1. Nutrient content of Harrington and Valier grown under irrigated or dryland environments.

	Harrington		Valier	
	Irrigated	Dryland	Irrigated	Dryland
Bulk density, kg/hL	70.3	60.6	79.7	56.8
DM, %	89.67	88.41	90.52	89.95
ADF, %	3.83	4.12	3.69	6.94
N, %	1.87	2.50	1.92	2.10
Starch, %	60.56	55.91	60.35	51.01

Table 2. Diet and nutrient composition (DM basis) of feedlot diets containing Harrington or Valier barley grown under irrigated or dryland conditions

	Harrington		Valier	
	Irrigated	Dryland	Irrigated	Dryland
Diet Composition				
Barley, %	71.63	70.81	72.29	72.06
Straw, %	5.45	5.38	5.48	5.46
Oil, %	2.72	2.69	2.74	2.73
Supplement, %	10.78	10.69	10.75	10.54
Nutrient Composition				
OM, %	95.34	95.62	95.99	95.55
N, %	2.12	2.25	2.39	2.49
ADF, %	6.97	9.88	8.55	7.81
Starch, %	48.05	45.51	45.42	44.64

Table 3. Effects of barley cultivar (Harrington vs. Valier) and Growing environment (Dryland vs. Irrigated) on animal performance and carcass characteristics of beef heifers consuming a finishing diet.

	Cultivar		Growing environment		SE	P-value		
	Harrington	Valier	Irrigated	Dryland		Cultivar	Growing environment	C x GE
Initial wt., kg	345.0	351.0	349.0	349.2	2.21	0.21	0.88	0.77
Final wt., kg	494.1	501.6	501.9	493.8	4.27	0.23	0.20	0.31
ADG, kg	1.75	1.80	1.82	1.72	0.047	0.46	0.17	0.18
DMI, kg/d	9.7	9.8	9.3	10.3	0.25	0.80	0.009	0.51
FE <sup>x</sup>	18.1	18.4	19.8	16.8	0.44	0.63	0.001	0.90
REA, cm <sup>2</sup>	73.2	73.7	75.2	71.7	1.86	0.84	0.21	0.26
Fat thickness, cm	1.5	1.3	1.5	1.3	0.10	0.14	0.19	0.47
KPH, %	2.2	2.2	2.3	2.1	0.08	0.78	0.06	0.78
Marbling score <sup>y</sup>	476	491	472	494	29.3	0.72	0.59	0.66
Quality grade <sup>z</sup>	2.1	2.3	2.2	2.2	0.13	0.51	1.0	1.0
Carcass, kg	312.4	310.4	311.9	310.9	7.89	0.90	0.93	0.23
Yield grade	3.4	3.2	3.3	3.3	0.13	0.22	0.76	0.29
Grain energy content								
NE <sub>m</sub> , Mcal/kg	2.39	2.43	2.58	2.24	0.052	0.63	0.001	0.71
NE <sub>g</sub> , Mcal/kg	1.62	1.66	1.79	1.49	0.046	0.56	0.001	0.68
Diet energy content								
NE <sub>m</sub> , Mcal/kg	2.01	2.06	2.16	1.90	0.037	0.36	0.001	0.63
NE <sub>g</sub> , Mcal/kg	1.35	1.40	1.49	1.26	0.033	0.36	0.001	0.63

<sup>x</sup>FE: Gain per 100 units of feed

<sup>y</sup>Marbling score: Slight = 300, Small = 400, Modest = 500, etc.

<sup>z</sup>Quality grade- 1= Prime; 2 = Choice; 3 = Select; 4 = Standard.

Table 4. In vivo digestibility of finishing diets containing Harrington or Valier barley grown under irrigated or dryland conditions

	Harrington		Valier		SE	P – value		
	Irrigated	Dryland	Irrigated	Dryland		Cultivar	Growing environment	C x I
DM, %	75.5	74.2	77.9	77.2	1.08	0.02	0.38	0.80
OM, %	77.7	76.8	80.3	79.6	1.08	0.02	0.47	0.95
N, %	74.7	74.8	73.1	79.8	1.76	0.34	0.06	0.07
ADF, %	14.1 <sup>a</sup>	30.9 <sup>b</sup>	30.2 <sup>b</sup>	22.9 <sup>ab</sup>	3.78	0.30	0.22	0.005
Starch, %	89.3	92.1	93.2	93.0	1.54	0.13	0.40	0.34

# EFFECTS OF IN VIVO INFUSION OF A NITRIC OXIDE DONOR ON PROGESTERONE SECRETION BY THE BOVINE CORPUS LUTEUM

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**ABSTRACT:** To determine if nitric oxide (NO) is involved in luteal function, the NO donor DETA NONOate was infused into the bovine corpus luteum (CL) via an ultrasound-guided needle, through a 7.5 MHz transvaginal probe and rectal palpation of the ovary. Crossbred beef heifers were synchronized via the CO-Synch protocol. Ultrasonography was performed to determine time of ovulation (d = 0) and 10 to 12 d CL were infused. Intraluteal treatments (300  $\mu$ L; n = 5) were: DETA NONOate (DN) at  $10^{-2}$  M,  $10^{-4}$  M, or  $10^{-6}$  M, in saline or  $10^{-2}$  M NaOH, control consisting of saline and  $10^{-2}$  M NaOH, and prostaglandin (PG)  $F_{2\alpha}$  (1.5 mg). Serum samples were collected at 0 (time of infusion), 1, 3, 6, 9, 12, 24, 36, 48, and 72 h. Serum was analyzed for progesterone ( $P_4$ ) concentrations by RIA and  $P_4$  concentrations were expressed as a percentage of the baseline (t = 0 h). Data were analyzed using MIXED procedures of SAS. There were no differences ( $P > 0.50$ ) in  $P_4$  concentrations among treatments delivered in saline vs NaOH through 72 h. Serum  $P_4$  concentrations declined ( $P = 0.01$ ) in CL treated with PGF $_{2\alpha}$  ( $45.9 \pm 26.6\%$ ) when compared to the controls ( $135.3 \pm 20.6\%$ ) through 72 h, confirming successful treatment delivery. Progesterone concentrations through 72 h were lower ( $P = 0.05$ ) between CL treated with PGF $_{2\alpha}$  than those treated with DN ( $98.4$ ,  $112.5$ , and  $92.5 \pm 14.6\%$  for  $10^{-2}$  M,  $10^{-4}$  M, and  $10^{-6}$  M, respectively). When compared to the control ( $132.6 \pm 18.7\%$ ), DN at all levels decreased ( $93.1$ ,  $92.0$ ;  $P < 0.10$  for  $10^{-2}$  M and  $10^{-4}$  M respectively; and  $86.3 \pm 13.2\%$ ;  $P < 0.05$  for  $10^{-6}$  M)  $P_4$  concentrations from 0 to 9 h. In addition, from 0 to 9 h, treatments were similar to PGF $_{2\alpha}$  ( $62.0 \pm 24\%$ ;  $P = 0.26$ ). However,  $P_4$  concentrations of CL infused with NO donor at all three levels returned to initial concentrations by 72 h. The initial decline in  $P_4$  concentrations in response to a NO donor, support a possible role for NO in luteolytic mechanisms within the CL.

Key Words: Corpus Luteum, Nitric oxide, Luteolysis

## Introduction

Nitric oxide (NO) is an ovarian regulatory factor having positive effects on follicular development in the mouse (Jablunka-Shariff and Olson, 2000) and ovulation in rats (Nakamura et al., 1999) while having a negative effect on luteal steroidogenesis in human, bovine, rat and rabbit (Johnson et al., 1999; Motta et al., 1999; Jaroszewski

and Hansel, 2000). Nitric oxide is synthesized from L-arginine by constitutive or inducible isoforms of nitric oxide synthase (NOS; Johnson et al., 1999). Johnson et al. (1999) detected both inducible NOS (iNOS) and endothelial NOS (eNOS) in the human CL. Messenger RNA encoding iNOS and eNOS were found in early and mid-cycle bovine corpus luteum (CL; Bryant et al., 2003).

Nitric oxide, a powerful vasodilator, has been implicated in regulation of the bovine CL function and lifespan by affecting luteal secretion of progesterone ( $P_4$ ) and prostaglandin (PG)  $F_{2\alpha}$  (Skarzynski and Okuda, 1999). Nitric oxide has a positive effect on the secretion of PGF $_{2\alpha}$  from the bovine CL in vivo (Jaroszewski and Hansel, 2000) and in vitro (Skarzynski and Okuda, 2000). Cyclooxygenase (COX) enzymes, are rate-limiting in the synthesis of PGF $_{2\alpha}$ , are activated by NO (Moncada et al., 1991). Nitric oxide directly inhibited  $P_4$  production in human granulosa cells (Van Voorhis et al., 1994) and cultured bovine luteal cells (Skarzynski and Okuda, 2000). Hanke et al. (1998) demonstrated that NO inhibited cytochrome P450 side-chain cleavage enzyme in humans. Jaroszewski and Hansel (2000) were able to prolong the functional life of the bovine CL by blocking NOS at d 17 of the cycle extending the life of the CL to at least 25 d, and increasing  $P_4$  on d 17. Therefore, the objective of this study was to determine the effect of in vivo infusion of the bovine CL with the NO donor DETA NONOate (DN) on  $P_4$  secretion. We hypothesized that intraluteal infusion of a NO donor would induce functional luteolysis, thereby decreasing  $P_4$  secretion.

## Materials and Methods

Cross-bred beef cows were synchronized in two groups via CO-Synch protocol: injection of 100  $\mu$ g (i.m.) GnRH (Cystorelin, Abbot Laboratories; North Chicago, IL) followed by 25 mg (i.m.) PGF $_{2\alpha}$  (Lutalyse, Pharmacia and Upjohn CO.; Kalamazoo, MI) 7 d later. Cows that failed to ovulate 48 h after PGF $_{2\alpha}$  injection received a second injection of GnRH (i.m.; 100  $\mu$ g). To determine d of ovulation (d = 0), transrectal ovarian ultrasonography using an Aloka 500v ultrasound console (Corometrics Medical Products; North Wallingford, CT) with a 7.5 MHz transducer was performed daily from d of PGF $_{2\alpha}$  injection until cows ovulated. Ovulation was defined as disappearance of the largest follicle.

Intraluteal treatments (300  $\mu$ L; n = 5) included: DN at  $10^{-2}$  M,  $10^{-4}$  M, or  $10^{-6}$  M in saline or  $10^{-2}$  M NaOH, controls of either saline or  $10^{-2}$  M NaOH, and PGF $_{2\alpha}$  (1.5 mg). Corpora lutea were randomly assigned to treatment on

d of infusion. Immediately prior to intraluteal infusion (d 10 to 12 post ovulation), cows were administered 10 mg (i.v.) Acepromazine (Vedco, Inc., St. Joseph, MO). A caudal epidural anesthetic was administered by inserting an 18-gauge, 1.5-cm needle between the last sacral and first coccygeal vertebrae until contact was made with the floor of the spinal canal followed by a 5 mL injection of 2% lidocaine (Vedco, Inc., St. Joseph, MO). Anesthetic effects were observed when the tail had no response to tactile stimuli and rectal contractions ceased.

A 50-cm transvaginal, needle-guided probe housing a 7.5 MHz transducer was covered with a non-sterile 4 x 30-cm latex transducer cover (Cook Veterinary Products, Inc., Bloomington, IN). The transducer cover was partially filled with a lubricant (Jorgenson Laboratories, Loveland, CO) to provide contact between the latex cover and transducer, thus allowing optimal visualization of CL images. Lubricant was also added to the exterior of the transducer cover to allow easier insertion of the probe into the vagina. After aseptic preparation of the cow's perineum, the ovary was palpated per rectum and placed on the transducer located in the vagina to visualize the CL on the ultrasound console. An 18-gauge, 53-cm aspiration needle with stylet (Cook Veterinary Products, Inc.; Bloomington, IN) was inserted into the needle-guided portion of the transvaginal probe. The vaginal wall was punctured and penetration of the CL by the needle was verified by visualization on the ultrasound monitor.

Treatments were slowly infused over a 10-s interval into the CL via aspiration needle, followed by 300  $\mu$ L of air to ensure the needle was cleared of treatment. Successful infusions were confirmed via ultrasound visualization. Serum samples were collected at 0, 1, 3, 6, 9, 12, 24, 36, 48, and 72 h. Serum was analyzed for  $P_4$  concentrations by solid phase RIA (Diagnostic Products Corporation, Los Angeles, CA) with modifications as described by Schneider and Hallford (1996). Intra- and interassay CV were 4.1% and 9.6% respectively. All animal procedures were approved by New Mexico State University Animal Care and Use Committee.

**Statistical Analysis:** Serum  $P_4$  concentrations were analyzed as a percentage of time 0 (preinfusion) to adjust for varying pretreatment basal concentrations of serum  $P_4$ . Serum  $P_4$  samples were analyzed using the MIXED procedures of SAS (SAS, Inst. Inc., Cary, NC) for repeated measures using compound symmetry as the covariate structure. The CL was the experimental unit and treatment, time, and treatment by time were included in the model statement. When differences were detected, means were separated with contrasts.

## Results

No differences were detected ( $P > 0.50$ ) in  $P_4$  concentrations between DN treatments delivered in either  $10^{-2}$ M NaOH or saline ( $93.8$  and  $106.6 \pm 20.6\%$  for  $10^{-2}$ M NaOH and saline, respectively) through 72 h. Therefore, treatments in  $10^{-2}$ M NaOH and saline were combined for each DN concentration ( $10^{-2}$ M,  $10^{-4}$ M, and  $10^{-6}$ M;  $n = 10$ ). Serum  $P_4$  concentrations declined ( $P \leq 0.05$ ) at 6 h in CL infused with  $\text{PGF}_{2\alpha}$  ( $68.4 \pm 24.7\%$ ) when compared to the

control ( $129.3 \pm 19.1\%$ ) and remained different ( $P \leq 0.01$ ) throughout 72 h (Figure 1).

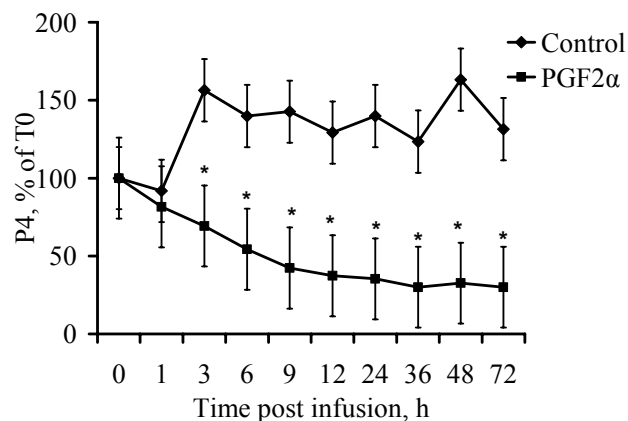


Figure 1. Effect of intraluteal infusion of  $\text{PGF}_{2\alpha}$  vs control on serum  $P_4$  concentrations from 0 (time of infusion) through 72 h in d 10 to 12 bovine CL. Control had greater ( $*P \leq 0.05$ ) serum  $P_4$  concentrations when compared to  $\text{PGF}_{2\alpha}$  (SE = 20.6%;  $n = 5$ ).

Mean serum  $P_4$  concentrations were lower ( $P \leq 0.10$ ; Figure 2) for DN  $10^{-6}$  M ( $88.1 \pm 13.5\%$ ) when compared to the control ( $129.3 \pm 19.1\%$ ) at 6 h and remained lower ( $P \leq 0.05$  for 9 and 12 h, and  $P \leq 0.10$  for 24 to 72 h) throughout 72 h ( $92.5 \pm 14.6\%$  and  $135.3 \pm 20.6\%$  for DN  $10^{-6}$  M and control, respectively). Mean serum  $P_4$  concentrations were also less ( $P \leq 0.10$ ) for  $10^{-2}$  M and  $10^{-4}$  M DN treatments vs. control at 9 h ( $93.1$  and  $92.0 \pm 13.2\%$  for  $10^{-2}$  M and  $10^{-4}$  M DN, respectively and  $132.6 \pm 18.7\%$  for control) and remained lower ( $P < 0.10$ ) for  $10^{-4}$  M DN through 24 h and  $10^{-2}$  M DN through 48 h post infusion.

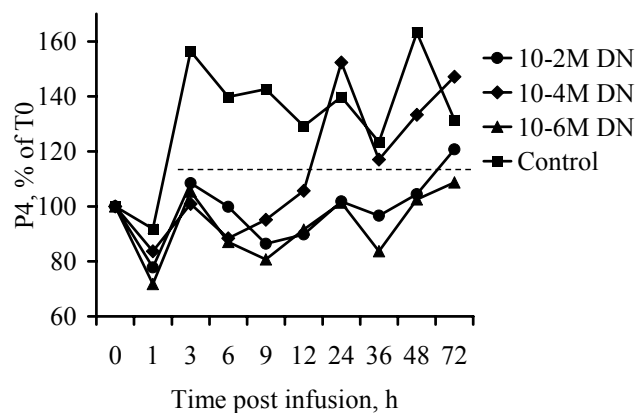


Figure 2. Effect of intraluteal infusion of DETA NONOate (DN) at  $10^{-2}$ M,  $10^{-4}$ M, and  $10^{-6}$ M vs control on serum  $P_4$  concentrations in the d 10 to 12 bovine CL. Serum  $P_4$  concentrations from DN-treated CL falling below the dotted line differ ( $P \leq 0.10$ ) from control (SE = 20.6%;  $n=5$  for control;  $n = 10$  for DN).

Serum  $P_4$  concentrations from the DN-treated CL became similar to those from CL treated with  $\text{PGF}_{2\alpha}$  at the same time the DN-treated serum  $P_4$  concentrations became different than controls. At 6 h, serum  $P_4$  concentrations

from cows with CL treated with DN  $10^{-6}$  M were similar ( $P = 0.48$ ; Figure 3) to those treated with  $\text{PGF}_{2\alpha}$  ( $68.4 \pm 24.7\%$ ) and remained similar ( $P = 0.14$ ) throughout 72 h. Also, at 9 h, serum  $\text{P}_4$  concentrations were similar ( $P > 0.25$ ) for  $10^{-2}$  M and  $10^{-4}$  M levels of DN and  $\text{PGF}_{2\alpha}$  ( $62.0 \pm 24.1\%$  for  $\text{PGF}_{2\alpha}$  and  $93.1$  and  $92.0 \pm 13.2\%$  for  $10^{-2}$  M and  $10^{-4}$  M respectively; Figure 3). Progesterone concentrations remained similar ( $P = 0.18$ ) to  $\text{PGF}_{2\alpha}$  for DN  $10^{-4}$  M treated CL through 12 h. For CL treated with DN  $10^{-2}$  M, serum  $\text{P}_4$  concentrations remained similar ( $P > 0.10$ ) to  $\text{PGF}_{2\alpha}$  through 48 h.

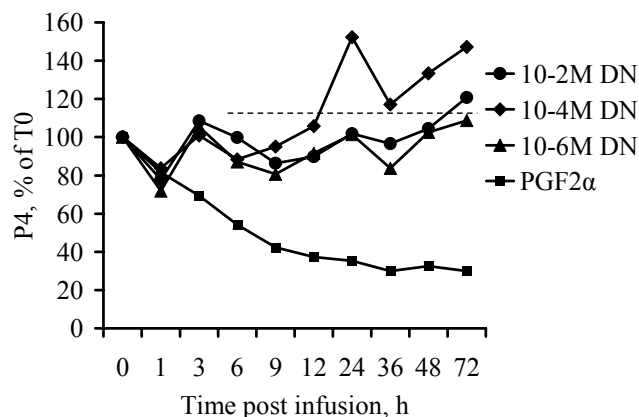


Figure 3. Effect of intraluteal infusion of DETA NONOate (DN) at  $10^{-2}$  M,  $10^{-4}$  M, and  $10^{-6}$  M vs.  $\text{PGF}_{2\alpha}$  on serum  $\text{P}_4$  concentrations from 0 (time of infusion) through 72 h in d 10 to 12 bovine CL. Serum  $\text{P}_4$  concentrations from DN-treated CL falling above the dotted line differ ( $P \leq 0.10$ ) from  $\text{PGF}_{2\alpha}$ -treated CL (SE = 26.6%;  $n = 5$  for  $\text{PGF}_{2\alpha}$ ;  $n = 10$  for DN).

## Discussion

Luteolysis consists of the functional (decreased  $\text{P}_4$  secretion) and structural (cellular demise) regression of the CL. Nitric oxide, a powerful vasodilator, has been implicated in regulation of the bovine corpus luteum (CL) function and lifespan by affecting luteal secretion of progesterone and  $\text{PGF}_{2\alpha}$  (Johnson et al., 1999; Jaroszewski and Hansel, 2000; Skarzynski and Okuda, 1999). Nitric oxide may play an important role at the ovarian level in inhibiting  $\text{P}_4$  production and initiating luteal regression as intraluteal infusion of the NO donor DN in the d 10 to 12 bovine CL had similar serum  $\text{P}_4$  concentrations as those treated with  $\text{PGF}_{2\alpha}$ . Although all three concentrations of DN decreased serum concentrations of  $\text{P}_4$  similar to  $\text{PGF}_{2\alpha}$ , only DN  $10^{-6}$  M sustained the effect through 72 h post infusion. This is in agreement with Motta et al. (2001) who found that when measuring luteal cell culture concentrations of glutathione, an enzyme involved in protection of the early CL against reactive oxygen species, the mid-CL of the rat treated with DN responded to  $10^{-6}$  M DN, but not to  $10^{-4}$  M or  $10^{-8}$  M.

Nitric oxide has also been shown to inhibit  $\text{P}_4$  production by blocking the cytochrome P450 side-chain cleavage enzyme most likely by binding to the heme groups of the cytochrome (Hanke et al., 1998). When NOS was blocked the lifespan of bovine CL was extended to 25 d and

an increase was seen in  $\text{P}_4$  on d 17, when luteolysis would normally have occurred (Jaroszewski and Hansel, 2000). Luteal  $\text{PGF}_{2\alpha}$ , in addition to uterine-derived  $\text{PGF}_{2\alpha}$ , is required for luteolysis (Diaz et al., 2002). Cyclooxygenase is activated by NO and is rate-limiting in  $\text{PGF}_{2\alpha}$  biosynthesis (Salvemini et al., 1993). Therefore, the ability of the CL to produce  $\text{PGF}_{2\alpha}$  may be dependent upon NO (Skarzynski et al. 2000). Motta and Gimeno (1997) found that L-NMMA, a NOS inhibitor, increased  $\text{P}_4$  production and diminished  $\text{PGF}_{2\alpha}$  synthesis in ovarian tissue in rats in the late luteal phase. In mid-luteal cells in the rabbit, Gobbetti et al. (1999) showed a decreased  $\text{P}_4$  secretion with a NO donor and reversed this effect with NOS inhibitors. In addition, a two- to threefold increase in the expression of eNOS protein occurred 12 h after  $\text{PGF}_{2\alpha}$  treatment in rabbits (Boiti et al., 2003). Boiti et al. (2003) also found  $\text{PGF}_{2\alpha}$  decreased luteal eNOS mRNA while eNOS proteins increased indicating that regulatory mechanisms affect protein synthesis for eNOS at the level of transcription or translation. Motta et al. (1999) were also the first to demonstrate an enhanced positive feedback mechanism exists between luteal  $\text{PGF}_{2\alpha}$  and NO during CL regression. Diaz et al. (2002) reported a positive feedback loop between uterine and luteal  $\text{PGF}_{2\alpha}$  synthesis. Together, these data imply that NO may have a role in luteolysis through luteal  $\text{PGF}_{2\alpha}$ .

## Implications

Infusions of bovine corpora lutea with the nitric oxide donor DETA NONOate caused a decline in serum progesterone. The ability of DETA NONOate to decrease serum progesterone concentrations similar to prostaglandin  $\text{F}_{2\alpha}$ -treated corpora lutea supports the hypothesis of nitric oxide's involvement in luteolysis. It has been shown that nitric oxide enhances prostaglandin synthesis by activating cyclooxygenases and inhibiting progesterone synthesis by inactivating cytochrome P450 side-chain cleavage. These results, combined with previous research, implicate nitric oxide in luteolytic mechanism in the bovine.

## Literature Cited

- Boiti, C., G. Guelfi, D. Zampini, G. Brecchia, A. Gobbetti, and M. Zerani. 2003. Regulation of nitric oxide synthase isoforms and role of nitric oxide during prostaglandin  $\text{F}_{2\alpha}$ -induced luteolysis in rabbits. *Reproduction* 125:807-816.
- Bryant, W. D. K. K. Kane, J. S. Nelson, A. Ramos III, and D. E. Hawkins. 2003. Relative amount of mRNA encoding endothelial and inducible nitric oxide synthase in the bovine corpus luteum. *Proc. West. Sec. Am. Soc. Anim. Sci.* 54:18-21.
- Diaz, F. J., L. E. Anderson, Y. L. Wu, A. Rabot, S. J. Tsai, and M. C. Wiltbank. 2002. Regulation of progesterone and prostaglandin  $\text{F}_{2\alpha}$  production in the corpus luteum. *Mol. Cell. Endocrinol.* 191:65-80.
- Gobbetti, A., C. Boiti, C. Canali, and M. Zerani. 1999. Nitric oxide synthase acutely regulates progesterone production by in vitro cultured rabbit corpora lutea. *J. Endocrinol.* 160:275-283.

- Hanke, J. C., J. G. Drewett, C. R. Myers, and W. B. Campbell. 1998. Nitric oxide inhibits aldosterone synthesis by a guanyl cyclase-independent effect. *Endocrinology* 139:4053-4060.
- Jaroszewski, J. J., and W. Hansel. 2000. Intraluteal administration of a nitric oxide synthase blocker stimulates progesterone and oxytocin secretion and prolongs the life span of bovine corpus luteum. *Proc. Soc. Exp. Med.* 224:50-55.
- Jablonka-Shariff, A., and L. M. Olson. 2000. Nitric oxide is essential for optimal meiotic maturation of murine cumulus-oocyte complexes in vitro. *Mol. Reprod. Dev.* 55:412-421.
- Johnson, M. C., H. A. Diaz, C. Stocco, A. Palomino, L. Devoto, and M. Vega. 1999. Antisteroidogenic action of nitric oxide on human corpus luteum in vitro. *Endocrine* 11:31-36.
- Moncada, S., D. D. Rees, R. Schulz, and R. M. J. Palmer. 1991. Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in vivo. 1991. *Physiol. Pharmacol.* 88:2166-2170.
- Motta, A. B., A. Estevez, T. Tognetti, M. A. F. Gimeno, and A. M. Franchi. 2001. Dual effects of nitric oxide in functional and regressing rat corpus luteum. *Mol. Hum. Reprod.* 7:43-47.
- Motta, A. B., A. Estevez, and M. A. F. Gimeno. 1999. The involvement of nitric oxide in corpus luteum regression in the rat: Feedback mechanism between prostaglandin  $F_{2\alpha}$  and nitric oxide. *Mol. Hum. Reprod.* 5:1011-1016.
- Motta, A. B., and M. A. F. Gimeno. 1997. Nitric oxide participates in the corpus luteum regression in ovaries isolated from pseudopregnant rats. *Can. J. Physiol. Pharmacol.* 75:1335-1339.
- Nakamura, Y., S. Kashida, M. Nakata, S. Takiguchi, Y. Yamagata, H. Takayama, and N. Sugino. 1999. Changes in nitric oxide synthase activity in the ovary of gonadotropin treated rats: The role of nitric oxide during ovulation. *Endocrine J.* 46:529-538.
- Salvemini, D., T. P. Misko, J. L. Masferrer, K. Seibert, M. G. Currie, and P. Neddlemen. 1993. Nitric oxide activates cyclooxygenase enzymes. *Pharmacology* 90:7240-7244.
- Schneider, F. A., and D. M. Hallford. 1996. Use of a rapid progesterone radio-immunoassay to predict pregnancy and fetal numbers in ewes. *Sheep & Goat Res. J.* 12:33-38.
- Skarzynski, D., S. Kobayashi, and K. Okuda. 2000. Influence of nitric oxide and noradrenaline on prostaglandin  $F_{2\alpha}$ -induced oxytocin secretion and intracellular calcium mobilization in cultured bovine luteal cells. *Biol. Reprod.* 63:1000-1005.
- Skarzynski, D., and K. Okuda. 1999. Sensitivity of bovine corpus lutea to prostaglandin  $F_{2\alpha}$  is dependent on progesterone, oxytocin, and prostaglandins. *Biol. Reprod.* 60:1292-1298.
- Skarzynski, D., and K. Okuda. 2000. Different actions of noradrenaline and nitric oxide on the output of prostaglandins and progesterone in cultured bovine luteal cells. *Prost. Other Lipid Med.* 60:35-47.
- Van Voorhis, B. J., M. S. Dunn, G. D. Snyder, and C. P. Weiner. 1994. Nitric oxide: An autocrine regulator of human granulosa-luteal cell steroidogenesis. *Endocrinology* 135:1799-1806.

**BREEDING PERFORMANCE OF FIRST-CALF SUCKLED BEEF COWS EXPOSED TO BULLS BEFORE, DURING, AND AFTER AN ESTROUS SYNCHRONIZATION PROTOCOL THAT INCLUDED CIDR, PGF<sub>2α</sub>, GnRH AND TIMED AI**

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**ABSTRACT:** Our objective was to determine if bull exposure before, during, and after an estrous synchronization protocol (ES) using CIDR, PGF<sub>2α</sub> and GnRH, alters AI or overall pregnancy rates in first-calf suckled beef cows. The hypotheses were: 1) proportions of cows cycling before the start of an ES protocol did not differ between cows exposed (BE) or not exposed to bulls (NE), and 2) AI and overall pregnancy rates did not differ among cows that received BE before, during, and after ES+AI; BE before but not during or after ES+AI (BENE); NE before, during, and after ES+AI; or BE during and after but not before ES+AI (NEBE). Fifty-three AxH cows stratified by calving date, calf BW, calf sex, BCS, and dystocia score were assigned to BE (n=26) or NE (n=27) 40 d before timed AI (d 0). Thirty d later, one half of BE and NE cows were assigned to the NE (BENE; n=13) and BE (NEBE; n=13) treatments, respectively, for the next 18 d. Each cow received a CIDR on d -10 then was given PGF<sub>2α</sub> (25 mg) 7 d later at CIDR removal. Cows detected in estrus for 60 h after PGF<sub>2α</sub> were bred by AI 12 h later; cows not detected in estrus by 60 h after PGF<sub>2α</sub> received GnRH (100 µg/hd) and timed AI at 72 h after PGF<sub>2α</sub>. Progesterone patterns (3-d intervals from d -40) were used to evaluate cycling activity. Pregnancy rates were determined transrectally 35 and 107 d after AI. Proportions of BE and NE cows cycling on d -40 did not differ ( $P>0.10$ ), but more ( $P<0.05$ ) BE than NE cows began cycling before the start of the ES protocol. There was no interaction ( $P>0.10$ ) for either AI or overall pregnancy rates among treatments. AI pregnancy rate for BE (BE and BENE) cows (80.8%) was greater ( $P<0.05$ ) than NE (NE and NEBE) cows (53.9%). We conclude that short-term bull exposure of first-calf beef cows during and after an estrous synchronization protocol that included CIDRs, PGF<sub>2α</sub> and GnRH with timed AI did not affect breeding performance; whereas, breeding performance was enhanced by exposing cows to bulls before implementation of this protocol.

**Key Words:** Biostimulation, Postpartum, Estrous Synchronization, Bovine

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**Introduction**

Prolonged postpartum anestrus is the major cause of cows failing to rebreed or breeding late in the breeding season. This is a particular problem in first-calf suckled cows that require 15 to 25 d longer to return to estrus than multiparous cows (Short et al., 1994). For this reason it can be a challenge to successfully synchronize estrus or ovulation in first-calf suckled beef cows.

Work reported in our laboratory showed that bulls decrease postpartum anestrus in first-calf suckled beef cows (Custer et al., 1990; Fernandez et al., 1993; Berardinelli et al., 2001). This biostimulatory effect could be used to reduce postpartum anestrus and improve estrous synchronization (ES) and AI pregnancy rates.

Berardinelli et al. (2001) reported that AI pregnancy rate in a GnRH-based synchronization protocol was not improved by bull exposure. Subsequently, we found that exposing cows to bulls may increase timed AI (TAI) pregnancy rates if bulls remained with cows throughout the ES protocol and for five d after AI (Anderson et al., 2002).

The objectives of this experiment were to determine if short-term bull exposure of first-calf suckled beef cows increases the number of cows cycling, and if bull exposure during and after ES alters AI and overall pregnancy rates using a GnRH-based ES protocol that included a controlled intravaginal drug release (CIDR) device. We tested the hypotheses that exposing first-calf suckled beef cows to bulls does not affect, 1) proportions of cows cycling before the start of an ES protocol, and 2) AI and overall pregnancy rates did not differ among cows that received bull exposure before, during, and after ES+AI; bull exposure before but not during or after ES+AI; no bull exposure before, during, and after ES+AI; or bull exposure during and after but not before ES+AI.

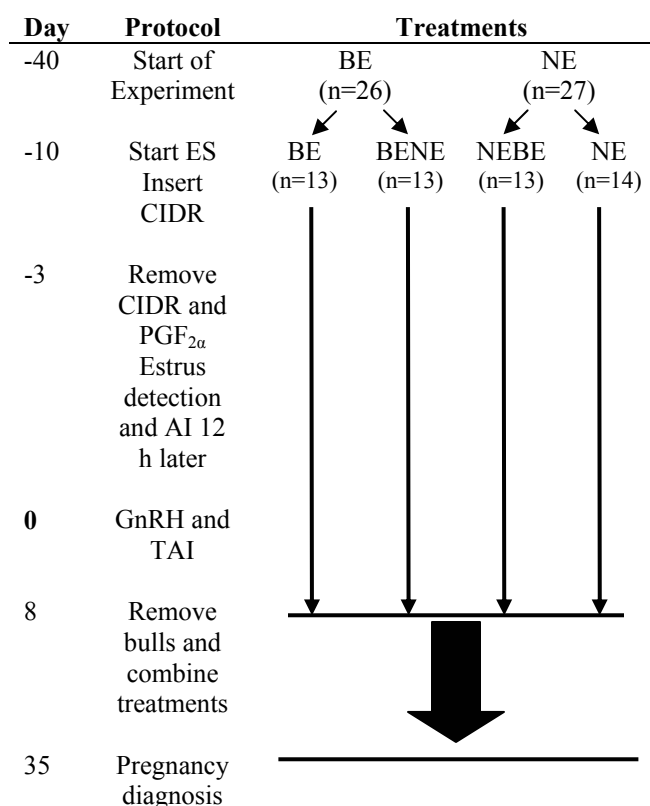
**Materials and Methods**

*Animals and Treatments*

Fifty-three spring-calving two-yr-old Angus X Hereford first-calf suckled beef cows and four epididectomized Angus X Hereford bulls were used in this experiment conducted at the Montana State University Livestock Teaching and Research Center. Animal care, handling, and protocols were approved by

the Montana State University Institutional Animal Care and Use Committee.

Cows and calves were maintained in a single pasture from calving until 40 d before TAI (d 0). Average calving date was Feb. 16, 2003. Cows and calves had no contact with bulls or their excretory products until the start of the experiment. One week before the start of treatment cows were stratified by calving date, cow body weight, calf birth weight, calf sex ratio, dystocia score, and body condition score. Once cows were stratified they were assigned randomly to one of two treatments; exposure to mature bulls (BE; n = 26) or no bull exposure (NE; n = 27). Ten d before TAI one-half of the BE cows were randomly assigned to no bull exposure (BENE; n = 13) and one-half of the NE cows were assigned to bull exposure (NEBE; n = 13). Bulls were removed and cows combined into one group eight d after TAI. Figure 1 depicts the experimental design and protocols.



**Figure 1.** Experimental design and protocols. BE=bull exposed, NE=no bull exposure.

#### Pen Areas

Cows were maintained in pens at the Bozeman Livestock Teaching and Research Center. Each pen is 48 m long by 18 m wide and is identical in configuration and aspect with access to shelter. The north pen area is 0.35 km away from the south pen area. Prevailing winds blow from the southwest to northeast so NE cows were housed in the south pen area and BE cows were housed in the north pen area. This reduced the chances of incidental

overlap exposure between treatments (Fernandez et al., 1996).

#### Nutrition

Cows had free access to good quality, chopped mixed-grass alfalfa hay, and any pasture grasses that were available. Once cows and calves were moved into pens they were given free access to the same hay, 0.5 kg•hd<sup>-1</sup>•d<sup>-1</sup> cracked barley, water, and a trace mineral-salt supplement. The TDN of the diet exceeded the NRC requirement for lactating beef cows with a mature weight of 545 kg by approximately 18% (NRC, 1996).

#### Estrous Synchronization and AI

Thirty d after the start of the experiment each cow was given exogenous progesterone via a controlled intravaginal drug release (CIDR) device ten d before TAI. Seven d later CIDRs were removed and cows were given PGF<sub>2α</sub> (25 mg/hd). Cows that showed estrus within 60 h after CIDR removal were bred by AI 12 h later. Cows that did not show estrus within 60 h were given GnRH (100 ug/hd) and bred AI 72 h after CIDR removal. Cows were exposed to natural service bulls 18 d later for 21 d.

#### Blood Sampling for Progesterone and Pregnancy Determination

Blood samples were collected from each cow by jugular venepuncture at three-d intervals from the start of the experiment. Serum was assayed for progesterone concentration using a solid-phase RIA kit (Diagnostic Systems Laboratories Inc., Webster TX) validated for bovine serum in our laboratory. The criterion for resumption of cycling activity was a rise in progesterone of greater than 0.5 ng/mL in three consecutive samples. Pregnancy was determined by transrectal ultrasonography of the uterine contents of each cow 35 and 107 d after TAI.

#### Statistical Analyses

Calving date, calf birth weight, calf sex, dystocia score, and body condition score were analyzed by analysis of variance for a completely random design using PROC GLM of SAS (SAS, Cary, NC). The model included treatment. Means were separated by PDIFF procedure of SAS (SAS, Cary, NC). Proportions of cows that resumed ovarian cycling activity before the start of the estrous synchronization protocol, AI pregnancy rates, and overall pregnancy rates were analyzed by contingency chi-square analyses (SAS, Cary, NC).

#### Results

Calving date, cow body weight, calf birth weight, calf sex ratio, dystocia score, and body condition score did not differ ( $P > 0.10$ ) among treatments.

Proportions of BE and NE cows cycling at the start of the experiment or 40 d before TAI did not differ (Table 1;  $P > 0.10$ ). More ( $P < 0.05$ ) cows exposed to bulls began cycling before the start of the ES protocol than cows not exposed to bulls (Table 1).

Table 1. Percentages of first-calf suckled beef cows exposed (BE) or not exposed (NE) to bulls that were cycling at the beginning of the experiment, and that resumed cycling activity before the start of the estrous synchronization (ES) protocol

Item	Treatment		$X^2$	$P$ value
	BE	NE		
n	26	27		
% Cycling at start of experiment	50.0% <sup>a</sup>	44.4% <sup>a</sup>	2.4	$> 0.10$
% Cycling at start of ES protocol	100% <sup>a</sup>	70.4% <sup>b</sup>	9.1	$< 0.05$

<sup>a,b</sup>Percentages in rows that lack a common superscript differ.

There was no interaction ( $P > 0.10$ ) for either AI or overall pregnancy rates among BE, BENE, NEBE, and NE cows. Therefore, data for AI and overall pregnancy rates were pooled for cows exposed to bulls or not exposed to bulls before the start of the ES protocol. Pregnancy rate to AI for cows exposed to bulls (BE and BENE) before estrous synchronization was greater ( $P < 0.05$ ) than that for cows not exposed to bulls (NE and NEBE; Table 2).

Table 2. AI and overall pregnancy rates for first-calf suckled beef cows exposed to bulls (BE and BENE) or not exposed to bulls (NE and NEBE) before the start of the estrous synchronization protocol

Item	Treatment		$X^2$	$P$ value
	BE and BENE	NE and NEBE		
n	26	27		
AI pregnancy rates	80.8% <sup>a</sup>	53.9% <sup>b</sup>	4.3	$< 0.05$
Overall pregnancy rates	88.5% <sup>a</sup>	92.3% <sup>a</sup>	1.0	$> 0.10$

<sup>a,b</sup>Percentages in rows that lack a common superscript differ.

## Discussion

Long-term bull exposure reduces postpartum anestrus in first-calf suckled beef cows (Custer et al.,

1990, Fernandez et al., 1993). The first objective of our experiment was to determine if short-term bull exposure increased the number of cows cycling before the start of the breeding season. We found that exposing cows to mature bulls for 30 d before ES and AI increased the proportion of anestrus cows that started to cycle by 30% relative to cows that were not exposed to bulls. Thus short-term bull exposure had the same effect as long-term exposure. Taken together these results indicate that the biostimulatory effect of the bull is an effective strategy to increase the proportion of first-calf suckled cows that are cycling at the beginning of the breeding season.

Anderson et al. (2002) reported that timed AI pregnancy rate was higher in bull-exposed cows if cows remained with bulls for five d after breeding. This indicates the possibility that the physical presence of a bull before, during, and after estrous synchronization (ES) and AI is necessary to enhance AI pregnancy rates. We tested this notion by exposing cows to bulls before, during, and after ES+AI, exposing cows to bulls before but not during and after ES+AI, not exposing cows before but during and after ES+AI, or not exposing cows to bulls. We found that pregnancy rate to AI for cows exposed to bulls before estrous synchronization was greater than that for cows not exposed to bulls. It seems that bull exposure is not effective in increasing AI pregnancy rates if cows are exposed to bulls only during and after a GnRH-based ES+AI that includes a progestin. However, these data indicate that bull exposure for at least 30 d before ES+AI improves pregnancy rates to AI. This result is not consistent with that reported by Berardinelli et al. (2001). The discrepancy between Berardinelli et al. (2001) and the present experiment may be related to the fact that Berardinelli et al. (2001) removed bulls from cows at the start of ES protocol. Therefore, it would appear that the biostimulatory effect of bulls is more beneficial for improving breeding performance if cows are exposed before, during, and after ES and AI. Another difference between Berardinelli et al. (2001) and our experiment is the use of a CIDR. Progestin was not used by Berardinelli et al. (2001) and recently, Stevenson et al. (2003) reported that progestin treatment concurrent with a GnRH-based ES protocol improves pregnancy rates in suckled beef cows after timed AI.

Overall pregnancy rates were not affected by bull exposure. This result is consistent with results reported by Custer et al. (1990), Fernandez et al. (1993), and Berardinelli et al. (2001).

We conclude that short-term bull exposure of first-calf beef cows increased the number of cows cycling before the beginning of the breeding season. Bull exposure during and after an estrous synchronization protocol that included progestin (CIDR), PGF<sub>2α</sub>, and GnRH with timed AI did not alter breeding performance for those cows not exposed to bulls before the start of the ES protocol. Whereas, breeding performance of first-calf suckled beef cows was enhanced by exposing cows to bulls before implementation of this protocol.

## Implications

The biostimulatory effect of bulls can be used to increase the number of cows cycling before the implementation of estrous synchronization protocols. Short-term bull exposure (30 d before ES+AI) is as effective as long-term exposure (> 40 d before ES+AI). Combining the biostimulatory effect of bulls with GnRH-based estrous synchronization protocols that include CIDR and timed AI can be an effective reproductive management strategy to improve overall reproductive efficiency in beef cattle production.

## Literature Cited

- Anderson, K. A., J.G. Berardinelli, P.S. Joshi, and B. Robinson. 2002. Effects of exposure to bull or excretory products of bulls on the breeding performance of first-calf restricted suckled beef cows using a modified CO-synch protocol. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 2002; 53:457-460.
- Berardinelli, J.G., B. Robinson, K. Anderson, and R. Adair. 2001. Effect of presence of bulls and restricted suckling on breeding performance of first-calf suckled beef cows using the OV-synch protocol. *Proc. West. Sec. Amer. Soc. Anim. Sci.*; 52:377-380.
- Custer, E.E., J.G. Berardinelli, R.E. Short, M. Wehrman, and R. Adair. 1990. Postpartum interval to estrus and patterns of LH and progesterone in first-calf suckled beef cows exposed to mature bulls. *J. Anim. Sci.* 68:1370-1377.
- Fernandez, D., J.G. Berardinelli, R.E. Short, and R. Adair. 1993. Temporal requirement for the presence of mature bulls to alter the postpartum period to resumption of ovarian cycling activity and reproductive performance in first-calf suckled beef cows. *Theriogenology* 39:411-419.
- Fernandez, D.L., J.G. Berardinelli, R.E. Short, and R. Adair. 1996. Acute and chronic changes in LH secretion, and postpartum interval to estrus in first-calf suckled beef cows exposed continuously or intermittently to mature bulls. *J. Anim. Sci.* 74:1098-1100.
- NRC. 1996. Pages 227 in *Nutrient Requirements of Beef Cattle*. 7th revised ed. Natl. Acad. Press, Washington, DC
- Short R.E., R.A. Bellows, R.B. Staigmiller, D.C. Adams, and J.G. Berardinelli. 1994. Effects of suckling on postpartum reproduction. In: M.J. Fields, and R.S. Sand RS (eds.), *Factors Affecting Calf Crop* p. 179-187. CRC Press, Boca Rotan, FL.
- Stevenson J. S., G. C. Lamb, S.K. Johnson, M. A. Medina-Britos, D. M. Grieger, K. R. Harmony, J. A. Carnill, S. Z. El-Zarkouny, C. R. Dahlen, and T. J. Marple. 2003. Supplemental norgestomet, progesterone, or melengesterol acetate increases pregnancy rates in suckled beef cows after timed inseminations. *J. Anim. Sci.* 81:571-586.

# GENETIC ANALYSIS OF TWO LINES OF RAMBOUILLET SHEEP DIVERGENTLY SELECTED FOR LAMBING RATE

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**ABSTRACT:** For 34 years, two lines of Rambouillet sheep have been and continue to be divergently selected for lambing rate at the Red Bluff Research Center near Norris, Montana. Selection is based on estimated breeding values calculated from an index of average number of lambs born per year over a ewe's lifetime. The model used for calculating estimated breeding values of the index includes fixed effects of year born and line. Selection for one ram lamb from each of the highest four indexing sire groups within the high lambing rate line (**HIGH**) and one ram lamb from each of the four lowest indexing sire groups within the low lambing rate line (**LOW**) is practiced to determine sires for each of the lines. Rams are first used at 18-mo of age and are only used for one breeding season. Originally, 30-35 ewes were selected each year based on having the highest indexing values within the HIGH line or lowest indexing values in the LOW line, regardless of sire group. Due to the decreased lambing rate in the LOW line, in recent years, no ewe selection has been performed on the LOW line, but ewe selection continues in the HIGH line. Ewes are bred first at 18-mo of age and remain in the lines for six years. Using data through the 2003 lambing season, direct heritability for the lambing rate index was estimated to be 0.30. Estimates of genetic trends through 2003 born lambs are 12.2 index units/year and -3.0 index units/year for the high and low lines, respectively. The average index breeding value for 2003 born lambs is 417.5 and -135.6 for the high and low lines, respectively. This equates to a difference of 0.55 lambs born per year per ewe between the two lines due to genetic effects. Selection for increased or decreased lambing rate will result in genetic change for number of lambs born.

Keywords: Divergent selection, lambing rate, Rambouillet

## Introduction

Reproductive traits are the most important group of traits to any production livestock operation. Without viable offspring, selection for other traits, such as growth, has no value.

In sheep, twin births is encouraged, but reproductive traits, such as number born, tend to be lowly heritable with estimates of direct heritability ranging from 0.07 – 0.12 (i.e., Bromley et al., 2000; and Rao and Notter, 2000; Hanford et al., 2004). However, selection for reproductive traits has been shown to result in genetic change in both sheep (Ercanbrack and Knight, 1998) and cattle (Echternkamp and Gregory, 1999) in long term selection projects. The purpose of this project was to estimate the genetic parameters and trends associated with

litter size in a population of Rambouillet sheep divergently selected for litter size.

## Materials and Methods

In 1970, selection for litter size born was started in a population of Rambouillet sheep at Montana State University's Red Bluff Research Ranch near Norris, Montana. The inbreeding level for this population is currently 12.2 to 14.2%, depending on line and the trends for inbreeding are shown in Figure 1.

Ewes were randomly assigned to one of two selection lines selected based on the estimated breeding value of a phenotypic index for increased (**HIGH**) or decreased (**LOW**) litter size. The index was:

$$I = \frac{(\text{Lifetime Number of Lambs born})}{(\text{Number of years in production})} \times 1000$$

Estimated breeding values were calculated each year using the following model:

$$y = X\beta + Za + e$$

where:

- y** is a vector of index values (1,666 in 2003);
- β** is a vector of fixed effects which includes year of birth and line;
- a** is a vector of direct genetic effects;
- e** is a vector of random error effects;
- X** is a known incidence matrix associating fixed effects with records in **y**; and
- Z** is a known incidence matrix associating random effects with records in **y** with zero columns associated with animals in the pedigree that do not have records.

Furthermore,

$$E[y] = X\beta; \text{ and}$$

$$\text{Var} \begin{bmatrix} \mathbf{a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A}\sigma_a^2 & 0 \\ 0 & \mathbf{I}\sigma_e^2 \end{bmatrix}$$

where **A** is the numerator relationship matrix of all animals included in the pedigree (3,758 animals in 2003), including those with no records, **I** is the identity matrix of the proper

order,  $\sigma_a^2$  is the variance due to additive genetic effects of the ewe, and  $\sigma_e^2$  is the variance due to random error.

In 1973, a control line (CTL) was also established. This line remained viable until 2001 at which time it was dispersed.

Each year, the three ram lambs with the highest EBVs from each of the four sire families represented within the HIGH line were selected to become potential sires in the HIGH line and the three with the lowest EBVs from each of the four sire families represented in the LOW line were selected as potential sires for the LOW line. The ram from each sire group with the highest or lowest EBV, depending on line, that remained at breeding time was used. Rams for the CTL line were randomly selected without regard for sire group. Rams were used at approximately 18 months of age and were used for only one breeding season.

Originally, approximately 30-35 ewe lambs were retained each year. The ewe lambs with the highest EBVs in the HIGH line were retained as dams for the HIGH line and the ewe lambs with the lowest EBVs in the LOW line were retained as dams for the LOW line regardless of sire group. In recent years, selection has continued in this way for the HIGH line, but the LOW line is currently producing so few lambs that all ewe lambs must be retained in order to sustain the line. Ewes are bred for the first time at approximately 18 months of age and remain in the lines for five breeding seasons.

All matings have been within lines through the 2003 lamb crop (2001 lamb crop for CTL) with the exception of the 1990 lamb crop. All ewes at the Red Bluff Research Ranch were bred to Merino rams in the fall of 1989, resulting in  $F_1$  Merino x Rambouillet lambs being born in the spring of 1990 from the ewes in the lambing rate selection project. None of these  $F_1$  lambs were retained for the HIGH, LOW, or CTL lines and no lambing data for 1990 was included in this dataset in order to avoid bias due to heterosis effects from the outcross matings. Additionally, sires of the 1991 lamb crop were approximately 30 months of age due to the interruption in selection.

Data analyzed for this project included all animals and index values from the foundation animals through the 2003 lamb crop. Breeding values were estimated using the multiple-trait derivative-free REML program (MTDFREML) of Boldman et al. (1995). The model used was the same as was used to estimate breeding values for the purpose of selection.

## Results and Discussion

Heritability for the lambing rate index was estimated to be 0.30. This is higher than the estimates typically obtained for reproductive traits that are measured only once (i.e., Okut et al., 1999; Hanford et al., 2002; Hanford et al., 2003). However, because this index includes information for multiple years of production, it is similar to a trait with repeated measures and the estimate of heritability is similar to the 0.38 estimate of heritability for ovulation rate obtained from an average of eight ovulation rate measures on cattle in the USDA twinning herd in Clay Center, Nebraska (Gregory et al., 1997).

Genetic trends for estimated breeding values of the index are shown in Figure 2. After more than three decades of selection, the difference between HIGH and LOW is 553.0 index units with the average EBV for index values for 2003 born animals being +417.5 and -135.6 index units for the HIGH and LOW lines, respectively. This equates to a difference in number of lambs born per year of 0.55 due to additive genetic effects.

Linear regression of EBV on year of birth was +12.2 and -3.0 index units for the HIGH and LOW lines, respectively, translating to an increase of +0.012 and -0.003 lambs born per year due to genetic effects for the HIGH and LOW lines, respectively. These trends are similar to those estimated by Saboulard et al. (1995). The EBV for the CTL also increased slightly with a linear trend of +1.4 which translates to an increase of +0.001 lambs per year due to genetic effects.

Selection for reproductive traits will result in genetic change in the same direction as selection.

## Implications

Reproduction is important in livestock enterprises, but due to the low estimates of heritability that are typically reported, it is often suggested that management or crossbreeding, utilizing heterosis, is a better way to improve reproduction compared to within breed selection. However, selection for increased reproduction will result in the desired response and an increased number of offspring born as shown by these results.

## Literature Cited

- Boldman, K.G., L.A. Kriese, L.D. Van Vleck, C.P. Van Tassell, and S.D. Kachman. 1995. A manual for use of MTDFREML. A set of programs to obtain estimates of variance and covariances [Draft]. U.S. Department of Agriculture, Agriculture Research Service.
- Bromley, C.M., G.D. Snowden, and L.D. Van Vleck. 2000. Genetic parameters among weight, prolificacy, and wool traits of Columbia, Polypay, Rambouillet, and Targhee sheep. *J. Anim. Sci.* 78:846-858.
- Echternkamp, S.E. and K.E. Gregory. 1999. Effects of twinning on postpartum reproductive performance in cattle selected for twin births. *J. Anim. Sci.* 77:48-60.
- Ercanbrack, S.K. and A.D. Knight. 1993. Responses to various selection protocols for lamb production in Rambouillet, Targhee, Columbia, and Polypay sheep. *J. Anim. Sci.* 76:1311-1325.
- Gregory, K.E., G.L. Bennett, L.D. Van Vleck, S.E. Echternkamp, and L.V. Cundiff. 1997. Genetic and environmental parameters for ovulation rate, twinning rate, and weight traits in a cattle population selected for twinning. *J. Anim. Sci.* 75:1213-1222.
- Hanford, K.J., L.D. Snowden, and G.D. Snowden. 2002. Estimates of genetic parameters and genetic change for reproduction, weight, and wool

characteristics of Columbia sheep. J. Anim. Sci. 80:3086-3098.

Hanford, K.J., L.D. Van Vleck, and G.D. Snowder. 2003. Estimates of genetic parameters and genetic change for reproduction, weight, and wool characteristics of Targhee sheep. J. Anim. Sci. 81:630-640.

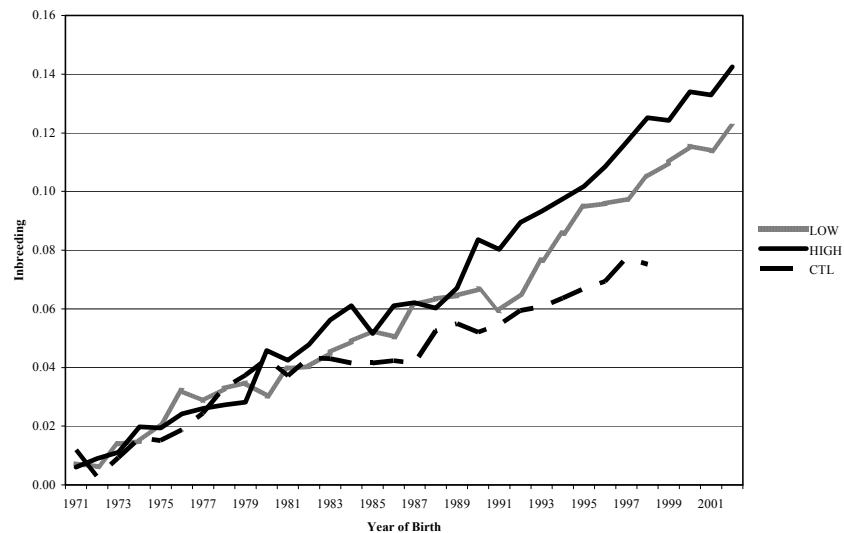
Hanford, K.J., L.D. Van Vleck, and G.D. Snowder. 2004. Estimates of genetic parameters and genetic change for reproduction, weight, and wool characteristics of Rambouillet sheep. J. Anim. Sci. *In press*.

Okut, H, C.M. Bromley, L.D. Van Vleck, and G.D. Snowder. 1999. Genotypic expression at different ages: I. Prolificacy traits of sheep. J. Anim. Sci. 77:2357-2365.

Rao, S. and D.R. Notter. 2000. Genetic analysis of litter size in Targhee, Suffolk, and Polypay sheep. J. Anim. Sci. 78:2113-2120.

Saboulard, M., W.C. Russell, and M.L. Riley. 1995. Selection for lambing rate and clean fleece weight in sheep. J. Anim. Sci. 73:3195-3198.

Figure 1. Inbreeding trends for the LOW, HIGH, and CTL lines<sup>a</sup>



<sup>a</sup> LOW – Selection line for decreased lambing rate; HIGH – Selection line for increased lambing rate; and CTL – Control line

Figure 2. Genetic trends for the LOW, HIGH, and CTL lines<sup>a</sup>



<sup>a</sup> LOW – Selection line for decreased lambing rate; HIGH – Selection line for increased lambing rate; and CTL – Control line

## CHANGES IN BODY WEIGHT, PELVIC MEASURES AND SEMEN CHARACTERISTICS OF HEREFORD CATTLE DUE TO SELECTION FOR SCROTAL CIRCUMFERENCE.

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**ABSTRACT:** Since 1995, a group of inbred Hereford cattle have been selected for increased scrotal circumference at the Northern Agricultural Research Center near Havre, MT. For 20 years prior, the cattle had been selected on an index of adjusted yearling weight minus 3.2 times adjusted birth weight. The current project was begun in an attempt to evaluate single trait selection on fertility of the herd sires, and the correlated response in other traits. This paper reports changes in body weight, pelvic measurements, and sperm characteristics due to single trait selection for increased scrotal circumference. Records from 2159 animals were evaluated for changes in estimated breeding values (EBV) for birth weight (BWT), weaning weight (WWT), yearling weight (YWT), and scrotal circumference (SC). Pelvic height (PH), pelvic width (PW), and sperm motility (MOT) were estimated using measurements beginning in 1996. Traits were analyzed with MTDFREML with fixed effects of year, age of dam and sex, and covariates of birth date, animal inbreeding and dam inbreeding included in the model, where appropriate. Estimates of direct heritability for BWT, WWT, YWT, and SC BV were 0.46, 0.21, 0.36 and 0.29, respectively. Estimates of maternal heritability for BWT, WWT, and YWT were 0.18, 0.17, and 0.12, respectively. Estimates of direct heritability for PH and PW were 0.22 and 0.21, respectively. The estimate of direct heritability for MOT was 0.09. Increases of 0.05, 0.50, 1.48, and 0.05 units per year were estimated for BWT, WWT, YWT, and SC direct genetic trends, respectively. There were slight increases in direct EBV for PH and PW since selection on scrotal circumference began. There is a trend toward larger direct EBV for all traits, indicating selection for larger scrotal circumference may also increase body weight and pelvic size.

Key Words: Beef Cattle, Selection, Scrotal Circumference

### Introduction

Efficient reproduction is a key element in animal production systems. Koots and Gibson (1998), using a bioeconomic model, found calf survival and fertility were traits vital to economic soundness for purebred systems. Dzuik and Bellows (1983) stated cows failing to become pregnant were the major reason for reduction of net calf crop. Selection for larger scrotal circumference (SC) has been shown to affect age at puberty in daughters (Bourdon and Brinks, 1986; Moser et al., 1996). Benefits in the production and viability of sperm cells have also been shown (Gipson, 1985; Coe, 1999). There have also been studies showing a relationship between SC and body size in

beef cattle (Bourdon and Brinks, 1986; Kriese et al., 1991). However, there is very little information on changes in size traits when using single trait selection based on a reproductive trait.

In 1995, in an effort to improve reproductive performance, a decision was made to begin selection for larger SC in the inbred Hereford line located at the Northern Agricultural Research Center (NARC) near Havre, Montana.

This study evaluates changes in birth weight (BWT), weaning weight (WWT), yearling weight (YWT), pelvic height (PH), pelvic width (PW), and sperm morphology (MOR) and motility (MOT) in Hereford bulls and heifers from a selection study based on larger scrotal circumference.

### Materials and Methods

In 1962 and 1963, cattle from the Fort Keogh Livestock and Range Research Laboratory, Miles City Line 1 Herefords, were transported to the NARC, Havre, Montana. The line was closed to outside breeding at this time. In 1976, selection began on an index of adjusted YWT minus 3.2 times adjusted BWT (Dickerson, 1974). This selection experiment continued until 1995 when the first bull calves were selected based on larger SC adjusted for age of dam and corrected to 365 d of age. Age of dam adjustment factors are shown in Table 1. This group of cattle has an average inbreeding coefficient (FX) of 0.24 at this time as shown in Figure 1. Breeding is by natural service for a 45 d breeding season with 4 sires and 100 dams per year. Bulls are used for breeding as 2-yr-olds, randomly mated with females with the restriction of no half-sib or son-dam matings. All heifers are exposed to bulls. Culling was based on pregnancy status, unsoundness (feet, legs, udders or cancer eye), or low calf performance records. Calves were weaned October 1 of each year at approximately 180 d of age. The calves grazed hay meadows for 45 d prior to being placed in drylots for a 140 d feeding period. Bull calves were fed to gain 1 kg/d, and heifers were fed to gain 0.68 kg/d. Data collected was weight, body height, pelvic measurements, and scrotal circumference for bulls as yearlings, and weight, body height, and pelvic measurements for heifers. Additional data was collected on heifers, cows and calves at birth and weaning. Number of records for BWT, WWT, YWT, SC, PH and PW, and MOT and MOR were 2220, 2008, 1860, 779, 461, and 178, respectively.

Breeding values and genetic parameters were estimated using MTDFREML (Boldman et al., 1995), with the model including fixed effects of year, age of dam, and sex, and linear and quadratic covariates for birth date, individual FX, and dam inbreeding (**DFX**) where appropriate. Initial models included direct genetic, maternal genetic, and permanent environment as random effects. Genetic trends for EBV are calculated from mean values.

## Results

The correction factors used for selection of breeding bulls are shown in Table 1. Evans et al. (1999) reported a similar day of age adjustment of 0.037, but age of dam adjustments were lower than those calculated for this study. Lunstra (1988) found age of dam to be important, mainly due to age of dam effects on body weight, but recommended using age adjustments rather than weight adjustments due to low genetic relationships found in their study. Similar estimates were obtained in this study with an estimated genetic correlation of 0.08 between SC and YWT. However, Makarechian (1984) found age was not more influential than weight for adjusting SC. Gipson (1985) also recommended use of linear body weight for adjusting SC.

Single trait analyses showed heritability estimates of 0.46, 0.21, 0.36, and 0.29 for BWT, WWT, YWT, and SC, respectively. Estimates of maternal genetic effects for BWT, WWT, and YWT were 0.20, 0.19, and 0.12, respectively. The estimates of direct-maternal genetic correlations were -0.08, -0.36, and -0.36, respectively. Permanent environmental effects for WWT were 0.19 and for YWT were 0.08. Estimates of heritability for BWT, WWT, and YWT are similar to those found by Alenda and Martin (1987). The estimate of heritability for SC is lower than other reports for yearling bulls (Coulter, 1987; Lunstra, 1988).

Estimates of heritability for PW and PH were 0.13 and 0.18, respectively. The heritability estimates for MOT was 0.09, however, a genetic estimate for MOR was not obtained. Moser et al. (1996) reported heritability estimates of 0.36 and 0.18 for MOT and MOR in lines selected for larger SC and fewer abnormalities and greater motility were found when bulls were selected based on phenotypic measurements for larger SC. A significant genetic correlation between SC and MOT were not found in this study.

With traits run in bivariate analyses, BWT and SC estimates of heritability were 0.61 and 0.30, respectively, with a genetic correlation of 0.08. Estimates of heritability for WWT and SC were 0.39 and 0.26, respectively with a genetic correlation of .38.

Genetic trends for EBV for estimates of BWT, WWT, and YWT showed increases of 0.05, 0.50, and 1.48 kg/yr, respectively, as shown in Figures 3-5. The genetic trend for SC was 0.05 cm/yr, shown in Figure 2. Genetic

trends for EBV for PH and PW were 0.005 and 0.01 cm/yr, respectively. There was a very slight increase in MOT EBV during this selection study.

## Implications

There were positive direct EBV values for all weight measures in this study. Direct EBV for PH and PW were also positive. This may indicate selecting only on increased scrotal size may also increase the size of the cattle in the herd. Age of dam is an important correction factor in adjusting SC in young bulls, but there is still debate on using age or weight of calf as correction factors.

## Literature Cited

- Alenda, R. and T. G. Martin. 1987. Genetic parameters and consequences of selection for growth traits in a beef herd selected for yearling weight. *J. Anim. Sci.* 64:366.
- Boldman, K. G., L. A. Kriese, L. D. VanVleck, C. P. Van Tassel, and S. D. Kachman. 1995. A manual for use of MTDFREML. A set of programs to obtain estimates of variance and covariance (Draft). U. S. Department of Agriculture, Agriculture Research Service.
- Bourdon, R. M. and J. S. Brinks. 1986. Scrotal circumference in yearling Hereford bulls: Adjustment factors, heritabilities and genetic, environmental and phenotypic relationships with growth traits. *J. Anim. Sci.* 62:958.
- Coe, P. H. 1999. Associations among age, scrotal circumference, and proportion of morphologically normal spermatozoa in young beef bulls during an initial breeding soundness exam. *J. Amer. Vet. Med. Assoc.* 214:1664.
- Coulter, G. H. 1987. Heritability of scrotal circumference in one- and two-year old bulls of different beef breeds. *Can. J. Anim. Sci.* 67:645.
- Dickerson, G. E., N. Kunzi, L. V. Cundiff, R. M. Koch, V. H. Arthaud, and K. E. Gregory. 1974. Selection criteria for efficient beef production. *J. Anim. Sci.* 39:659.
- Dzuik, P. J. and R. A. Bellows. 1983. Management of reproduction of beef cattle, sheep and pigs. *J. Anim. Sci.* 57(suppl.2):355.
- Evans, J. L., B. L. Golden, R. M. Bourdon, and K. L. Long. 1999. Additive genetic relationships between heifer pregnancy and scrotal circumference in Hereford cattle. *J. Anim. Sci.* 77:2621.

Gipson, T. A. 1985. Associations of scrotal circumference with semen traits in young beef bulls. *Theriogenology*. 24:217.

Koots, K. R. and J. P. Gibson. 1998. Economic values for beef production traits from a herd level bioeconomic model. *Can. J. Anim. Sci.* 78:29.

Kriese, L. A., J. K. Bertrand and L. L. Benyshek. 1991. Age adjustment factors, heritabilities and genetic correlations for scrotal circumference and related growth traits in Hereford and Brangus bulls. *J. Anim. Sci.* 69:478.

Lunstra, D. D. 1988. Heritability estimates and adjustment factors for the effects of bull age and age of dam on yearling testicular size in breeds of bulls. *Theriogenology*. 30:127.

Makarechian, M. 1984. Relationships between growth parameters and scrotal circumference in young beef bulls. *Theriogenology* 22:667

Moser, D. W., J. K. Bertrand, L. L. Benyshek, M. A. McCann, and T. E. Kiser. 1996. Effects of selection for scrotal circumference in Limousin bulls on reproductive and growth traits of progeny. *J. Anim. Sci.* 74:2052.

Table 1. Age of dam correction factors and regression of scrotal circumference on age

Age of dam (yr)	Correction factor (cm)
2	+1.60
3	+0.76
4-10	0
11+	+0.30

Regression of scrotal circumference on age = 0.031 cm/d

Figure 1. Direct inbreeding coefficients

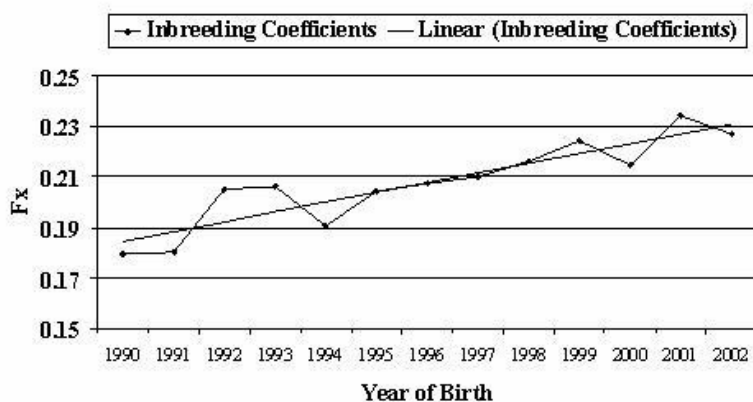


Figure 2. Direct scrotal circumference EBV

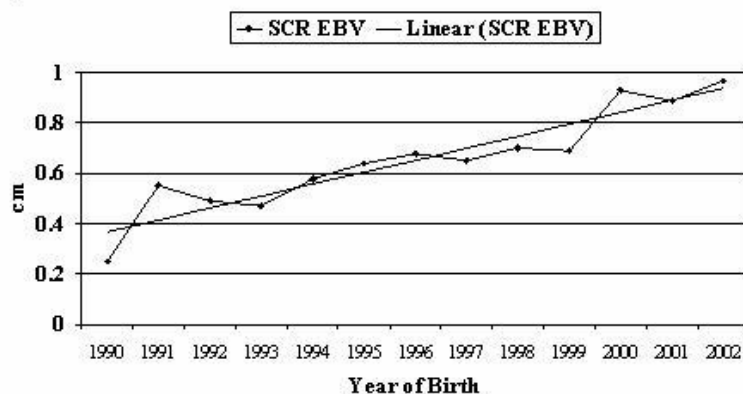


Figure 3. Direct EBV for birth weight

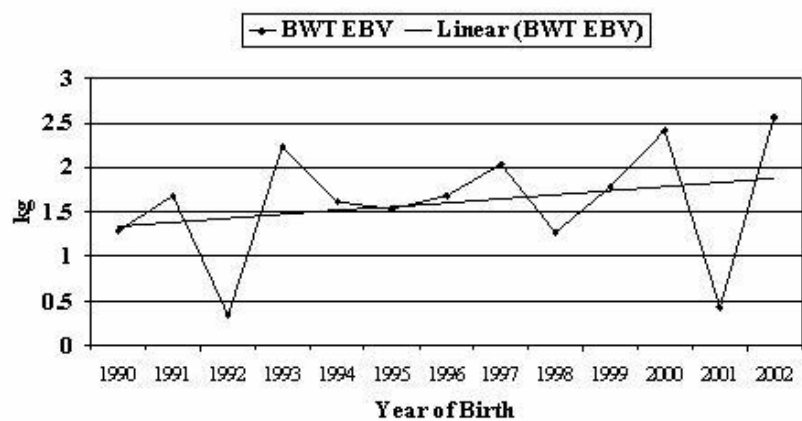


Figure 4. Direct EBV for weaning weight

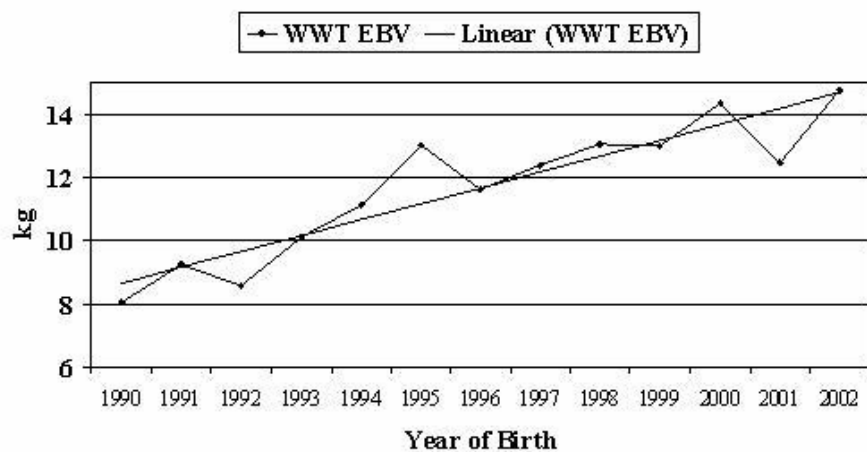
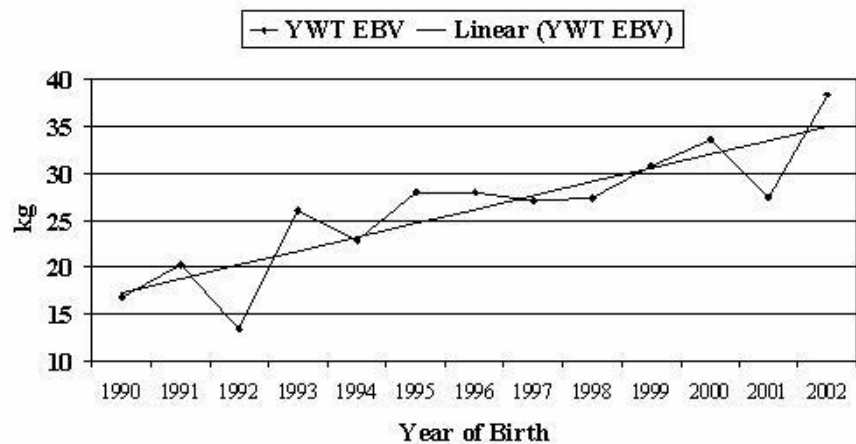


Figure 5. Direct EBV for yearling weight



## GENETIC CORRELATIONS OF GROWTH WITH CARCASS TRAITS FROM CHAROLAIS FIELD DATA

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**ABSTRACT:** Genetic parameters for three growth and five carcass traits were estimated for Charolais using a combination of carcass progeny test, purebred field performance and pedigree data. Heritabilities and genetic correlations were derived from variance components for birth weight (**BWT**,  $n = 54,221$ ), 205-d weaning weight (**WT205**,  $n = 31,384$ ), postweaning gain (**PWG**,  $n = 19,403$ ), hot carcass weight (**HCW**,  $n = 6,958$ ), average subcutaneous fat thickness (**FAT**,  $n = 6,866$ ), longissimus muscle area (**REA**,  $n = 6,863$ ), marbling score (**MAR**,  $n = 6,903$ ) and estimated carcass lean yield percentage (**PLY**,  $n = 6,852$ ) with an animal model ( $n = 78,728$ ) and restricted maximum likelihood. Breed of dam and contemporary group appropriate to each trait were included as fixed effects in the model, whereas random effects included direct genetic for all traits, maternal genetic for BWT and WT205, and maternal permanent environmental for WT205. Carcass traits were adjusted to a constant harvest age of 425 d. Heritability estimates of 0.53, 0.22, and 0.21 were obtained for direct components of BWT, WT205, and PWG, respectively, and maternal heritabilities were 0.16 and 0.10 for BWT and WT205, respectively. Direct  $\times$  maternal genetic correlations for BWT (-0.49) and WT205 (-0.35) were negative. Heritabilities for HCW, FAT, REA, MAR, and PLY were 0.33, 0.39, 0.43, 0.34, and 0.46, respectively. Genetic correlations among direct effects for growth traits were moderately positive and generally uncorrelated with maternal effects across traits. Lean and fat in the carcass generally had negative genetic correlations, although improvement in lean yield and marbling score may not be strongly antagonistic. Genetic correlations of direct and maternal components of growth traits with carcass traits suggested that selection for increased growth rate would not be antagonistic to improvement in carcass yield or meat quality.

**Key Words:** Carcass, Charolais, Genetic parameters, Growth

### Introduction

As one of several breeds conducting carcass merit national cattle evaluations (**NCE**), the Canadian Charolais Association (**CCA**) annually publishes EPD predicted for hot carcass weight (**HCW**), subcutaneous fat thickness (**FAT**), longissimus muscle area (**REA**), marbling score (**MAR**), and estimated carcass lean yield percentage (**PLY**) using carcass data from their Conception to Consumer

(**CtoC**) progeny test. Carcass trait EPD are augmented by the inclusion of adjusted birth weight (**BWT**), adjusted 205-d weaning weight (**WT205**), and adjusted postweaning gain (**PWG**) records from the purebred CCA performance and pedigree databases. Estimates of heritabilities and genetic and residual correlations are population specific parameters required to predict EPD in **NCE** and should be periodically updated to account for potential change over time due to selection and management (Koots et al., 1994a,b).

Growth from birth to weaning, from weaning to yearling, and carcass traits constitute groups of economically important phenotypes in the design of comprehensive genetic improvement programs. Genetic correlations among these groups of traits indicate potential synergies and antagonisms to multiple trait selection, but are generally lacking in the recent literature (Crews and Kemp, 1999; Splan et al., 2002; Meyer et al., 2004), especially with regard to large field populations. The objective of this study was to estimate heritabilities and genetic correlations among growth and carcass traits using CCA field data.

### Materials and Methods

The CCA CtoC carcass database contained records on steers and heifers ( $n = 6,968$ ) harvested in 22 years between 1975 and 2003. Market progeny were sired by 349 Charolais bulls mated to commercial and purebred cows ( $n = 4,063$ ) representing 48 distinct breed types. Sires of calves with carcass data were identified and used to extract growth and/or pedigree data from the (purebred) CCA performance database (**CHARM**) corresponding to: 1) their own record, 2) their male and female contemporaries born in the same year and herd, 3) their parents and grandparents, and 4) all available progeny. The combined growth and carcass data set represented 2,122 sires and 26,883 dams. Adjustments for BWT, WT205, and PWG were made according to Beef Improvement Federation guidelines (BIF, 2002), whereas carcass traits were adjusted to a constant slaughter age of 425 d. Breed of dam was defined by the concatenation of breed of sire, maternal grand sire, and maternal grand dam, as provided by CtoC commercial cooperators, or assigned the purebred Charolais breed of dam code. Genetic models for growth and carcass traits included fixed contemporary group and breed of dam effects, and random effects appropriate to each phenotype. Direct genetic effects were fit for all traits, BWT and WT205 included maternal genetic effects with non-zero

direct  $\times$  maternal genetic covariances, and WT205 included maternal permanent environmental effects.

Initial univariate models provided starting values for subsequent multiple trait models, however, a complete 8-trait model was not fit due to computing limitations. Genetic correlations of growth with carcass traits were estimated with a series of 15 bivariate models with individual growth and carcass traits included in pairs. Within growth and carcass trait groups, multivariate models were fit with the exception that PLY was not included in the multivariate carcass model due to a linear dependency among PLY, FAT, and REA. (Co)variances involving PLY were estimated with seven bivariate models with other traits. Models were fit, (co)variance components estimated and genetic parameters computed with the ASREML software package (VSN International Ltd., Hemel Hempstead, UK) which employs REML with an average information algorithm. Standard errors associated with genetic parameters were also computed by the software and used to evaluate their relative significance.

## Results and Discussion

Summary statistics are reported in Table 1. The largest number of records were available for BWT, with fewer records available for WT205 and PWG. Even though CCA has adopted mandatory cow-based (i.e., whole herd) reporting, only 36% of calves with BWT records also had records for PWG. The average carcass in the CCA database adjusted to 425 d of age weighed 324.42 kg, had 10.15 mm of subcutaneous fat, a longissimus muscle area of 86.72 cm<sup>2</sup>, and was expected to yield 59.58% lean. The average marbling score was 76.30, corresponding to the lower portion of the “AAA” Canadian quality grade, or the lower one-third of the USDA “Choice” quality grade. Among the 6,903 carcasses with marbling score, 60% had sufficient marbling to receive the Canadian “AAA” or USDA “Choice” quality grade, while approximately 5% had less than a “Slight” degree of marbling.

Table 1 contains multivariate estimates of heritabilities and genetic correlations among growth and carcass traits. The direct heritability estimate for BWT ( $0.53 \pm 0.02$ ) was high, and higher than the weighted average reported by Koots et al. (1994a) while the estimate for WT205 ( $0.22 \pm 0.02$ ) was moderate and very similar to the summary by Koots et al. (1994a). Maternal heritability estimates were  $0.16 \pm 0.01$  and  $0.10 \pm 0.02$  for BWT and WT205, respectively, and were similar to the mean estimates of 0.14 for maternal BWT and 0.13 for maternal WT205 in the summary of Koots et al. (1994a). These results indicate that for growth up to weaning in Charolais, direct effects account for a considerably higher proportion of phenotypic variance than do maternal effects. Twelve percent of WT205 phenotypic variance was attributed to maternal permanent environmental effects. For both BWT and WT205, direct  $\times$  maternal covariances and correlations ( $-0.49 \pm 0.03$  and  $-0.35 \pm 0.07$ , respectively) were negative and similar to the results reported in numerous recent studies, implying an antagonism between direct and maternal components of early growth. The heritability estimate for PWG was  $0.21 \pm 0.02$  and generally within the

range of values reported in the recent literature. Direct BWT was moderately and positively correlated with direct WT205 (0.33) and PWG (0.29) as was direct WT205 with PWG (0.39). It appears that direct and maternal effects were negatively correlated within early growth traits, however, in general, direct and maternal effects were uncorrelated between pairs of growth traits.

Heritability estimates were moderate to high for the five carcass traits, ranging from 0.33 for HCW to 0.46 for PLY. In general, heritability estimates for carcass traits were similar to or higher than those reported in the recent literature based on large field data sets (e.g., Wilson et al., 1992; Woodward et al., 1992; Meyer et al., 2004). The genetic correlation of HCW with REA was high and positive (0.47), but was smaller with both FAT (0.18) and PLY (-0.05). Carcass weight and MAR had a negative genetic correlation estimate of -0.21, which was moderate and indicated that increased HCW was associated with increased MAR, because the marbling score scale assigned lower scores to carcasses with higher amounts of intramuscular fat. Carcass FAT had a negative genetic correlation with REA (-0.35) indicating that muscle and fat were antagonistic, which was reflected in the strongly negative genetic correlation of FAT with PLY (-0.88) which would be expected given the intuitive antagonism between FAT and fat-free lean. Intramuscular (MAR) and subcutaneous (FAT) fat measurements were negatively correlated, indicating that selection for increased MAR would result in correlated increases in FAT, however, the low to moderate magnitude of this correlation suggests that genetic potential for MAR could be increased without large increases in FAT. Both MAR and PLY had positive genetic correlations with REA, although the correlation with MAR would be considered weak and should be judged near zero. In the case of REA and PLY, a strongly positive genetic correlation would be predicted on the basis of the lean yield percentage definition. These results suggest, in general, that increases in weight and muscling were positively associated, but not strongly correlated with deposition of fat, either in subcutaneous or intramuscular depots. Genetic improvement in carcass lean yield could be accomplished nearly independent of genetic change in either body size (e.g., HCW) or quality grade (e.g., MAR).

Table 1 also contains estimates of genetic correlations of growth with carcass traits. A total of 4,805 animals in the final data set had both growth and carcass traits, therefore, genetic correlations were estimated from about two-thirds of all animals that could possibly have both types of data, as well as though additive relationships. Not all animals with carcass data also had growth data because such a data requirement is enforced in neither the CtoC program nor in the Charolais carcass national evaluation.

Direct effects on BWT were positively correlated with HCW ( $0.39 \pm 0.08$ ) and REA ( $0.21 \pm 0.08$ ) but negatively correlated with FAT ( $-0.21 \pm 0.08$ ), suggesting that direct BWT was a reasonable predictor of lean growth, also evidenced by a moderately positive genetic correlation with PLY ( $0.21 \pm 0.08$ ). Generally strong and positive genetic correlations between BWT and HCW have been reported in several studies (MacNeil et al., 1984; Johnston et al., 1994; Crews and Kemp, 1999) as well as in a summary of

published genetic correlations (Koots et al., 1994b). Also, MacNeil et al. (1984), Koots et al. (1994b) and Crews and Kemp (1999) found negative correlations ranging from  $-0.07$  to  $-0.44$  between BWT and FAT which are supported by the results of the present study. The genetic correlation found here between direct BWT and REA was similar to the mean ( $0.31$ ) reported by Koots et al. (1994b).

Direct WT205 had a stronger positive genetic correlation ( $0.79 \pm 0.05$ ) with HCW than either direct BWT or PWG. Recent studies have also shown high genetic correlations between direct weaning weight and HCW, ranging from  $0.70$  (Splan et al. 2002) to  $0.92$  (Splan et al., 1998). Direct WT205 had a moderately positive genetic correlation with FAT of  $0.30 \pm 0.10$ , but a lower positive genetic correlation with REA of  $0.19 \pm 0.10$ . These associations led to a genetic correlation of  $-0.25 \pm 0.10$  between WT205 and PLY. Recent studies (Splan et al., 1998; 2002) have shown moderately positive genetic correlations of  $0.26$  to  $0.30$  between direct WT205 and FAT, which are supported by the results of the present study. Overall, direct WT205 appeared to be less strongly related to lean components of the carcass than direct BWT.

Few studies in the recent literature have reported genetic correlations of PWG with carcass traits. The genetic correlation of  $0.49 \pm 0.11$  between PWG and HCW was strong and positive, which is in general agreement with results from Johnston et al. (1992) and Splan et al. (1998) who both reported strongly positive genetic correlations of HCW with yearling weight. Genetic correlations of PWG were moderate and positive with both FAT ( $0.21 \pm 0.14$ ) and REA ( $0.38 \pm 0.13$ , but near zero ( $0.06 \pm 0.14$ ) with PLY. Splan et al. (1998) reported genetic correlations of yearling weight with FAT ( $0.34$ ) and REA ( $0.29$ ) that were moderate and similar to the present results involving PWG.

Direct components of BWT and WT205 tended to have positive and therefore unfavorable genetic correlations with MAR, however, the genetic correlation between PWG and MAR ( $-0.38 \pm 0.15$ ) was negative and therefore favorable. The standard errors associated with these correlations were relatively large and therefore do not support any strong conclusions. The recent literature similarly provides no clear indication of the genetic association of growth with MAR across experimental and field populations. For example, Woodward et al. (1992) reported a near-zero genetic correlation ( $0.05$ ) between BWT and MAR whereas marbling adjusted to a constant fat end point in Johnston et al. (1992) had an unfavorable genetic correlation of  $-0.26$  with BWT. Further, Woodward et al. (1992) and Splan et al. (1998) reported positive genetic correlations of WT205 with MAR, conflicting with negative estimates of  $-0.55$  and  $-0.12$  reported by Johnston et al. (1992) and Splan et al. (2002), respectively. Five studies summarized by Koots et al. (1994b) had a mean genetic correlation of  $-0.17$  between direct WT205 and MAR, which is unfavorable and supported by the results of the present study.

Genetic correlations of maternal components of BWT and WT205 with carcass traits were low and generally near zero. There was a weak trend for maternal BWT to have a positive genetic correlation with FAT ( $0.13 \pm 0.10$ ) and therefore a negative genetic correlation with PLY ( $-0.12 \pm 0.10$ ). Genetic correlations of maternal WT205 were near

zero with FAT ( $-0.02 \pm 0.14$ ) and MAR ( $-0.07 \pm 0.15$ ) and weakly positive with PLY ( $0.13 \pm 0.14$ ). Maternal WT205 further had a moderately positive genetic correlation with HCW ( $0.27 \pm 0.10$ ) but this correlation was lower than an analogous parameter ( $0.61$ ) reported by Splan et al. (2002). The study of Crews and Kemp (1999) found a negative genetic correlation of  $-0.23$  between maternal BWT and REA. Splan et al. (2002) found that maternal WT205 had a moderately positive genetic correlation of  $0.29$  with REA, results generally supported by the present study. The corresponding genetic correlation of maternal WT205 with REA reported by Crews and Kemp (1999) was positive as well.

## Implications

Comprehensive genetic improvement programs for beef cattle should consider not only direct and maternal components of growth, but end product traits as well. Heritability estimates for growth and carcass traits were moderate to high, suggesting that selection should be used to improve these traits. Genetic correlations among direct components of growth were moderately positive although genetic correlations between direct and maternal effects for growth were either negative (i.e., within traits) or near zero (i.e., across traits). Genetic correlations of components carcass traits related to muscling with those related to fat were generally negative. Genetic correlations indicated that selection for growth rates or weights would not be strongly antagonistic to improvement in carcass lean yield or meat quality.

## Literature Cited

- BIF. 2002. Guidelines for Uniform Beef Improvement Programs, 8th Ed. Animal and Dairy Science Department, University of Georgia, Athens.
- Crews, D. H., Jr. and R. A. Kemp. 1999. Contributions of preweaning growth information and maternal effects for prediction of carcass trait breeding values among crossbred beef cattle. *Can. J. Anim. Sci.* 79:17-25.
- Johnston, D. J., L. L. Benyshek, J. K. Bertrand, M. H. Johnson, and G. M. Weiss. 1992. Estimates of genetic parameters for growth and carcass traits in Charolais cattle. *Can. J. Anim. Sci.* 72:493-499.
- Koots, K. R., J. P. Gibson, C. Smith, and J. W. Wilton. 1994a. Analyses of published genetic parameter estimates for beef production traits. 1. Heritability. *Anim. Breed. Abstr.* 62:309-337.
- Koots, K. R., J. P. Gibson, and J. W. Wilton. 1994b. Analyses of published genetic parameter estimates for beef production traits. 2. Phenotypic and genetic correlations. *Anim. Breed. Abstr.* 62:825-833.
- MacNeil, M. D., L. V. Cundiff, C. A. Dinkel, and R. M. Koch. 1984. Genetic correlations among sex-limited traits in beef cattle. *J. Anim. Sci.* 58:1171-1180.

- Meyer, K., D. J. Johnston, and H.-U. Graser. 2004. Estimates of the complete genetic covariance matrix for traits in multi-trait genetic evaluation of Australian Hereford cattle. *Aust. J. Ag. Res.* 55:195-210.
- Splan, R. K., L. V. Cundiff, and L. D. Van Vleck. 1998. Genetic parameters for sex-specific traits in beef cattle. *J. Anim. Sci.* 76:2272-2278.
- Splan, R. K., L. V. Cundiff, M. E. Dikeman, and L. D. Van Vleck. 2002. Estimates of parameters between direct and maternal genetic effects for weaning weight and direct genetic effects for carcass traits in crossbred cattle. *J. Anim. Sci.* 80:3107-3111.
- Wilson, D. E., R. L. Willham, S. L. Northcutt, and G. H. Rouse. 1992. Genetic parameters for carcass traits estimated from Angus field records. *J. Anim. Sci.* 71:2365-2370.
- Woodward, B. W., E. J. Pollak, and R. L. Quaas. 1992. Parameter estimation for carcass traits including growth information of Simmental beef cattle using restricted maximum likelihood with a multiple trait model. *J. Anim. Sci.* 70:1098-1109.

Table 1. Summary statistics and genetic parameters<sup>a</sup> among growth<sup>b</sup> and carcass<sup>c</sup> traits

Item	BWT		WT205 <sup>d</sup>		PWG	HCW	FAT	REA	MAR <sup>e</sup>	PLY
n	54,221		31,384		19,403	6,958	6,866	6,863	6,903	6,852
Mean	45.89		292.41		191.71	324.42	10.15	86.72	76.30	59.58
SD	5.40		39.73		56.45	40.69	3.63	11.62	9.84	3.73
Trait	BWTd	BWTm	WT205d	WT205m	PWG	HCW	FAT	REA	MAR	PLY
BWTd <sup>f</sup>	0.53									
BWTm <sup>g</sup>	-0.49	0.16								
WT205d <sup>f</sup>	0.33	0.10	0.22							
WT205m <sup>g</sup>	-0.16	0.33	-0.35	0.10						
PWG	0.29	0.06	0.39	0.01	0.21					
HCW	0.39	0.12	0.79	0.27	0.49	0.33				
FAT	-0.21	0.13	0.30	-0.02	0.21	0.18	0.39			
REA	0.21	0.03	0.19	0.22	0.38	0.47	-0.35	0.43		
MAR	0.03	-0.03	0.14	-0.07	-0.38	-0.21	-0.19	0.10	0.34	
PLY	0.21	-0.12	-0.25	0.13	0.06	-0.05	-0.88	0.72	0.24	0.46

<sup>a</sup> In the lower portion of the table, heritability estimates are on the diagonal, while genetic correlation estimates are below the diagonal. Standard errors for heritability estimates were 0.02 for growth traits, and 0.04 for carcass traits. Standard errors for genetic correlation estimates were approximately 0.05 among growth traits, approximately 0.08 among carcass traits, and approximately 0.11 for growth × carcass trait pairs.

<sup>b</sup> Growth traits: BWT = adjusted birth weight, kg, WT205 = adjusted 205-d weaning weight, kg, PWG = adjusted postweaning gain, kg.

<sup>c</sup> Carcass traits: HCW = hot carcass weight, kg, FAT = subcutaneous fat thickness, mm, REA = longissimus muscle area, cm<sup>2</sup>, MAR = marbling score, PLY = estimated carcass lean yield, %. Carcass traits were adjusted to a constant slaughter age of 425 d.

<sup>d</sup> The proportion of WT205 phenotypic variance attributed to maternal permanent environmental effects ( $c^2$ ) was  $0.12 \pm 0.01$ .

<sup>e</sup> Marbling score: 90.00 to 99.99 = Traces, 80.00 to 89.99 = Slight, 70.00 to 79.99 = Small, 60.00 to 69.99 = Modest.

<sup>f</sup> BWT<sup>d</sup> and WT205<sup>d</sup> refer to direct genetic effects on BWT and WT205, respectively.

<sup>g</sup> BWT<sup>m</sup> and WT205<sup>m</sup> refer to maternal genetic effects on BWT and WT205, respectively.

# GENETIC ANALYSIS OF MATURE COW WEIGHTS IN A POPULATION OF INBRED HEREFORD CATTLE

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**ABSTRACT:** Weights were measured on 642 cows in the Line 4 inbred Hereford herd at the Northern Agricultural Research Center in Havre, Montana from 1976 to 1997. The Line 4 Hereford herd descended from Miles City Line 1. The herd had been selected based on an index of adjusted yearling weight minus 3.2 times adjusted birth weight from 1976 to 1995 and had reached an average inbreeding of 20.7% at the conclusion of this selection experiment. Over the course of the study, there was a maximum of seven weigh dates per year. Weights were measured prior to calving, after calving, at two different milk test dates, prior to breeding, after breeding, and at calf weaning. Cows averaged 14.5 cow weight records during their lifetime and 3.8 records per year. Maternal genetic effects were determined to be statistically significant for mature cow weight and were included in the model with direct genetic effects, direct-maternal genetic covariance, and the proportion of variance due to direct permanent environmental effects. Weights were analyzed individually as repeated records within seasons across years as well as combined in a single univariate analysis combining all seasons. Estimates of genetic parameters (and associated s.e.) from the single analysis including all seasons were 0.64 (0.13), -0.64 (0.20), 0.07 (0.05), and 0.15 (0.08) for direct heritability, direct-maternal genetic correlation, maternal heritability, and proportion of variance due to direct permanent environmental effects, respectively. Over the course of the experiment, genetic trends were small and estimated to be 0.38 kg/yr and -0.15 kg/yr for direct and maternal breeding values, respectively. Selection for increased yearling weight and decreased birth weight in this index does not appear to have an effect on mature cow weight.

Keywords: Birth weight, correlated response, mature weight, yearling weight

## Introduction

Mature cow weight (MW) has become an important trait in recent years because MW has been shown to be an indicator for maintenance energy requirements (McMorris and Wilton, 1986; Montaño-Bermudez et al., 1990) in which larger mature weights would be expected to result in higher maintenance energy requirements.

Previous estimates of direct heritability for mature weight have been moderate to high (i.e., Johnson et al., 1990; Northcutt and Wilson, 1993; Kaps et al., 1999) with estimates of 0.60 to 0.70 generally. Maternal effects are not generally included in models used to analyze MW, but have been estimated to be low, but significant with maternal

heritability estimates of 0.09 to 0.21 and estimates of the proportion of variance due to maternal permanent environmental effects to be 0.00 to 0.06 (Rumph et al., 2002b).

The objective of this research was to determine the genetic parameters associated with cow weight and the genetic trends for cow weight when selection is based on an index of increasing yearling weight and decreasing birth weight.

## Materials and Methods

The Line 4 Hereford line is descended from the Miles City Line 1 Hereford line and has been maintained as a closed herd at the Northern Agricultural Research Center since 1963. The inbreeding trend for this herd is shown in Figure 1 and is increasing by 0.4% per year.

From 1976 to 1995, sires were selected using an index that incorporated adjusted birth weight (BW) and adjusted yearling weight (YW). Birth weight was adjusted for sex of calf and age of dam and yearling weight was adjusted for sex of calf, age of dam, and age at measurement. The index used was:

$$I = \text{Adj. YW} - 3.2 (\text{Adj. BW})$$

A total of 9321 cow weight records were measured on 642 cows a maximum of seven times per year: prior to calving (PREC), after calving (PSTC), at two different milk test dates (MLK1 and MLK2), prior to breeding (PREB), after breeding (PSTB), and at calf weaning (WEAN). Cows averaged 14.5 measures of weight in their lifetime and 3.8 measures within a given year. Number of records and means for each season are shown in Table 1.

Genetic parameters and breeding values to calculate genetic trends were estimated using the following model:

$$y = X\beta + Z_a a + Z_m m + Z_c c + e$$

where:

y is a vector of observed cow weights;  
 $\beta$  is a vector of fixed effects;  
a is a vector of direct genetic effects;  
m is a vector of maternal genetic effects;  
c is a vector of direct permanent environmental effects;  
e is a vector of random error effects;  
X is a known incidence matrix associating fixed effects with records in y; and

$Z_a$ ,  $Z_m$ , and  $Z_c$  are known incidence matrices associating random effects with records in  $y$  with zero columns associated with animals in the pedigree that do not have records.

Furthermore,

$$E[y] = X\beta; \text{ and}$$

$$\text{Var} \begin{bmatrix} a \\ m \\ c \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_a^2 & A\sigma_{am} & 0 & 0 \\ A\sigma_{am} & A\sigma_m^2 & 0 & 0 \\ 0 & 0 & I\sigma_c^2 & 0 \\ 0 & 0 & 0 & I\sigma_e^2 \end{bmatrix}$$

where  $A$  is the numerator relationship matrix of all 2079 animals included in the pedigree, including those with no records,  $I$  are the identity matrices of the proper order,  $\sigma_a^2$  is the variance due to additive genetic effects of the cow,  $\sigma_m^2$  is the variance due to maternal genetic effects,  $\sigma_c^2$  is the variance due to permanent environmental effects of the cow, and  $\sigma_e^2$  is the variance due to random error.

In separate analyses of each of the seven individual seasons, fixed effects included were year of measure, age of cow at measure, and weigh code. Weigh code identified whether a cow was pregnant, open, aborted, sick, nursing her own calf, nursing a grafted calf, or dry and were specific for the weigh date that they were associated with. Linear and quadratic covariates of weigh date and individual inbreeding were also included with the exception of the PSTC weight in which weigh date was unknown for all weight observations.

A combined analysis including all seasons except PSTC, due to lack of weigh date information, was also performed. In the combined analysis, an additional fixed effect of season was included.

Genetic parameters were estimated using the multiple-trait derivative-free REML program (MTDFREML) of Boldman et al. (1995) modified by Dodenhoff et al. (1998) for calculation of standard errors of estimates of genetic parameters for certain models.

## Results and Discussion

Genetic parameters estimated for each analysis are shown in Table 2. In the combined analysis, direct heritability was estimated to be 0.64 (0.13) and ranged from 0.19 to 1.00 across seasons. Extreme estimates were obtained for PSTC, MLK1, and MLK2 weights. For all three analyses, the direct-maternal genetic correlation was estimated to be at the boundaries (-1.00 or 1.00) and is likely to be the reason the estimates vary from other seasons and the combined analysis. The remainder of estimates ranged from 0.71 to 0.90 and are in agreement with those previously reported in literature (i.e. Brinks et al., 1962; Benyshek and Marlowe, 1973; Jenkins et al., 1991).

Although typically not estimated in cow weight data, maternal genetic effects were found to be significant in most analyses and are similar to those estimated by

Rumph et al. (2002b) with an estimate of 0.07 (0.05) in the combined analysis and a range of estimates from 0.05 to 0.10 across seasons.

The direct-maternal correlation was estimated to be -0.64 (0.20) in the combined analysis and ranged from -1.00 to 1.00 across seasons with the majority of estimates highly negative. This is similar in sign, but more extreme than estimates reported for other weight traits (i.e., Lee and Pollak, 1997; Dodenhoff et al., 1999; Splan et al., 2002).

The proportion of variance that can be attributed to permanent environmental effects was included to account for repeated records, both across years within a season and across seasons within a year, and was estimated to be significant at 0.15 (0.08) in the combined analysis, but was generally not significant in the analyses within seasons with a range across seasons of 0.00 to 0.47. The estimate of 0.47 was obtained from the PSTC weight which had the smallest number of observations and tended to differ from the rest of the analyses for all estimates.

The direct and maternal genetic trends for MW are shown in Figures 2 and 3, respectively. The trend for direct genetic effects is slight and positive with an increase of 0.38 kg/year. This is in agreement with the estimate of genetic trends by Rumph (2000) in a control and three closed selection lines of Hereford cattle selected for increased weaning weight, yearling weight, and an index of yearling weight and muscling score at the USDA Meat Animal Research Center in Clay Center, Nebraska. The estimated trend for maternal genetic effects was small and negative with an estimate of -0.15 kg/year. Selection for this index did not appear to have an effect on mature weight in the herd. This may be due to the fact that selection pressure was for an increase in one growth trait and a decrease in another, both traits having been shown to have positive genetic correlations with mature weight (Rumph, 2002a).

## Implications

Selection for growth traits can be expected to increase all growth traits due to positive genetic correlations, but when one growth trait is selected for increased weight and another for decreased weight, the effect on other correlated growth traits can be small. Selecting for decreased weight early in life and increased weight later in life, which is typical of current beef cattle production systems may not adversely affect mature cow weight.

## Literature Cited

- Benyshek, L.L. and T.J. Marlowe. 1973. Estimating heritability of Hereford cow weights. *J. Anim. Sci.* 36:854-861.
- Boldman, K.G., L.A. Kriese, L.D. Van Vleck, C.P. Van Tassell, and S.D. Kachman. 1995. A manual for use of MTDFREML. A set of programs to obtain estimates of variance and covariances [Draft]. U.S. Department of Agriculture, Agriculture Research Service.

- Brinks, J.S., R.T. Clark, N.M. Kieffer, and J.R. Quesenberry. 1962. Mature weight in Hereford range cows – heritability, repeatability, and relationship to calf performance. *J. Anim. Sci.* 23:711-716.
- Dodenhoff, J., L.D. Van Vleck, and D.E. Wilson. 1999. Comparison of models to estimate genetic effects of weaning weight of Angus cattle. *J. Anim. Sci.* 77:3176-3184.
- Dodenhoff, J., L.D. Van Vleck, S.D. Kachman, and R.M. Koch. 1998. Parameter estimates for direct, maternal, and grandmaternal genetic effects for birth weight and weaning weight in Hereford cattle. *J. Anim. Sci.* 76:2521-2527.
- Jenkins, T.G., M. Kaps, L.V. Cundiff, and C.L. Ferrell. 1991. Evaluation of between- and within-breed variation in measures of weight-age relationships. *J. Anim. Sci.* 69:3118-3128.
- Johnson, Z.B., C.J. Brown, and A.H. Brown, Jr. 1990. Evaluation of growth patterns of beef cows. *Arkansas Agric. Exp. Sta. Bull.* 623, Fayetteville.
- Kaps, M., W.O. Herring, and W.R. Lamberson. 1999. Genetic and environmental parameters for mature weight in a Angus cattle. *J. Anim. Sci.* 77:569-574.
- Lee, C. and E.J. Pollak. 1997. Influence of sire misidentification on sire x year interaction variance and direct-maternal genetic covariance for weaning weight in beef cattle. *J. Anim. Sci.* 75:2858-2863.
- McMorris, M.R. and J.W. Wilton. 1986. Breeding system, cow weight, and milk yield effects on various biological variables in beef production. *J. Anim. Sci.* 63:1361-1372.
- Montaño-Bermudez, M., M.K. Nielsen, and G.H. Deutscher. 1990. Energy requirements for maintenance of crossbred beef cattle with different genetic potential for milk. *J. Anim. Sci.* 68:2279-2288.
- Northcutt, S.L. and D.E. Wilson. 1993. Genetic parameter estimates and expected progeny differences for mature size in Angus cattle. *J. Anim. Sci.* 71:1148-1153.
- Rumph, J.M. 2000. A comparison of models used for estimation of genetic parameters for mature weight of Hereford cattle. M.S. Thesis, Univ. Nebraska, Lincoln.
- Rumph, J.M. 2002a. Genetic analysis of production traits for improvement of national beef cattle genetic evaluations: mature weight, body condition score, hip height, birth weight, and weaning weight. Ph.D. Diss., Univ. Nebraska, Lincoln.
- Rumph, J.M., R.M. Koch, K.E. Gregory, L.V. Cundiff, and L.D. Van Vleck. 2002b. Comparison of models for estimation of genetic parameters for mature weight of Hereford cattle. *J. Anim. Sci.* 80:583-590.
- Splan, R.K., L.V. Cundiff, M.E. Dikeman, and L.D. Van Vleck. 2002. Estimates of parameters between direct and maternal genetic effects for weaning weight and direct genetic effects for carcass traits in crossbred cattle. *J. Anim. Sci.* 80:3107-3111.

Figure 1. Inbreeding trend for Havre Line 4 Hereford herd

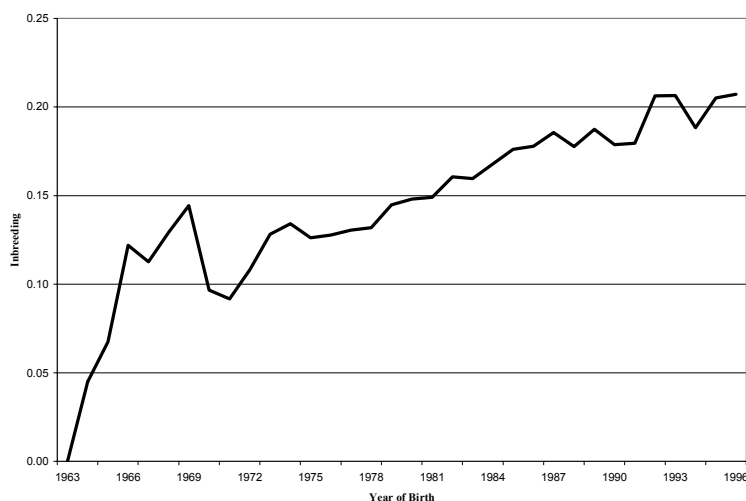


Table 1. Number of records and mean observations for cow weight (kg) at each season<sup>a</sup> and combined across six seasons

	PREC	PSTC	MLK1	MLK2	PREB	PSTB	WEAN	COMBINED
Mean	519.8	398.1	471.3	547.2	465.0	506.0	530.8	506.7
Number	1988	270	861	783	1744	1741	1933	9051

<sup>a</sup> PREC – Precalving weight; PSTC – Postcalving weight; MLK1 – Weight on first milk test day; MLK2 – Weight on second milk test day; PREB – Prebreeding weight; PSTB – Postbreeding weight; WEAN – Cow weight at weaning of calf; and COMBINED – All seasons except PSTC

Table 2. Estimates of genetic parameters (and associated s.e.) from univariate analyses within season<sup>a</sup> and in a combined analysis across six seasons

Parameter <sup>b</sup>	PREC	PSTC	MLK1	MLK2	PREB	PSTB	WEAN	COMBINED
$h_a^2$	0.78 (0.15)	0.19 (0.20)	0.95 (0.22)	1.00 (0.20)	0.90 (0.18)	0.77 (0.17)	0.71 (0.15)	0.64 (0.13)
$r_{am}$	-0.83 (0.18)	1.00 (2.38)	-1.00 (0.27)	-1.00 (0.39)	-0.91 (0.16)	-0.90 (0.22)	-0.75 (0.18)	-0.64 (0.20)
$h_m^2$	0.07 (0.05)	0.06 (0.18)	0.08 (0.08)	0.05 (0.06)	0.10 (0.07)	0.07 (0.06)	0.09 (0.06)	0.07 (0.05)
$c^2$	0.04 (0.09)	0.47 (0.16)	0.01 (0.14)	0.00 (0.13)	0.00 (0.11)	0.07 (0.10)	0.12 (0.10)	0.15 (0.08)
$e^2$	0.30 (0.03)	0.17 (0.02)	0.24 (0.03)	0.18 (0.02)	0.27 (0.03)	0.29 (0.03)	0.27 (0.02)	0.28 (0.02)
$\sigma_p^2$	2144.2	985.4	1969.8	2672.4	1931.6	2039.6	2098.8	2251.8

<sup>a</sup> PREC – Precalving weight; PSTC – Postcalving weight; MLK1 – Weight on first milk test day; MLK2 – Weight on second milk test day; PREB – Prebreeding weight; PSTB – Postbreeding weight; WEAN – Cow weight at weaning of calf; and COMBINED – Analysis across all seasons except PSTC

<sup>b</sup>  $h_a^2$  – Direct heritability;  $r_{am}$  – Direct-Maternal genetic correlation;  $h_m^2$  – Maternal heritability;  $c^2$  – Proportion of variance due to permanent environmental effects;  $e^2$  – Proportion of variance due to random error; and  $\sigma_p^2$  – Phenotypic variance ( $kg^2$ )

Figure 2. Genetic trend for direct breeding value

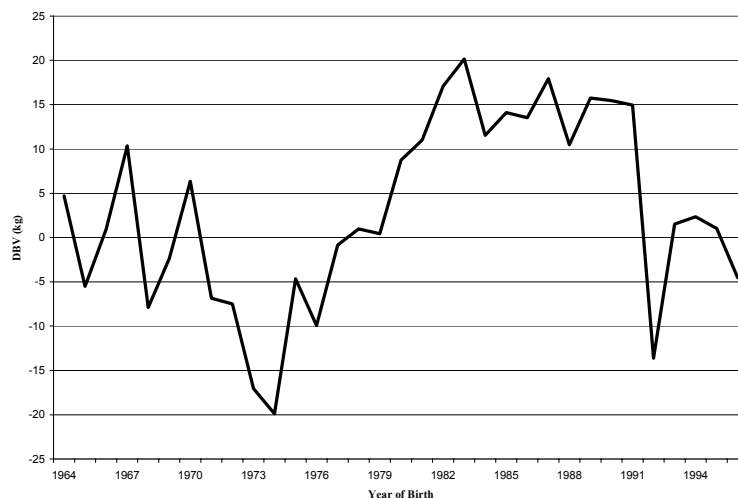


Figure 3. Genetic trend for maternal breeding value



## THE STATUS OF EQUINE GENETIC EVALUATION

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**ABSTRACT:** The use of genetic evaluation in the equine industry is reviewed and potential problems identified. The U.S. equine industry has been slow in adopting genetic evaluation as a tool for animal selection, in comparison to many European breed associations. Research has shown that many important traits are moderate to highly heritable indicating that genetic progress from selection is possible. Heritabilities for: cutting ability (0.04-0.19), trotting (0.25-0.3), Thoroughbred race times (0.15-0.2), Quarter Horse race times (0.34-0.38), jumping (0.19-0.31), and dressage (0.1-0.2) have been reported in the literature. In Dutch Warmbloods, the high genetic correlation (0.75) between performances at different ages implies that selection at an early age may be effective in making genetic progress at all ages. Studies in Warmbloods have shown a high genetic correlation (0.84) between observations at station performance testing of stallions and competition results of their offspring. A genetic trend of 3.2% of a phenotypic standard deviation per year was reported in standardized accumulated transformed earnings in Norwegian trotters. The trade of live stallions and semen across countries allows greater access to superior breeding stock and is an important part of the economy for many countries. Several problems exist in the international trade of stallions and semen: the lack of a common database, the lack of precisely defined breeding goals, the various methods of performance testing and breeding value estimation. Interstallion was created in 1998 to address these issues by establishing an international database, harmonizing traits within riding disciplines and standardizing performance testing. A Universal Equine Life Number has been implemented to link international performance data. The standardization of performance testing, reporting of data and breeding values will greatly enhance the usefulness of genetic evaluations in the equine industry.

Keywords: Equine, Genetic Evaluation

### Introduction

Large-scale genetic evaluation, using Best Linear Unbiased Prediction (BLUP) methodology, is used by most livestock industries. The United States equine industry, unlike many European breed associations, has been slow to adopt genetic evaluation as a tool for animal selection. Research has shown that many economically important traits are moderately to highly heritable indicating that genetic progress can be made through selection. Genetic change due to selection is affected by selection intensity, genetic variation, accuracy of selection and the generation interval. Progeny testing can increase

accuracy but this can lengthen the generation interval which in horses is between eight and twelve years. The generation interval can be influenced, as far as genetic improvement, by how early in life a trait can be measured (Ström and Philipsson, 1978). Sampling young stallions and making use of test results for young progeny can increase accuracy without prolonging the generation interval. The high genetic correlation (0.75) between performances at different ages implies that selection on performance at an early age would be effective in making genetic progress at all ages (Huizinga and van der Meij, 1989). Bruns (1981) and Meinardus and Bruns (1988) found repeatabilities of 0.42 and 0.45 for show jumping and dressage respectively, suggesting that early records are a good indicator of later performance. In 1986 the Icelandic Toelter horse became the first breed to officially publish breeding values estimated using BLUP procedures (Sigurdsson et al., 1997). The objective of this paper is to review the use of genetic evaluation in the equine industry.

### Review of Literature

*Cutting.* In cutting contests, each horse is judged for two and a half minutes; during this time the rider guides the horse into a herd of cattle and pushes a small group of cows until one cow is left. The rider then drops the reins, allowing the horse to work independent of rider cues. Usually a horse and rider cut two to three cattle in the allotted time. Hintz (1980) found that cutting ability was lowly (0.04) heritable. Later studies estimated higher heritabilities of 0.19 (Ellersieck et al., 1985) and 0.12 (Willschau, 1994). Cutting success is somewhat heritable and thus genetic progress can be made when genetically superior animals are chosen as parents. Genetic evaluation is currently not used by any cutting horse associations.

*Trotting.* Trotting is a form of harness racing where a horse pulls a driver in a light, two-wheeled sulky. Trotters move with a diagonal gait, the left front and the right hind legs and the right front and left hind legs move in unison. Trotters cannot break into a canter or gallop during a race.

The following heritabilities have been estimated for best racing time in trotters: Minkema (1975) 0.36, Hintz (1980) 0.25 and Saastamoinen and Nylander (1996) 0.27. Árnason et al. (1982) found that the heritability of best time varied in three different populations and reported values of 0.36 in Dutch trotters, 0.12 in North-Swedish trotter and 0.18 in Russian trotters. Ojala and Van Vleck (1981) found simple correlations between best and average time for a year were greater than 0.90,

suggesting that there is a very strong relationship between the two traits. The authors cite similar correlations in the literature of 0.83 (Linner, 1975), 0.72 (Neisser, 1976) and 0.85 (Katona, 1979) and note that large correlations for best time with average time in a year imply that best time is a good measure of average speed in a year.

Two studies have estimated the heritability and repeatability of time at finish: Ojala and Van Vleck (1981) 0.30 ( $r = 0.70$ ) and Thuneberg-Selonen (1999) 0.23-0.28 ( $r = 0.50$ -0.57).

Minkema (1975) estimated a heritability of 0.26 for total earnings. Klemetsdal (1994) used Accumulated Transformed and Standardized Earnings (ATSE) as a measure of performance in Norwegian Trotters. ATSE was found to be lowly to moderately heritable (0.14 to 0.22 depending on age). Saastamoinen and Nylander (1996) found the heritability of earnings in Finnish Standardbred Trotters to be 0.33 when non-starters were included and 0.39 when non-starters were excluded. Thuneberg-Selonen (1999) found that earnings were lowly heritable (0.05-0.09). These findings suggest that best time may be a more appropriate measure of performance as it tends to be more heritable than earnings. Since 1992, breeding values estimated using BLUP animal model have been published for Swedish Trotters.

*Thoroughbred Racing.* Thoroughbred Racing is comprised of flat racing and jump racing. Flat racing is prevalent worldwide, while jump racing is popular in European countries with relatively few jump races run in the USA. Jump racing includes national hunt racing (hurdling), steeplechasing and point-to-point. The obstacles in national hunt racing are smaller and the distances are shorter than in steeplechasing. Point-to-point racing is steeplechasing for amateurs. Several studies have looked at the genetic component of performance in Thoroughbred racehorses using timeform rating, race time, handicap weight and earnings as measures of performance.

Timeform rating is the merit of a horse expressed in pounds, other things being equal, the horse with the highest timeform rating is the most likely to win (Timeform, 2004). Timeform rating is revised each season to maintain a comparable mean from year to year (Field and Cunningham, 1976). The heritability of timeform rating using regression of timeform rating on sire has been estimated as 0.74 (More O'Ferrall and Cunningham, 1974) and 0.76 (Gaffney and Cunningham, 1988). Gaffney and Cunningham (1988) estimated that the predicted rate of genetic change is 0.92 timeform units per year.

Cunningham (2002) reports the heritability of racing based on handicap weight to be 0.30. Handicap weight is the weight carried by horses in a race. Superior horses will generally carry more weight than poorer performing individuals.

Race time has also been used as a measure of performance in Thoroughbred racehorses. The heritability of race time was reported as 0.09 to 0.11, depending on race distance (Moritsu et al., 1994). Park

and Lee (1999) estimated a higher heritability of 0.27 to 0.30 for race time.

Watanabe (1974) used number of lengths each horse finished behind the first horse as a measure of performance and estimated a heritability of 0.64.

Langlois et al. (1996) estimated the heritability of log of yearly earnings to 0.28 in flat races and 0.25 in jump races. Foye et al. (1972) found a repeatability of 0.37 for earnings per start suggesting that an individual should not be culled based on one racing year.

Race time is an inferior method of measuring performance as non-runners are excluded and according to Cunningham's paradox best time seems to have reached a genetic plateau. Cunningham (1975) estimated that winning time showed a two percent improvement per decade up to about 1900 but has remained stagnant since then despite intense selection, this is known as Cunningham's paradox. Earnings are also a poor measure of performance as they are lowly heritable and do not account for the level of race. Tavernier (1991) and Langlois (1996) recommend using rank as it accounts for level of competition, number of horses etc.

*Quarter Horse Racing.* Few studies have examined the genetic component of racing in the American Quarter Horses (AQH). Buttram et al. (1988) estimated a heritability of approximately 0.37 for racing time in AQHs after adjusting for age and sex. Wilson et al. (1988), using heritabilities and variance components estimates from Buttram et al. (1988), found an average rate of genetic change of -0.0067 seconds per year in racing AQHs.

*Arabian Racing.* Several studies have estimated the heritability of earnings: Sobczyńska and Kownacki (1997) 0.22, Sobczyńska and Lukaszewicz (2002) 0.19 ( $r = 0.46$ ) and Belhajyahia et al. (2002) 0.09 ( $r = 0.25$ ).

Sobczyńska and Kownacki (1997) found rank at finish to be moderately (0.25) heritable. Later studies reported lower heritabilities: Sobczyńska and Lukaszewicz (2002) 0.18 ( $r = 0.45$ ) and Belhajyahia et al. (2002) 0.12 ( $r = 0.35$ ).

Earnings and rank have very similar heritabilities suggesting that either trait could be used as a measure of performance.

*Competition traits.* Competition traits include dressage, show jumping and cross-country jumping. Dressage is a competitive equestrian sport that uses classical training comprising of movements that have been developed over centuries. Show jumping is a sport where the horse and rider jump a set course of obstacles usually within a time period. In cross-country jumping horses and riders jump (at a gallop) a series of obstacles over varied terrain.

Dressage has been found to be moderately heritable: Meinardus and Bruns (1988) 0.16, Huizinga and van der Meij (1989) 0.10 and Koenen et al. (1995) 0.17. Wallin et al. (2003) estimated the heritability of cumulative placing in dressage as 0.16.

The heritability of jumping has been estimated as 0.18 (Meinardus and Bruns, 1988), 0.31 (Huizinga et al., 1991) and 0.19 (Koenen et al., 1995). Wallin et al. (2003)

found a heritability of 0.27 for cumulative placing in jumping.

Gerber Olsson et al. (2000) citing Árnason et al. (1997) noted that the genetic trend, based on breeding values estimated from a BLUP analysis, increased rapidly from the late 1980s to the late 1990s. Gerber Olsson et al. (2000) found an annual genetic progress for gaits and jumping of 0.05 and 0.03 genetic standard deviations per year respectively for Swedish Warmbloods born between 1988 and 1992.

Performance testing is carried out by many breed associations and can last up to 100 days; animals are trained and judged under uniform conditions that minimize the effects of rider and pre-training (Huizinga et al., 1991). Station performance testing of progeny allows for the estimation of breeding values on stallions three to four years earlier than from progeny competition results (Christmann, 1995). Station performance testing seems to be an appropriate measure as it is highly heritable. Ricard et al. (2000) estimated heritabilities of 0.40 to 0.60 for station performance testing observations. High genetic correlations between station performance testing and competition data also suggest that station observations are appropriate measures of performance. Ricard et al. (2000) found a genetic correlation of 0.70 to 0.90 for station performance testing observations and competition data. Wallin et al. (2003) found a genetic correlation between performance testing in the Swedish Riding Horse Quality Test and in show jumping and dressage to be 0.83 to 0.93 and 0.63 to 0.75, respectively. These high correlations suggest that there is a strong positive relationship between station performance testing observations and later competition results.

Interstallion was formed by the European Association for Animal Production, the World Breeding Federation of Sport Horses and the International Committee for Animal Recording in 1998 (Koenen and Aldridge, 2002). The aims of Interstallion are to describe breeding values, testing procedures and genetic evaluation methods of Warmblood breeding organizations so that genetic evaluations can be compared across countries. A major difficulty in this has been the identification of horses across breeds and countries. A Universal Equine Life Number has been implemented which can be used alongside the studbook of birth number.

*Longevity.* Numerous studies have examined factors that affect career longevity or length of productive live in equine athletes. These factors include conformation, training, environmental conditions, type of competition, age and sex.

Wallin et al. (2001) found that orthopedic scores had the greatest influence on longevity. Horses that scored a nine or ten were half as likely to be culled as those that scored a six or below. Poor conformation predisposes a horse to injury. Rossdale et al. (1985) found that lameness is the most significant cause of loss in young racehorses. Wallin et al. (2000) cite several studies that have found that musculoskeletal injury is the major cause of culling in performance horses (Clausen et al., 1990; Heisle, 1995 and Hommerich, 1995).

Training has been found to have a significant impact on the risk of injury and thus career longevity. Verheyen and Wood (2001) note that 80% of all fractures in flat racehorses occurred during training. Proudman et al. (2004) state that training has a significant effect on the risk of falls and the likelihood that a horse would complete the race.

Several studies have examined the relationship between track condition, risk of injury and performance. Tracks with some moisture seem to reduce the risk of injury while dry tracks and very wet tracks seem to increase the risk of injury. Rooney (1983) states that trace amounts of rainfall protects against lameness. Bailey et al. (1997a) and Bailey et al. (1997b) found that tracks with lower water content were associated with a greater risk of injury. Proudman et al. (2004) notes that good-to-soft ground resulted in significantly fewer falls and increased the likelihood that a horse would complete the race in comparison to soft ground.

The rates of fatal musculoskeletal injuries reported in the USA have been considerably higher than in Europe, which may be a result of differences in track surface. Racing in the USA is predominantly on dirt tracks whereas most races occur on turf in Europe. Mohammed et al. (1991) observed that horses racing on dirt tracks have a higher risk of serious musculoskeletal injury compared with horses racing on turf. Buttram et al. (1988) found that racetrack alone accounted for between 11% and 32% of the variation depending on race distance.

Type of competition has been evaluated as a potential risk factor associated with longevity of career. Horses in hurdle races are four times more likely to suffer a musculoskeletal breakdown, while horses in steeplechases are eight times more likely to suffer an injury compared with horses racing on the flat (Bailey et al., 1998). The presence of barriers likely explains this; Bourke (1995) found that the majority of jumping fatalities were associated with a fall.

Age also has a significant impact on career longevity. High levels of loss occur in the first or second racing seasons (Mason and Bourke, 1973; Mohammed et al. 1991; Bourke, 1995). Several studies have found that the risk of injury increases with age (Bailey 1997; Robinson et al., 1988; Mohammed et al., 1991) but starting to compete at an older age has a negative impact on career duration (Bourke, 1995; Ricard and Fournet-Hanocq, 1997).

Wallin et al. (2001) notes that males were twice as likely to be culled as females when studying longevity in Swedish Warmbloods. Bailey et al. (1999) found that females were less likely to race and Bourke (1995) found that males raced on average one season longer than females. This may be because females tend to win less money (Minkema, 1975) and males are faster than females (Leroy et al., 1989; Thuneberg-Selonen et al., 1999 and Röhe et al., 2001).

## Implications

Many important traits are moderately to highly heritable and thus much genetic progress can be made through selection. The Danish Warmblood, Irish Sport

Horse, Dutch Warmblood, French Sport Horse, Swedish Warmblood, Holstein, Hanoverian, Icelandic Toelter, German Trotter and Swedish Trotter Associations all use breeding values estimated using BLUP techniques and have shown an increase in genetic progress since its implication.

## Literature Cited

- Árnason, Th., Darenius, A. and Philipsson, J. 1982. Genetic selection indices for Swedish Trotter broodmares. *Livest. Prod. Sci.* 8:557-565.
- Árnason, Th. 1997. The selection intensity in Standardbred Trotters in Sweden as measured by BLUP animal model index. Page 380 in *Proc. Eur. Assoc. Anim. Prod.*, 48<sup>th</sup> Annual Meeting, Vienna.
- Bailey, C. J., Rose, R. J., Reid, S.W. and Hodgson, D. R. 1997a. Wastage in the Australian Thoroughbred racing industry: a survey of Sydney trainers. *Aust. Vet. J.* 75(1):64-66. *Aust. Vet. J.* 75(1):64-66.
- Bailey, C. J., Reid, S. W., Hodgson, D. R., Bourke, J. M. and Rose, R. J. 1997b. A retrospective case-control study of musculoskeletal racing injuries in Australian Thoroughbreds. *Epidémiol. Santé Anim.* Pages 31-32.
- Bailey, C. J., Reid, S.W., Hodgson, D. R., Bourke, J. M. and Rose, R. J. 1998. Flat, hurdle and steeple racing: risk factors for musculoskeletal injury. *Equine Vet. J.* 30(6):498-503.
- Bailey, C. J., Reid, S.W., Hodgson, D. R. and Rose, R. J. 1999. Factors associated with time until first race and career duration for Thoroughbred racehorses. *AJVR.* 60(10):1196-1200.
- Belhajyahia, T., Blouin, C. and Langlois, B. 2002. Breeding evaluation of Arab horses from their racing results in Tunisia by a BLUP animal model. Page 253 in *Proc. 53<sup>rd</sup> Annual Meeting of Eur. Assoc. Anim. Prod.*, Cairo.
- Bourke, J.M. 1995. Wastage in Thoroughbreds. Pages 107-119 in *Proc. Animal Seminar, Equine Branch, New Zealand Veterinary Association. Foundation for Continuing Education*, no.167.
- Bruns, E. 1981. Estimation of the breeding values of stallions from the tournament performance of their offspring. *Livest. Prod. Sci.* 8:465-473.
- Buttram, S. T., Willham, R. L. and Wilson, D. E. 1988. Genetics of racing performance in the American Quarter Horse: II. Adjustment factors and contemporary groups. *J. Anim. Sci.* 66:2800-2807.
- Christmann, L., Bruns, E. and Schade, W. 1995. Survey on the mare performance test in the Hanoverian breed. Page 3551 in *Proc. Eur. Assoc. Anim. Prod.*, 46<sup>th</sup> Annual Meeting, Prague.
- Cunningham, E. P. 1975. In *Proc. Int. Sym. On genetics and horse breeding. Royal Dublin Soc.*, Ireland.
- Cunningham, E. P. 2002. The horse genome – past, presence and future. Available: <http://www.usyd.edu.au/su/rirdc/articles/genetics/cunningham.htm>. Accessed Mar. 29, 2004.
- Ellersieck, M. R., Lock, W.E., Vogt, D. W. and Aipperspach, R. 1985. Genetic Evaluation of cutting scores in horses. *Equine Vet. Sci.* 5(5):287-289.
- Field, J.K. and Cunningham, E. P. 1976. A further study of the inheritance of racing performance in Thoroughbred horses. *J. of Hered.* 67:247-248.
- Foye, D. B., Dickey, H. C and Sniffen, C. J. 1972. Heritability of racing performance in the Thoroughbred horse. *J. Anim. Sci.* 35(6):1141-1145.
- Gaffney, B. and Cunningham, E. P. 1988. Estimation of genetic trends in racing performance of Thoroughbred horses. *Nature.* 332:722-724.
- Gerber Olsson, E., Árnason, Th., Näsholm, A. and Philipsson, J. 2000. Genetic parameters for traits at performance test of stallions and correlations with traits at progeny tests in Swedish Warmblood horses. *Livest. Prod. Sci.* 65:81-89.
- Hintz, R. L. 1980. Genetics of performance in the horse. *J. Anim. Sci.* 51(3):582-594.
- Huizinga, H. A. and van der Meij, G. J. 1989. Estimated parameters of performance in jumping and dressage competition of the Dutch Warmblood horse. *Livest. Prod. Sci.* 21:333-345.
- Huizinga, H. A., van der Werf, J. H., Krover, S. and van der Meij, G. J. 1991. Stationary performance testing of stallions from the Dutch Warmblood riding horse population. 1. Estimated genetic parameters of scored traits and the genetic relation with dressage and jumping competition form offspring of breeding stallions. *Livest. Prod. Sci.* 27:231-244.
- Klemetsdal, G. 1994. Application of standardized, accumulated transformed earnings in breeding of Norwegian Trotters. *Livest. Prod. Sci.* 38:245-253.
- Koenen, E. P., van Veldhuizen, A. E. and Brascamp, E. W. 1995. Genetic parameters of linear scored conformation traits and their relation to dressage and show-jumping performance in the Dutch Warmblood riding horse population.
- Koenen, E .P and Aldridge, L.I. 2002. Testing and genetic evaluation of sport horses in an international perspective. Pages 1-5 in *Proc. 7<sup>th</sup> World Congress Applied to Livest. Prod.*, Montpellier.
- Langlois, B., Blouin, C. and Tavernier, A. 1996. Nouveaux resultants d'estimation de l'héritabilité des gains en course des pur-sang en France. *Genet. Sel. Evol.* 28:275-283.
- Leroy, P. L., Kafidi, N. and Bassleer, E. 1989. Estimation of breeding values of Belgian Trotters using an animal model. Pages 3-17. *Eur. Assoc. Anim. Prod. Publ. No. 42.* London, England.
- Mason, T. A. and Bourke, J.M. 1973. Closure of the distal radial epiphysis and its relationship to unsoundness in two year old Thoroughbreds. *Australian Vet. J.* 49:221-228.
- Meinardus, H. and Bruns, E. 1988. Use of riding event records for selection of riding horses 1<sup>st</sup> communication: Selection criteria and genetic parameters. Eingegangen am 30.6.98.
- Minkema, D. 1975. Studies on the genetics of trotting performance in Dutch Trotters. *Ann. Génét. Sél. Anim.* 7(1):99-121.

- Mohammed, H.O., Hill, T. and Lowe, J. 1991. Risk factors associated with injuries in Thoroughbred horses. *Equine Vet. J.* 23(6):445-448.
- More O'Ferrall, G. J. and Cunningham, E. P. 1974. Heritability of racing performance in Thoroughbred horses. *Livest. Prod. Sci.* 1:87-97.
- Moritsu, Y., Funakoshi, H. and Ichikawa, S. 1994. Genetic evaluation of sires and environmental factors influencing best racing times of Thoroughbred horses in Japan. *J. Equine Sci.* 5(2):53-58.
- Ojala, M.J. and Van Vleck, L. D. 1981. Measures of racetrack performance with regard to breeding evaluation of Trotters. *J. Anim. Sci.* 53(3):611-619.
- Park, K. D. and Lee, K. J. 1999. Genetic evaluation of Thoroughbred racehorses in Korea. *Kor. J Anim Sci.* 41(2):135-140.
- Proudman, C., Pinchbeck, G., Clegg, P. and French, N. 2004. Risk of horses falling in the Grand National. *Nature.* 428:385-386.
- Ricard, A. and Fournet-Hanocq, F. 1997. Analysis of factors affecting length of competitive life of jumping horses. *Genet. Sel. Evol.* 29: 251-267.
- Ricard, A., Bruns, E., and Cunningham, E. P. 2000. Page 411-438 in *The Genetics of the Horse*. A., T. Bowling and A., Ruvinsky,. CAB International, Oxon, UK.
- Robinson, R. A. and Gordon, B. 1988. American Association of Equine Practitioners track breakdown studies – horse results. Pages 385 –394 in *Proc. of the 7<sup>th</sup> International Conference of Racing Analysts and Veterinarians*, Kentucky.
- Röhe, R., Savas, T., Brka, M., Willms, F. and Kalm, E. 2001. Multiple-trait genetic analyses of racing performance of German Trotters with distanglement of genetic and driver effects. *Arch. Tierz, Dummerstorf.* 44(6):579-587.
- Rooney, J. R. 1983. Track condition in relationship to fatigue and lameness in Thoroughbred racehorses. *Equine Vet. Data.* May 10:134-135.
- Rossdale, P. D., Hopes, R., Wingfield Digby, N. J. and Offord, K. 1985. Epidemiological study of wastage among racehorses 1982 and 1983. *Vet. Rec.* 116:66-69.
- Saastamionen, M. T. and Nylander, A. 1996. Genetic and phenotypic parameters for age at starting to race and racing performance during early career in Trotters. *Livest. Prod. Sci.* 45:63-68.
- Sigurdsson, A., Hugason, K. and Árnason, Th. 1997. Breeding strategies and genetic progress in the Iceland toelter population. Page 377 in *Proc. 48<sup>th</sup> Annual Meeting of Eur. Assoc. Anim. Prod.*, Vienna.
- Sobczyńska, M. and Kownacki, M. 1997. Genetic aspects of racing performance in Polish pure bred Arab horses. II. Genetic trends. *J. Appl. Genet.* 38(3):303-308.
- Sobczyńska, M. and Lukawzewicz, M. 2002. Estimation of genetic parameters for racing performance in Polish Arab horses. Page 254 in *Proc. 53<sup>rd</sup> Annual Meeting, Eur. Assoc. Anim. Prod.*, Cairo.
- Ström, H. and Philipsson, J. 1978. Relative importance of performance tests and progeny tests in horse breeding. *Livest. Prod. Sci.* 5:303-312.
- Tavernier, A. 1991. Genetic evaluation of horses based on ranks in competitions. *Genet. Sel. Evol.* 23:159-173.
- Thuneberg-Selonen, T., Pösö, J., Mäntysaari, E. and Ojala, M. 1999. Use of individual race results in the estimation of genetic parameters of trotting performance for Finnhorse and Standardbred trotters. *Agricultural and Food Science in Finland.* 8:353-363.
- Timeform. 2004. How Timeform handicaps horses. Available: [http://www.timeform.com/show\\_article.asp?num=166](http://www.timeform.com/show_article.asp?num=166) Accessed Mar. 29, 2004.
- Verheyen, K. and Wood, J. 2001. Survey of racecourse injuries and fatalities. Available: <http://www.aht.org.uk>. Accessed Mar. 29, 2004.
- Watanabe, Y. 1974. Performance rates of Thoroughbreds as criterion of racing ability. *Jap. J. Zootech. Sci.* 45(7):408-411.
- Wallin, L., Strandberg, E., Philipsson, J. and Dalin, G. 2000. Estimates of longevity and causes of culling and death in Swedish Warmblood and Coldblood horses. *Livest. Prod. Sci.* 63:275-289.
- Wallin, L., Strandberg, E. and Philipsson, J. 2001. Phenotypic relationship between test results of Swedish Warmblood horses as 4-year-olds and longevity. *Livest. Prod. Sci.* 68:97-105.
- Wallin, L., Strandberg, E. and Philipsson, J. 2003. Genetic correlations between field test results of Swedish Warmblood riding horses as 4-year-olds and lifetime performance results in dressage and showjumping. *Livest. Prod. Sci.* 82:61-71.
- Willschau, C. L. 1994. Parameter estimation and genetic prediction of cutting performance in horses. Master thesis, Colorado State University, Fort Collins, CO.
- Wilson, D. E., Willham, R. L., Buttram, S. T., Hoekstra, J. A. and Luecke, G.r. 1988. Genetics of racing performance in the American Quarter Horse: IV. Evaluation using a reduced animal model with repeated records. *J. Anim. Sci* 66:2817-2825.

## **SAS<sup>®</sup> Tools to Facilitate QTL Discovery<sup>1</sup>**

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**ABSTRACT:** The objective was to develop data management tools using SAS<sup>®</sup> that facilitate searching for quantitative trait loci (QTL). Genotypes were surveyed for six F<sub>1</sub> bulls and 159 markers selected after being found informative in at least four bulls and providing an inter-marker interval less than 20 cM. Genotypes (N=162,816) generated using PCR<sup>®</sup> and Li-COR GeneReader 4200<sup>®</sup> are scored by two people. Seven SAS<sup>®</sup> applications were developed: A1) marker\_in, A2) animal\_in, A3) plate\_in, A4) genotype\_in, A5) compare, A6) crimap\_in, and A7) simplify. A1-A4 generate a series of data files. The file from A1 is keyed by marker name and contains its chromosome number and map position. Files from A2-A4 are keyed by animal number. The file from A2 contains sire, dam, and sex of each animal. The file from A3 contains the microtiter plate number and cell containing DNA from each animal and the electrophoresis gel lane assigned to that animal. A4 facilitates entry of genotypic scores generating separate files for each scorer; 1XYZgen or 2XYZgen (XYZ is marker name). A5 compares the files from A4 and produces four new files. The “zero” file identifies animals with genotypes not scored by either scorer. The “discrepancy” file identifies animals with genotypes not agreed upon by both scorers. The “zero” and “discrepancy” files also contain the microtiter plate location for that animal’s DNA. The “good” file identifies animals and their genotype when both scorers agreed. The “good-zero” file merges data from the “good” and “zero” files. A6 assembles data for a chromosome from the “good-zero” files and creates an input file for CRI-MAP. A7 simplifies CRI-MAP output for resolving non-inheritances using animal ID and marker to generate a file containing animal ID, related marker genotype, sire and dam ID and genotypes. WINDOW, DISPLAY, and TRUNCOVER statements and macro facility, CALL SYMPUT were used in several of these applications. Applications of SAS<sup>®</sup> described here save labor and improve data integrity in conducting whole-genome searches for QTL.

**Key Words:** Computer Programming, Genome Analysis, Information Systems

## **Introduction**

Advances in computer technology and genetic research go hand in hand. Over the last 50 years our knowledge has greatly increased beyond the basic structure of DNA and proteins to sequencing genes and complete genomes. These advances can be greatly tied to advances of various technologies. Sophisticated machines have tremendous power and speed that allow integration of DNA sequence information. Sequencing DNA used to take several years over a decade ago, but can now be accomplished in a matter of days or weeks.

Genetic linkage maps have been developed for several species, with the intention to aid the identification of chromosomal regions that may influence traits of economic importance. The information gained will lead to improved genetic selection practices by identifying animals with superior genotypes for specific traits and aid in identifying genes contributing to phenotypic variation.

Computers are integral to the research and development of these studies. Large amounts of data are generated and computer software applications are essential for accurate, efficient, and timely completion of the research effort. Our objective was to develop data management tools using SAS<sup>®</sup> (SAS Institute, Inc., Cary NC) that facilitate searching, management, evaluation, analysis, and distribution of genotypic data.

## **Materials and Methods**

A herd of F<sub>1</sub> Wagyu X Limousin cattle was developed. Genotypes for six F<sub>1</sub> bulls were surveyed for 240 initial markers. A marker was deemed informative if four of the six bulls were heterozygous for that marker. A marker spacing of approximately 20 cM was also desired. Based on these criteria, a suite of 159 markers were chosen for use in a whole-genome search for QTL.

Blood was collected from each dam and resulting offspring for a period of four years (n=512) with DNA later extracted. Genotypes for each animal were generated using PCR<sup>®</sup> and Li-COR GeneReader 4200<sup>®</sup> and were scored by two people. The sheer number of initial genotypes (n=81,408), redundant scoring and entry, and additional redone genotypes created a need for convenient and easy data entry and management procedures so data could be subsequently processed. Thus, a series of seven SAS<sup>®</sup> applications were developed; A1) marker\_in, A2) animal\_in, A3) plate\_in, A4) genotype\_in, A5) compare, A6) crimap\_in, and A7) simplify, for data entry and storage.

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<sup>1</sup> Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, the Montana Agric. Exp. Sta. or the authors, and does not imply its approval to the exclusion of other products that may also be suitable.

The A1 application created a file keyed by marker name and also storing, chromosome number, centamorgan position (cM) for a pre-existing genome map, and source of each marker. This application (and most applications described subsequently) makes use of A WINDOW statement that creates customized windows to display text and accept input.

#### *window entry*

```
#3 @5 'Marker Name'    @20 mn $10. c=blue a=rev_video
#5 @5 'Chromosome No.' @20 cn $2. c=blue a=rev_video
#7 @5 'Position, cM'    @20 ps $6. c=blue a=rev_video
#9 @5 'Source'          @20 sr $1. c=blue a=rev_video
#13 @5 'end?'          @20 stopkey $1. c=red a=rev_video;
display entry;
```

The window definition is only effective for the DATA step containing the WINDOW statement. Many arguments and field definitions can be used within a WINDOW statement. A color for the variable string is specified, by default the color is white, but options are available to define and make the strings easier to see (c=blue). In conjunction with the choice of color we utilize an attribute called rev\_video (a=rev\_video), which displays the field in reverse video. Following the WINDOW statement the screen is defined with row designations preceded by “#” and column designations preceded by “@”. Internally the program uses shorthand variable names (mn, cn, ps, sr, and stopkey).

The DISPLAY statement displays the window created in the WINDOW statement. When the window is displayed it contains fields identified by text prompts (Marker name; Chromosome No.; Position, cM; Source; and end?) where values can be entered. The DISPLAY statement is executed within a DO WHILE loop conditioned on the value of stopkey. In this scenario, data entry continues while the stopkey equals the default value “n” until “y” is entered.

```
do while (stopkey ne 'y');
  display entry;
.
.
.
end;
```

The applications A2 and A3 are similar in structure to A1. Both A2 and A3 produce files keyed by animal identification number. Sire and dam identification numbers and sex are input using A2 and stored in a file (animal.dat). The address coordinates of each animal's DNA in a specified microtiter plate and electrophoresis gel lane are input using A3 and stored in a file (plates.dat).

The A4 application facilitates redundant entry of genotypes. The resulting data files are named dynamically based on the marker name (mn) and scorer identity (fn, 1=original and 2=verifier),

```
f_name = fn||trim(mn)||'gen';
file genes filevar=f_name mod;
```

which are input. The TRIM function removes trailing blanks from its argument as the concatenation operator (||) does not remove trailing blanks. The file f\_name was then written to a temporary location in which a FILEVAR= was used to call it back. The FILEVAR= option was needed in order to read from one file, then close it and open another, the MOD function again allows output lines to be written following existing data already in the output file. The number of the plate being scored is also input to allow retrieval of animal identification numbers from the file produced by A3. Like the previous applications, a data entry window is defined and displayed in a DO WHILE loop.

The application A5 is used to compare the two files of genotypes for a marker produced by A4. A5 produces four new files. The intent is to find differences in genotype scoring between the two scorers and place them in new files for resolution and/or evaluation. A “discrepancy” file contains genotypes that are not agreed upon by both scorers which could result in one score being a base pair different or one scorer did not score the genotype while the other did. Matching “zero” (i.e. unscored) genotypes agreed upon by both scorers are placed in another file. A “good” genotype file contains genotypes agreed upon by both scorers. A fourth file merges the good and zero genotypes and is used by A6.

In A5 a WINDOW/DISPLAY statement pair queries the user for the marker name and five file names are dynamically created as in A4. An explicit OUTPUT statement tells SAS® to write the current observation to a data set immediately and rather than at the end of the data step. Subsequently, repeated invocation of the SYMPUT routine creates macro variables whose values are information from the DATA step. SYMPUT is a DATA step interface, and part of the SAS® macro facility. SAS® macro facilities are tools that extend and customize the SAS® system and reduce the amount of text that's to be entered in order to do common tasks.

#### *window entry*

```
#3 @5 'Marker Name'    @20 mn $10.
display entry;
fname1 = "1"||trim(mn)||"gen.*";
fname2 = "2"||trim(mn)||"gen.*";
fname3 = "3"||trim(mn)||"dsp";
fname4 = "4"||trim(mn)||"zer";
fname5 = "5"||trim(mn)||"good";
fname6 = "6"||trim(mn)||"zerogood";
output;
call symput ("fname1", trim(fname1));
call symput ("fname2", trim(fname2));
call symput ("fname3", trim(fname3));
call symput ("fname4", trim(fname4));
call symput ("fname5", trim(fname5));
call symput ("fname6", trim(fname6));
```

A FILENAME statement before a new DATA step temporarily associates a valid SAS® name with an external file or an output device. Once a file reference name is associated with an external file, it can be referenced shorthand for that file in other SAS®

statements such as FILE or INFILE that access external files. The association of file reference names and external files only lasts for the duration of the SAS<sup>®</sup> session or until it is changed or ended with another FILENAME statement.

***filename fname1 "C:\My SAS Files\&fname1";***

Within the ensuing DATA step, an INFILE statement references the fname1 file. The TRUNCOVER option causes data to be read only from a single record rather than continuing to read data from the next record if more variables are specified in the INPUT statement than there are data values in the record.

***infile fname1 truncover;***

The final step in A5 is to merge the two files produced by A4 and the file from A3, keyed by animal identification number. A series of IF-THEN statements compare the genotype scores and determine the file to assign each record. Based on the output of A5, discrepancies for all markers on a particular chromosome have to be resolved before moving forward.

Application A6 is very similar in design to A5 especially with regard to WINDOW/DISPLAY statements and the dynamic identification of data files. It compiles the required information to create a file named chr(#)gen with “chr” signifying chromosome for input into CRIMAP (Green et al., 1990). In our research, CRIMAP (Green et al., 1990) is used for detecting inconsistencies (non-inheritances) in the data errors and construction of multi-locus linkage maps.

CRIMAP (Green et al., 1990) builds several output files. The temp file among many things provides a list of non-inheritances for animals organized by marker number specific to the chromosome. Application A7 simplifies the results from the CRIMAP (Green et al., 1990) “prepare” option using animal ID and marker to generate a new file with extension “temp-simp” that contains animal ID, the related marker genotypic scores along with their sire and dam ID’s and related genotypic scores. To illustrate the concepts employed in developing this suite of software tools the SAS<sup>®</sup> code for application A7 is shown in Figure 1.

## **Discussion**

A WINDOW and DISPLAY statement was used in every application to facilitate the user interface. This software development effort has been process oriented and modular in structure. The applications have become more complex as the project has progressed from data accumulation and entry to verification and ultimately to mapping. Following on database design principles (Martin, 1985); redundancy across data files has been minimized.

SAS is used as a platform for managing the data owing to its power, implementation across numerous operating systems and computing platforms, and

widespread use in the animal science research community. Certain SAS arguments proved instrumental to these efforts. For instance, SAS macro facilities (CALL SYMPUT) allowed us to customize programming language in order to reduce the amount of text entered which attributed to faster data turnover. We were able to package large amounts of text into specific units with names, i.e. the marker name. So from that point onward we could work with names rather than the paths identifying the files themselves.

Additionally, TRUNCOVER was influential in apportioning out values to correct places. When the INPUT statement encountered a shortened line the TRUNCOVER option takes what is left and assigns it to an appropriate value.

Every application used in this analysis built upon the previous one. Files generated by various applications were used again and again in other applications.

## **Implications**

Computing is an integral part of genetic research and SAS<sup>®</sup> as an integrated application system helps to optimize research components.

## **Literature Cited**

- Davis, G.P. and S.K. DeNise. 1998. The impact of genetic markers on selection. J. Anim. Sci. 76:2331-2339.
- Green, P., K. Falls, and S. Crook. 1990. Documentation for CRIMAP. <http://biobase.dk/Embnetut/Crimap> Accessed March 8, 2000.
- Martin, J. 1985. System Design from Provably Correct Constructs. Prentice-Hall Inc., Englewood Cliffs, NJ.

Figure 1. SAS programming statements for application to simplify output from CRIMAP (Green et al., 1990) and aid in resolution of discrepancies among marker genotypes. Note, while the actual implementation considers many more markers the illustration shown here only uses two markers.

---

```

data temp;

window initial
  #3 @5 'Crompic File' @20 cn $2. c=blue a=rev_video;

display initial;

cname1 = "0"||trim(cn)||"temp-A";
cname1 = "0"||trim(cn)||"temp-simp";

file 'c:\scratch6'; put cn 1-3;

filename cname1 'c:\crimap\&cname1';
filename cname13 'c:\simple\&cname13';

data two;

window entry
  #5 @5 '1ST marker name'
    @28 mn1 $9. c=blue a=rev_video
  #7 @5 '2ND marker name'
    @28 mn2 $9. c=blue a=rev_video
  #9 @5 'end?' @20 stopkey $1. c=red a=rev_video;

do while (stopkey ne 'y');
display entry;

  fname1 = "1"||trim(mn1)||"gen.*";
  fname2 = "2"||trim(mn2)||"gen.*";
output;
  call symput ("fname1", trim(fname1));
  call symput ("fname2", trim(fname2));
end;

file 'c:\scratch7'; put mn1 mn2;

filename cname1 'c:\crimap\&cname1';

data file;
infile cname1 truncover;
input non_ih $ fam $ WL $ indiv $ anid locus $ number;

proc sort nodup; by number anid;

data file2; set file;
put number anid;

filename fname1 'c:\crimap\&fname1';
filename fname2 'c:\crimap\&fname2';

data m1; infile fname1 truncover;
input anid 1-6 a1 8-10 a2 12-15;
proc sort; by anid;

data m2; infile fname2 truncover;
input anid 1-6 a3 8-10 a4 12-15;

```

```

proc sort; by anid;

data plates;
infile plates.dat;
input pn 1 pp 3-4 pc $ 6-7 anid 9-14;

proc sort; by anid;

data animals;
infile animal.dat;
input anid sire dam sex;
proc sort; by anid;

data check;
merge m1 m2 plates animals; by anid;
file fname13;
if number = . then delete;
if number = 0 then put "Marker is;" mn1;
put number 1-3 anid 5-10 sire 12-17 dam 19-24 a1 30-32
    a2 34-35 pn 38-40 pp 42-44 pc 46-48;
if number = 1 then put "Marker is;" mn2;
put number 1-3 anid 5-10 sire 12-17 dam 19-24 a3 30-32
    a4 34-35 pn 38-40 pp 42-44 pc 46-48;

run;

```

## EFFECT OF USING LINEAR MEASUREMENTS AND WEIGHT TO PREDICT FUTURE PRODUCTION OF HEIFER CALVES

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**Abstract:** Numerous linear measurements have been taken over the years and used in various forms and combinations to make predictions in beef cattle production. The object of this study was to evaluate the accuracy, importance, and effect linear measurements taken at 8-, 12-, and 18-mo have on predicting future production of heifers. Data were collected on linebred and crossbred heifers (n = 240) raised at the Northern Agricultural Research Center. Measurements (n = 17) were taken on heifer calves for 2 yr. Measurements included in stepwise regression and principal component (PC) analyses were heart girth circumference, flank girth circumference, rump length, topline length, 2/3 topline length, rump width, shoulder width, wither height, hip height, thurl depth, head width, head length, weight at measurement, cannon bone length, pelvic height, pelvic width, and pelvic area. Principal components were calculated for each age group separately. Production traits to be predicted were puberty date (PD), first calf birth weight (BW), calving difficulty at first parturition (CD), weaning weight of first calf (WW), and the female's weight at 2- (CW2) and 3-yr (CW3) of age. The first PC accounted for 50-56% of the variation in body measurements and described the heifer's size. The first PC was strongly related to CW2 and CW3, but explained little of the variation in PD, BW, CD, and WW. The second PC accounted for an additional 8-15% of variation in body measurements, described as shape of the heifer and was not an important predictor of her future performance. In a stepwise regression analysis, individual body measurements accounted for a small amount of variation in future performance ( $R^2 < .25$ ).

**Key Words:** Linear measurements, Prediction, Beef Cattle

### Introduction

Body measurements involving linear measurements and weights have been promoted as a means for producers to select cattle for fertility and producing ability. Previous estimates of repeatability of linear measurements taken postweaning on bull and heifer calves were reported moderate to high by Doornbos et al. (1984) and Doornbos et al. (1986). Pelvic and other physical measurements have been reported as poor predictors of calving difficulty without including birth weight (i.e., Bellows et al., 1971; Johnson et al., 1988; Basarab et al., 1993). Brown et al. (1973) concluded from a principal component analysis that selection on the basis of size at young ages may yield bulls which differ in

shape at older ages. Johnson et al. (2000) indicated that body measurements taken at birth would be useful in predicting mature weight and maturing rate of Angus and Charolais cows. Looper et al. (2002) reported on traits that are indicators of growth and body composition in replacement beef heifers and subsequent calving rates for those heifers, but limited information is available on the use of body measurements taken on heifers and predicting future production potential. The object of this study was to evaluate the accuracy, importance, and effect of body measurements taken at 8-, 12-, and 18-mo of age have on predicting future performance traits and size of beef heifers.

### Materials and Methods

Body measurements (n = 17) were taken on straightbred Hereford and crossbred heifers sired by Charolais or Tarentaise sires (n = 240) over a 2-yr period at the Northern Agricultural Research Center, Havre, Montana. Measurements used in this study were taken by the same technician as part of a repeatability study for accuracy within technician and between technicians (Doornbos et al., 1984; Doornbos et al. 1986). Heifer calves were weaned October 1 at approximately 180-d of age and after a short grazing and warm up period were placed in a dry lot and fed to gain 0.68 kg/d for 140 days. Body measurements were taken at postweaning (8-mo of age), prebreeding (12- to 13-mo of age), and in the fall before winter feeding (18-mo of age). Puberty date (PD) was recorded on all heifers plus pregnant heifers (n = 155) were retained and calved with birth weight (BW), calving difficulty (CD), and weaning weight (WW) recorded on their first calf plus their own weight as 2- (CW2) and 3-yr-old (CW3) was recorded.

Measurements taken on heifers included heart girth circumference (HGC), flank girth circumference (FGC), rump length (RL), topline length (TL), 2/3 topline length (2/3T), rump width (RW), shoulder width (SW), wither height (WH), hip height (HH), thurl depth (TD), head width (HW), head length (HL), weight at measurement (WT), cannon bone length, (CBL), and taken at 12- and 18-mo were pelvic height (PH), pelvic width (PW), and pelvic area (PA) which was the product of PH and PW. Doornbos et al. (1984) gives a complete description of the measurements.

Statistical analyses were conducted using the principal component and stepwise regression procedures of SAS (SAS Inst. Inc., Cary, NC). Principal components (PC) from the residuals of the 17 body measurements

adjusted for age at measurement and breed group (BG) (Hereford or crossbred) were calculated for each age at measurement class separately. Stepwise regression analysis with BG forced into the equation was used to identify variables with the highest relationship to PD, BW, CD, WW, CW2, and CW3.

## Results and Discussion

Means and standard deviations for traits measured in the three age groups are in Table 1. Significant differences in measured traits were found between BG with crossbred heifers having larger ( $P<0.05$ ) measurements than straightbred heifers except for 18-mo HW.

Results of principal component analysis appear in Table 2. The first principal component was very similar in all three age group data sets. The first principal component accounted for greater than 49% of total variation. It provided a means of contrasting animals according to overall size and weight because all coefficients were positive. Animals with large positive values for PC1 would tend to be above average for all traits, the reverse being true for individuals with large negative values. The remaining PC allow contrasts of different shapes. Principal component 2 accounted for 8.7-14.6% of variation while PC3 accounted for 6.5-7.7% of variation. Total variation explained by the first 3 PC was 66.5-73.1%. Principal component 2 of traits measured at 8-mo was mainly a contrast of heifers wide at hip and shoulders and wide heads with those that were average weight and shorter at hips and withers. Principal component 2 of traits measured at 12-mo was mainly a contrast of heifers wide at shoulders and hips and big heads with those that were average weight, shorter length, and small pelvic area. Principal component 2 of traits measured at 18-mo was mainly a contrast of tall heifers with a large pelvic area with those that were slightly below average weight, narrow girth, narrow width at shoulder and hips, and a small head. Principal component 3 was more variable among the three sets of data, but this component provided a contrast of shapes, as did CP2.

Coefficients of multiple determination ( $R^2$ ) for BW, CD, WW, PD, CW2, and CW3 for the three age groups measured are presented in Table 3. In all equations, BG was forced into the stepwise regression first with all dependent variables freely to enter at  $P<1$ . Most of the variation explained in the equations for WW and PD was due to BG which did account for a significant size difference in all measurements between the two groups. The amount of variation explained in future production traits (BW, CD, and WW) from measurements taken at 8-mo was small, usually 3.1 to 5.9%. As the heifers became older, the accuracy increased for most traits measured. The highest coefficients of determination were for CW2 and CW3 from measurements taken at 12- and 18-mo of age with WT being the greatest influence. This would be expected due to the large positive

correlation of 12- and 18-mo weight to mature weight (Brinks et al., 1964).

## Implications

Body measurements taken at 8-, 12-, and 18-mo of age are poor predictors of the heifer's future production. The largest amount of variation explained in these measurements is due to size and weight of heifers with a smaller amount of variation due to shape. Shape of heifer was not an important predictor of future performance and individual body measurements accounted for a small amount of variation.

## Literature Cited

- Basarab, J. A., L. M. Rutter, and P. A. Day. 1993. The efficacy of predicting dystocia in yearling beef heifers: II. Using discriminant analysis. *J. Anim. Sci.* 71:1372.
- Bellows, R. A., R. E. Short, D. C. Anderson, B. W. Knapp, and O. F. Pahnish. 1971. Cause and Effect relationship associated with calving difficulty and calf birth weight. *J. Anim. Sci.* 33:407.
- Brinks, J. S., R. T. Clark, N. M. Kieffer, and J. J. Urick. 1964. Estimates of genetic, environmental and phenotypic parameters in range Hereford females. *J. Anim. Sci.* 23:711.
- Brown, J. E., C. J. Brown, and W. T. Butts. 1973. Evaluating relationships among immature measures of size, shape and performance of beef bulls: I. Principal components as measures of size and shape in young Hereford and Angus bulls. *J. Anim. Sci.* 36:1010.
- Doornbos, D. E., D. C. Anderson, and D. D. Kress. 1984. Repeatability of linear body measurements in beef cattle. *Proc. West. Sect. Amer. Soc. Anim. Sci.* 35:116.
- Doornbos, D. E., D. C. Anderson, D. E. Kress, and K. J. Hanford. 1986. Repeatability of linear body measurements in beef cattle. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 37:157.
- Johnson, S. K., G. H. Deutscher, and A. Parkhurst. 1988. Relationship of pelvic structure, body measurements, pelvic area and calving difficulty. *J. Anim. Sci.* 66:1081.
- Johnson, Z. B., A. H. Brown, C. F. rosenkrans, and J. A. Hornsby. 2000. Prediction of mature weight and maturing rate from body measurements taken on Angus and Charolais calves at birth. *The Prof. Anim. Sci.* 16:213.
- Looper, M. L., C. F. Rosenkrans, Z. B. Johnson, A. H. brown, J. A. Hornsby, and J. L. Perkins. 2002. Relationships among growth characteristics of replacement Angus heifers. *The Prof. Anim. Sci.* 18:120.

TABLE 1. MEANS AND STANDARD DEVIATIONS OF BODY MEASUREMENTS TAKEN ON HEIFERS AT 8, 12, AND 18 MONTHS OF AGE

Variable <sup>1</sup>	Age at measurement					
	8-months		12-months		18-months	
	Mean	SD	Mean	SD	Mean	SD
HGC, cm	138.8	8.3	159.3	7.3	176.3	6.2
FGC, cm	147.2	9.6	166.6	8.4	184.4	7.4
RL, cm	38.2	3.3	42.2	2.5	47.0	2.3
TL, cm	147.2	9.1	162.8	7.7	179.6	7.4
2/3T, cm	101.2	6.5	114.6	6.0	128.3	6.7
RW, cm	40.8	3.3	47.0	3.2	51.3	2.6
SW, cm	34.8	3.0	41.9	3.7	44.2	2.6
WH, cm	100.2	5.0	109.5	4.3	118.9	3.8
HH, cm	107.2	5.5	116.7	4.9	125.7	4.3
TD, cm	13.1	1.7	16.4	1.6	18.3	1.5
HW, cm	17.0	2.7	19.3	1.3	19.6	0.8
HL, cm	36.0	1.5	41.4	2.1	44.2	1.5
WT, kg	225.8	39.6	321.8	41.6	426.7	43.5
CBL, cm	36.2	1.7	37.6	1.6	39.4	1.5
PH, cm			13.9	0.9	16.1	1.0
PW, cm			10.9	1.0	13.9	0.9
PA, cm			152.2	19.9	223.8	24.2

<sup>1</sup> Description of variables at bottom of table 2.

TABLE 2. COEFFICIENTS OF PRINCIPAL COMPONENTS FOR BODY MEASUREMENTS TAKEN ON HEIFERS AT 8, 12 AND 18 MONTHS OF AGE

Variable <sup>1</sup>	Coefficients for principal component number								
	8 mo			12 mo			18 mo		
	1	2	3	1	2	3	1	2	3
HGC	0.301	-.135	-.141	0.265	-.024	-.261	0.281	-.206	0.088
FGC	0.299	0.000	-.148	0.267	0.054	-.272	0.251	-.272	0.184
RL	0.226	0.139	0.123	0.234	0.005	-.097	0.226	-.056	0.086
TL	0.306	-.141	0.118	0.282	-.055	-.023	0.250	0.035	-.198
2/3T	0.301	-.100	0.179	0.231	-.313	-.188	0.269	0.117	0.146
RW	0.235	0.463	-.260	0.243	0.227	-.189	0.227	-.323	0.132
SW	0.257	0.300	-.357	0.237	0.300	-.192	0.225	-.412	0.135
WH	0.300	-.151	0.323	0.279	-.106	-.057	0.252	0.217	-.304
HH	0.308	-.165	0.200	0.299	-.068	0.117	0.266	0.131	-.386
TD	0.199	-.135	-.553	0.167	0.208	0.488	0.174	0.231	0.076
HW	0.007	0.747	0.310	0.168	0.472	0.178	0.187	-.298	-.004
HL	0.258	0.054	-.102	0.213	0.401	0.126	0.225	-.052	-.391
WT	0.331	-.012	-.010	0.313	-.034	-.220	0.314	-.193	0.003
CBL	0.264	0.160	0.381	0.225	-.077	-.112	0.226	0.158	-.399
PH				0.216	-.055	0.495	0.190	0.358	0.407
PW				0.185	-.431	0.124	0.253	0.253	0.161
PA				0.238	-.332	0.340	0.265	0.358	0.323
Eigenvalue	7.89	1.43	0.91	8.41	2.48	1.24	8.51	1.49	1.31
% total variance	56.4	10.2	6.5	49.5	14.6	7.3	50.1	8.7	7.7

<sup>1</sup> HGC=heart girth circumference, FGC=flank girth circumference, RL=rump length, TL=topline length, 2/3T=2/3 topline length, RW=rump width, SW=shoulder width, WH=wither height, HH=hip height, TD=thurl depth, HW=head width, HL=head length, WT=weight at measurement, CBL=cannon bone length, PH=pelvic height, PW=pelvic width, PA=pelvic area.

TABLE 3. COEFFICIENTS OF MULTIPLE DETERMINATION ( $R^2$ ) OF CALF BIRTH WEIGHT, CALVING DIFFICULTY, WEANING WEIGHT, PUBERTY AGE, 2-YR OLD WEIGHT, AND 3-YR-OLD WEIGHT

$R^2$ for body measurements taken at 8 mo of age <sup>a</sup>						
Variable <sup>b</sup>	1st calf BW	CD	1st calf WW	Puberty age	2-yr-old wt	3-yr-old wt
BG	0.099	0.062	0.229	0.416	0.035	0.001
HGC	0.032					
FGC			0.031			
2/3T						0.034
SW				0.029		0.101
WH					0.112	0.035
HH				0.022		
TD		0.042	0.059	0.042		
Total $R^2$	0.131	0.104	0.319	0.509	0.147	0.171

$R^2$ for body measurements taken at 12 mo of age <sup>a</sup>						
Variable <sup>b</sup>	1st calf BW	CD	1st calf WW	Puberty age	2-yr-old wt	3-yr-old wt
BG	0.101	0.066	0.214	0.418	0.037	0.001
FGC					0.008	
TL				0.045		
RW						0.016
SW	0.119					
WH			0.015	0.014		
HH				0.014		0.014
TD			0.019			
HW	0.029					0.055
HL	0.029		0.109	0.065		
WT					0.651	0.517
PA		0.022				
Total $R^2$	0.278	0.088	0.357	0.556	0.696	0.603

$R^2$ for body measurements taken at 18 mo of age <sup>a</sup>						
Variable <sup>b</sup>	1st calf BW	CD	1st calf WW	Puberty age	2-yr-old wt	3-yr-old wt
BG	0.093	0.014	0.225	0.429	0.001	0.031
FGC	0.029					
2/3T		0.035		0.064		
SW	0.049					
HH					0.025	
TD	0.021					
HL	0.169		0.095	0.026		
WT					0.652	0.582
CBL		0.038				
PH						0.013
PW			0.126			0.022
PA		0.042				
Total $R^2$	0.361	0.129	0.446	0.519	0.678	0.648

<sup>a</sup>BG included first in all equations. Remaining values entered equation at  $P < .10$

<sup>b</sup>BG=straightbred Hereford or crossbred, HGC=heart girth circumference, FGC=flank girth circumference, TL=topline, 2/3T=2/3 topline length, RW=rump width, SW=shoulder width, WH=withers height, HH=hip height, TD=thurl depth, HW=head width, HL=head length, WT=body weight at measurement, CBL=cannon bone length, PH=pelvic height, PW=pelvic width, PA=pelvic area.

## DETECTION OF QUANTITATIVE TRAIT LOCI FOR MARBLING AND BACKFAT IN WAGYU X LIMOUSIN F<sub>2</sub> CROSSES USING A CANDIDATE GENE APPROACH<sup>1</sup>

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**ABSTRACT:** Marbling commonly describes the presence of white flecks or streaks of fatty tissue between the muscle fibers in meat. Backfat refers to the amount of fat over the animal's back, usually measured between the twelfth and thirteenth rib in beef. Both traits have attracted a great deal of interest, since these two quantitative traits affect carcass quality and value in beef cattle. In this study, 247 F<sub>2</sub> Wagyu x Limousin animals with recorded phenotypes for marbling and backfat were genotyped for four candidate gene markers: thyroglobulin (*TG*), leptin (*LEP*), diacylglycerol O-acyltransferase (*DGAT1*) and growth hormone 1 (*GHI*) genes. The markers were assayed on a C/T substitution in *TG*, a C/T substitution in *LEP*, a C/A substitution in *DGAT1* and an *MspI* polymorphism in *GHI*, respectively. The backfat measurements on those animals varied from 0.1 to 1.3 inches and marbling scores ranged from 4 to 9.5. Frequencies of allele C in *TG*, allele C in *LEP*, allele A in *DGAT1* and allele 1 in *GHI* were 0.61, 0.68, 0.58 and 0.10, respectively. Analysis of variance using a generalized linear model did not show any significant differences among genotypes in *LEP* and *GHI* genes. However, the *DGAT1* gene had a significant additive effect on backfat ( $P = 0.036$ ), while the *TG* gene showed a dominant effect on marbling that approached significance ( $P = 0.061$ ). Our results indicate that not all genes previously identified as affecting marbling and backfat significantly impacted these phenotypes in crosses between Wagyu and Limousin. Given the variability of these traits and their economic importance in the marketing of beef in the U.S., further effort to identify QTL affecting marbling and backfat in crosses of Wagyu and Limousin is warranted.

Key words: beef, candidate genes, carcass and meat quality.

## Introduction

Marbling and backfat thickness are two important quantitative traits that affect carcass quality and production efficiency in beef cattle (Elias Calles et al., 2000). Selection for marbling and against backfat has long been recognized as an important objective for production of high quality beef. In beef cattle, traditional selection to increase marbling and reduce backfat is not simple owing to a potential genetic antagonism between the traits. Generally speaking, selection for carcass traits requires significant effort, expense, and time. Rapid development of molecular genetic marker technology in recent years has provide a means to identify and use genes that contribute to the genetic variation in marbling and fat thickness, and hence to molecular farming by marker-assisted selection.

Previous efforts have identified candidate genes responsible for marbling and backfat in beef. Barendse and colleagues (1997 and 2001) developed a TG5 polymorphism that occurs in the 5' promotor region of the thyroglobulin (*TG*) gene. This marker had a genotypic association with marbling score in long-fed cattle. Leptin is a 16-kilodalton protein produced by the obesity (*ob*) gene. Mutations in the leptin (*LEP*) gene cause beef cattle to reach slaughter weight sooner and develop more marbling in the carcass (Buchanan et al., 2002). *DGAT1* encodes diacylglycerol O-acyltransferase, a microsomal enzyme that catalyzes the final step of triglyceride synthesis (Grisart et al. 2002). A nonconservative K232A substitution in the *DGAT1* gene has been shown to affect milk fat content in dairy and intramuscular fat deposition in beef (Grisart et al. 2002 and Thaller et al. 2003). Interestingly, a strong association ( $P = 0.0058$ ) was detected between a microsatellite marker (CSSM066) lying approximately mid-way between the *TG* and *DGAT1* genes and the backfat EBV (Moore et al., 2003). Growth hormone (GH) is a major participant in the control of several physiological processes, including growth and metabolism. Therefore, considering the wide range of GH effects, its coding gene, *GH*, has been suggested as a putative candidate for variability of traits related to meat production and quality.

As a direct result of the Japanese grading system and substantial premiums paid for the highest quality meat, the Wagyu breed has been selected for generations

<sup>1</sup> Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, Montana AES, or the authors and does not imply its approval to the exclusion of other products that may also be suitable. USDA, ARS, Northern Plains Area, is an equal opportunity/ affirmative action employer. All agency services are available without discrimination.

for superior meat yield, marbling, and quality of meat. In contrast, the Limousin breed is recognized for leanness and muscularity. The objective of this study was to verify if the genes described above were associated with both marbling and backfat thickness in a Wagyu x Limousin  $F_2$  population.

### Materials and Methods

**Animals:** A population of Wagyu x Limousin cross cows and bulls was developed at Washington State University and transferred to the Fort Keogh Livestock and Range Research Laboratory, ARS, USDA. This reference population includes 6  $F_1$  bulls, 113  $F_1$  dams and 246  $F_2$  progeny. The  $F_2$  population resulted from inter se mating  $F_1$  Wagyu x Limousin sires and dams. Each calf was weighed within 24 h after birth and again at weaning when the calves averaged approximately 180 d of age. After weaning, the calves were returned to native range pastures and were supplemented with 0.7 kg per calf per day of both barley cake and alfalfa pellets. In mid-January, the calves were moved from the range and were fed silage and chopped hay to achieve anticipated gains of 0.5 to 0.8 kg per day. They were then placed on finishing diet for approximately 150 days followed by slaughter. Growth rate and carcass and meat quality data were collected on all  $F_2$  calves. The backfat measurements on  $F_2$  animals varied from 0.1 to 1.3 inches and marbling scores ranged from 4 to 9.5. DNA was extracted from blood samples.

**Primer sequences:** The primer sequences designed for genotyping a C/T substitution in the *TG* were: forward, 5' GGGGATGACTACGAGTATGACTG 3' and reverse 5' GTGAAAATCTTGTGGAGGCTGTA 3'. Four PCR primers were designed to genotype a C/T transition in exon 2 of the *LEP* gene using a tetra primer amplification refractory mutation system based PCR (tetra primer ARMS-PCR). The two outer primers were 5' GACGATGTGCCACGTGTGGTTTCTTCTGT3' and 5' CGGTTCTACCTCGTCTCCAGTCCCTCC3', while the two inner primers were: 5' TGTCTTACGTGGAGGC TGTGCCAGCT3' for the T allele and 5' AGGGTTTTG GTGTCATCCTGGACCTTTCG3' for the C allele. A lysine/alanine polymorphism of the *DGATI* gene was detected by primers derived from GenBank accession number AF318490. The forward primer was: 5'- TGGGCTCCGTGCTGGCCCTGATGGTCTA-3', and the reverse primer: 5'- TTGAGCTCGTAGCACAGGGTGG GGGCG A-3'). Primers for the *GH* gene (forward: 5' TGGGGTGGGGAGGGTTCCGAATAAGGCGG3' and reverse 5' T GAGGAAGTGCAGGGGCCCAAGC CACGA3') were designed based on GenBank accession number M57764 to amplify a fragment of 492 bp spanning the third intron region.

**Genotyping:** Genomic DNA (~50 ng) was amplified in a final volume of 10  $\mu$ l that contained 5 pmol of each primer, 200 nM dNTPs, 2.5 mM  $MgCl_2$ , 50 mM KCl, 10 mM Tris HCl, 0.1% Triton X-100 and 0.5 U of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA). After denaturation at 94°C for 3 min, 30 amplification cycles were performed as follows:

denaturation at 94°C for 30 sec, annealing at 63°C for 30 sec and extension at 72°C for 30 sec, followed by a further 5-min extension at 72°C. The genetic polymorphisms in the *TG*, *DGATI* and *GH* genes were revealed by digestions with restriction enzymes *Bst*YI, *Crf*I and *Msp*I, respectively. PCR products or PCR-digested products were analyzed using 1.6% agarose gels, stained with ethidium bromide and photographed.

**Statistical analysis:** To derive age-, live weight-, and fat-depth-constant phenotypes, the observed phenotypes were analyzed by least squares, using a model that included fixed effects for year of birth (2000, 2001 or 2002), sex (heifer or steer), linear effects of either age (days), live weight (kilograms), or fat depth (centimeters), as appropriate, and all possible interactions. Residuals from these analyses were used in subsequent analyses for genetic effects. The following regression model was used to estimate the genetic effects of the four candidate loci on backfat and marbling score.

$$y = \mu + a x + d z + \varepsilon$$

where,  $y$  is the vector of phenotypic values,  $\mu$  is the overall mean,  $a$  is the additive effect,  $d$  is the dominance effect,  $x$  and  $z$  are vectors that contain dummy variables linking individuals to the additive ( $a$ ) and dominance effect ( $d$ ), respectively, of the candidate locus, and  $\varepsilon$  is the vector of random residuals. Given the candidate genotype of an individual (say  $j$ ),  $x_j = 1$  or  $x_j = -1$  for either of the homozygous genotypes and  $x_j = 0$  for the heterozygous genotype while  $z_i = 1$  for the homozygous genotypes and  $z_i = -1$  for the heterozygous genotype. Denoting  $X = (1, x, z)$  and  $\beta = (\mu, a, d)'$ , equation (1) becomes

$$y = X\beta + e$$

The parameter estimates were obtained using weighted least-squares criteria

$$\hat{\beta} = (X'WX)^{-1}(X'Wy)$$

with

$$Var(\hat{\beta}) = (X'WX)^{-1}s^2$$

where the mean square error  $s^2$  estimates the variance of error  $\sigma^2$  and  $W$  is a diagonal weight matrix. Student  $t$  distributions were then constructed for the significance test of each estimated model parameter, as below

$$t = \frac{\hat{\beta}_i}{\sqrt{(X'WX)^{-1}_{ii}s^2}}$$

### Results and Discussion

***TG* gene:** A fragment of 548 bp was amplified to flank a C/T polymorphism at position 1696 of the bovine thyroglobulin sequence (GenBank Acc no. M358823). The products contain one common and one polymorphic cut site for restriction enzyme *Bst*YI. The enzyme digestion yielded a common band of 75 bp, and three polymorphic bands of 178 bp, 295 bp and 473 bp,

respectively (Figure 1). A total of 242 F<sub>2</sub> animals were successfully genotyped with this marker, including 94 homozygous CC animals, 41 homozygous TT and 107 heterozygous CT animals. The frequencies of allele C and allele T in the population were 0.61 and 0.39, respectively. This marker was not significantly associated with marbling score or backfat thickness in the population, although it showed a dominant effect on marbling that approached significance (Table 1 and Table 2).

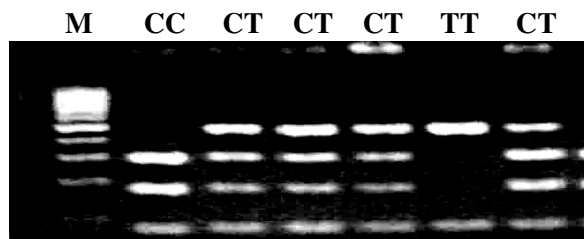


Figure 1: Genotypes of the bovine *TG* gene.  
CC: 295 + 178 + 75 bp; CT: 473 + 295 + 178 + 75 bp;  
and TT: 473 + 75 bp.

*LEP* gene: A C/T transition in exon 2 of the bovine *LEP* gene was genotyped. Tetra primer ARMS-PCR based amplification was used to amplify a common product of 239 bp for all animals by two outer primers. The product size for the T allele is 131 bp, whereas the product size for the C allele is 164 bp (Figure 2). Among 246 animals genotyped, the frequencies of different genotypes were 45% for CC, 46% for CT and 9% for TT, respectively. The frequencies of allele C and T in the population were 0.68 and 0.32, respectively. This gene failed to show any significant effect on marbling and backfat (Table 1 and Table 2).

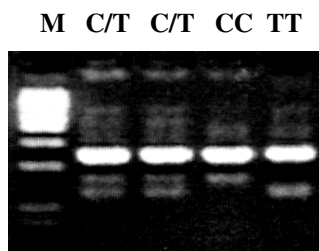


Figure 2: Genotypes of the bovine *LEP* gene.  
CC: 239 + 164 bp; CT: 239 + 164 + 131 bp and TT: 239 + 131 bp.

*DGAT1* gene: To detect a nonconservative lysine to alanine substitution (K232A) in the bovine *DGAT1* gene, a fragment of 405 bp was amplified using primers described above. Cleavage of the products by *Cfr*I yielded two fragments of 230 and 175 bp (Figure 3). The frequencies of AA, AC, and CC genotypes for *DGAT1* were 34%, 48% and 18%, respectively. Frequency for A in *DGAT1* was 0.58. This gene was significantly associated with backfat thickness ( $P < 0.05$ ), but not with marbling in the Waygu x Limousin F<sub>2</sub> population. Therefore, our study supports the hypothesis that *TG-DGAT1* region harbors a quantitative trait locus

(QTL) for backfat in beef cattle, as discussed by Moore and colleagues (2003).

Table 1. Additive and dominance effects of four genes on backfat

Locus	$\mu$	$a$	$d$
TG	-0.002±0.015	-0.002±0.015	0.013±0.021
LEP	-0.020±0.018	0.019±0.018	0.037±0.023
DGAT1	-0.004±0.015	0.032**±0.015	0.004±0.021
GH1	0.011±0.056	-0.001±0.056	-0.043±0.061

\*  $p < 0.10$ ; \*\*  $p < 0.05$

Table 2. Additive and dominance effects of four genes on marbling

Locus	$\mu$	$a$	$d$
TG	0.115±0.094	-0.074±0.093	-0.253*±0.134
LEP	0.002±0.116	0.075±0.116	-0.088±0.150
DGAT1	-0.077±0.095	0.092±0.095	0.099±0.134
GH1	-0.116±0.351	-0.161±0.351	-0.138±0.382

\*  $p < 0.10$ ; \*\*  $p < 0.05$

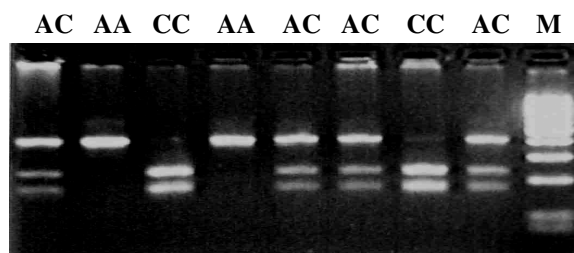


Figure 3: Genotypes of the bovine *DGAT1* gene  
AA: 405 bp; AC: 175 + 230 + 405 bp and CC: 175 + 230 bp.

*GH1* gene: The *Msp*I polymorphism represents a C/G substitution in the third intron of the bovine growth hormone gene (Zhang et al., 1993). A fragment of 492 bp was amplified to genotype this marker. Of the 243 animals genotyped, only two were homozygotes with *Msp*I(-), while 44 were heterozygotes and 197 were homozygotes with *Msp*I(+) (Figure 4).

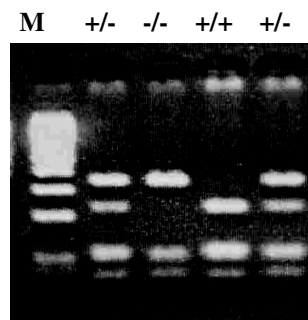


Figure 4: Genotypes of the bovine GH gene.  
+/-: 223 + 109 + 97 + 63 bp; -/-: 332 + 97 + 63bp; and +/+ : 332 + 223 + 109 + 97 + 63 bp.

The frequency of the *MspI* (-) allele was low (10%) in this population. The bovine growth hormone gene was associated with neither marbling score nor with backfat thickness (Table 1 and Table 2) in the population studied.

Our results indicate that not all genes previously identified as affecting marbling significantly impacted these phenotypes in crosses between Wagyu and Limousin. Previously reported genome scans using microsatellite markers have revealed two significant and four suggestive QTLs for marbling on bovine chromosomes 2 and 3, and on bovine chromosomes 8, 16, 17 and 27, respectively (Casas et al. 2000, 2001 and 2002; MacNeil and Grosz, 2002). However, none of the candidate genes examined in this study map to these QTL regions for marbling.

Given the variability of marbling and backfat thickness that existed in these crosses of Wagyu and Limousin and the economic importance of these traits in the marketing of beef in the U.S., further effort to identify QTL affecting marbling and backfat in crosses of Wagyu and Limousin is, therefore, warranted.

### Implications

The identification and utilization of alleles for the high marbling and low backfat of the Wagyu breed in American beef crossbreeding programs would have obvious commercial value. Undoubtedly, these molecular markers will allow the marker-assisted selection of breeding stock, or the marker-assisted sorting of feeder cattle for high marbling and hence reduce overall costs of production. Economically, DNA diagnostic technology will benefit both beef producers and marketers. Consumers will also benefit from the improved quality and consistency of beef. Further study on genes responsible for marbling and backfat may allow producers to make management decisions on how to feed out animals by realizing their maximal genetic potential and gaining a more consistent carcass at the end of a feeding period.

### Literature Cited

Barendse, W. 1997. Assessing lipid metabolism. Patent Application WO9923248 PCT/AU98/00882.

Barendse, W., R. Bunch, M. Thomas, S. Armitage, S. Baud, and N. Donaldson. 2001. The TG5 DNA marker test for marbling capacity in Australian feedlot cattle. Marbling Symposium 2001.

Buchanan, F.C., C.J. Fitzsimmons, A.G. Van Kessel, T.D. Thue, D.C. Winkelman-Sim, and S.M. Schmutz. 2002. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. *Genet Sel Evol.* 34:105-16.

Casas, E., J.W. Keele, S.D. Shackelford, M. Koohmaraie, T.S. Sonstegard, T.P. Smith, S.M. Kappes, and R.T. Stone. 1998. Association of the muscle hypertrophy locus with carcass traits in beef cattle. *J. Anim. Sci.* 76:468-73.

Casas, E., S.D. Shackelford, J.W. Keele, R.T. Stone, S.M. Kappes, and M. Koohmaraie. 2000. Quantitative trait

loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin. *J. Anim. Sci.* 78:560-9.

Casas, E., R.T. Stone, J.W. Keele, S.D. Shackelford, S.M. Kappes, and M. Koohmaraie. 2001. A comprehensive search for quantitative trait loci affecting growth and carcass composition of cattle segregating alternative forms of the myostatin gene. *J. Anim. Sci.* 79:854-60.

Elias Calles, J.A., C.T. Gaskins, J.R. Busboom, S.K. Duckett, J.D. Cronrath, J.J. Reeves, and R.W. Wright Jr. 2000. Differences among Wagyu sires for USDA carcass traits and palatability attributes of cooked ribeye steaks. *J. Anim. Sci.* 78:1710-5.

Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford, P. Berzi, N. Cambisano, M. Mni, S. Reid, P. Simon, R. Spelman, M. Georges, R. Snell. 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Res.* 12:222-31.

MacNeil, M.D., and M.D. Grosz. 2002. Genome-wide scans for QTL affecting carcass traits in Hereford x composite double backcross populations. *J. Anim. Sci.* 80:2316-24.

Moore, S.S., C. Li, J. Basarab, W.M. Snelling, J. Kneeland, B. Murdoch, C. Hansen, and B. Benkel. 2003. Fine mapping of quantitative trait loci and assessment of positional candidate genes for backfat on bovine chromosome 14 in a commercial line of *Bos taurus*. *J. Anim. Sci.* 81:1919-25.

Thaller, G., C. Kuhn, A. Winter, G. Ewald, O. Bellmann, J. Wegner, H. Zuhlke, and R. Fries. DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. *Anim. Genet.* 34:354-7.

Zhang, H.M., D.R. Brown, S.K. DeNise, and R.L. Ax. 1993. Rapid communication: polymerase chain reaction-restriction fragment length polymorphism analysis of the bovine somatotropin gene. *J. Anim. Sci.* 71:2276.

# ECONOMIC SELECTION INDEXES TO IMPROVE FIBER PRODUCTIVITY OF CASHMERE GOATS IN MONGOLIA

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**ABSTRACT:** The objective of this study was to identify indicator traits that facilitate selection for decreased **FD** and to quantify the superiority of an economic selection index applied to simultaneously decrease **FD** and increase **CFWT** in Cashmere goats. **FD** is the most important factor determining the value per unit weight of cashmere fleece. International market prices are established according to the **FD** and the color of fiber. Cashmere fleece weight (**CFWT**) and live weight (**LWT**) are easy traits for cashmere producers to measure whereas fiber diameter (**FD**) and length (**FL**) are expensive and time-consuming to collect. The accuracy of prediction for indicator traits- **CFWT**, **LWT** and combinations of **FD** and **FL** were compared to predict the economically relevant traits **FD** and **CFWT** using Best Linear Prediction. Seven combinations of indicator traits were considered to predict **CFWT** and **FD**: 1- **FD** alone; 2- **CFWT** alone; 3- **CFWT** & **FL**; 4- **FD** & **FL**; 5- **CFWT** & **LWT**; 6- **CFWT**, **FD** & **FL**; 7- **CFWT**, **LWT**, **FD** & **FL**. Economic selection indexes combining the two economically relevant traits as in  $I = 10 G_{CFWT} - 100 G_{FD}$  were derived from published genetic and phenotypic parameters using relative economic weights of 10 for **CFWT** and -100 for **FD**. **CFWT** and **LWT** were not good indicators of **FD** as it only decreased by 0.01 microns per year. Using all of **CFWT**, **LWT**, **FD** and **FL** decreased **FD** by 0.40 micron per year, however **CFWT** hardly changed (- 0.34 g per year). The results suggested that **FD**, a major determinant of cashmere value, should be measured directly so that efficient selection indexes can be constructed despite higher **FD** measurement cost. Economic selection indexes can simultaneously improve genetically antagonistic traits- **CFWT** and **FD** in the desired direction in an integrated cashmere production enterprise. Such indexes may reduce the rate of improvement for individual traits compared to single trait selection but increase overall productivity and profitability.

Key Words: Fiber characteristics, Economic selection index, Cashmere goats

## Introduction

The need to consider **FD** in selection programs in addition to **CFWT** has increased in the last decade, because **FD** in many Cashmere populations has

deteriorated as a result of intensive selection on fleece weight.

Considering **FD** in selection programs is problematic because of its antagonistic relationship with fleece weight. **FD** and **CFWT** are highly and positively correlated. The reported genetic correlations between **FD** and **CFWT** range from 0.12 to 0.83 for combed (Zagdsuren et al., 1994; Ning et al., 1995; Zhou et al., 2002) and shorn fleece (Pattie et al., 1989; Bigham et al., 1993; Bishop et al., 1996). Selection for fleece weight, ignoring **FD**, could deteriorate fiber quality. Pattie et al. (1989a,b), Ponzoni et al. (1990), and Baker et al. (1991) have stated that the three most important traits for maximizing financial returns from Cashmere goats are yearling **LWT**, down weight and **FD**. However, the costs of determining **FD** and yield of down are high.

Numerous studies have been conducted to quantify the influence of **FD** in selection programs and concluded that strategies to maintain **FD** are required. Pattie et al. (1989 b) stated that **FD** should be accurately predicted from direct measurement. Bishop et al. (1996) stated that **FD** selection could be accurately predicted by considering the functionally related fiber traits on the log scale. Herrmann et al. (1997) pointed out that the length-based model using Wildman/Bray formula could be effectively used. Diaz et al. (1999) stated that the use of linear programming techniques and restricted BLUP was found to be the most appealing strategy. However, little is known about simultaneous selection for **CFWT** and **FD** for combed fleece from indigenous goats. The objective of this study was to identify indicator traits that facilitate selection for decreased **FD** and to quantify the superiority of an economic selection index applied to simultaneously decrease **FD** and increase **CFWT** in Cashmere goats.

## Materials and Methods

Published genetic and phenotypic parameters for fiber traits (Table 1) were from Zagdsuren et al. (1994) and Zhou et al. (2002). There are no genetic parameters published for down characteristics from combed cashmere fleece. Selection index methodology (Hazel, 1943) was used to evaluate the expected responses to selection for the aggregated breeding goal and the individual traits. Seven combinations of indicator traits were considered to predict **CFWT** and **FD**: 1- **FD** alone; 2- **CFWT** alone; 3- **CFWT** & **FL**; 4- **FD** & **FL**; 5- **CFWT** & **LWT**; 6- **CFWT**, **FD** & **FL**; 7- **CFWT**, **LWT**, **FD** & **FL**.

Three alternative selection strategies were compared. These included direct selection on estimated breeding values (EBV) for **CFWT** ( $G_{CFWT}$ ); direct selection on EBV for **FD** ( $G_{FD}$ ) or index selection on the economic index combining the two economically relevant traits as in  $I = 10 G_{CFWT} - 100 G_{FD}$ .

Two multiple-trait selection criteria were of particular interest for use in the economic index. These were combination 5 which included **CFWT** and **LWT**, the easy to collect traits as selection criteria and combination 7 which included the easy and difficult traits as selection criteria.

Table 1. Trait means and estimates of genetic (above diagonal) and phenotypic correlations (below diagonal) between fiber traits and live weight for Cashmere goats

	CFW T (g)	LWT (kg)	FD ( $\mu$ )	FL (mm)
Phenotypic Mean	350	30	15.0	65
Standard Deviation	83.9	5.5	1.1	12
Fleece Wt (CFWT)	<b>0.28</b>	0.25	0.10	0.40
Live Wt (LWT)	0.33	<b>0.10</b>	0.35	0.30
Fiber diameter (FD)	0.25	0.44	<b>0.30</b>	0.15
Fiber length (FL)	0.17	0.17	0.07	<b>0.23</b>

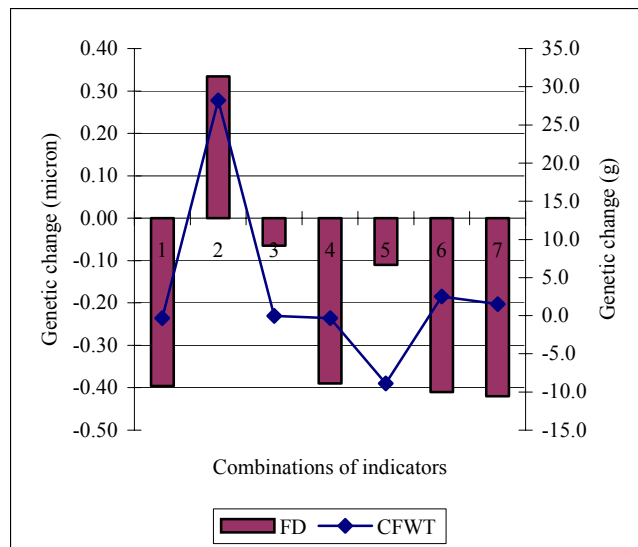
The predicted responses to direct and index selection were compared. Selection intensity was assumed to be 1.2, based on selection of the top 28% of bucks and does.

## Results and Discussion

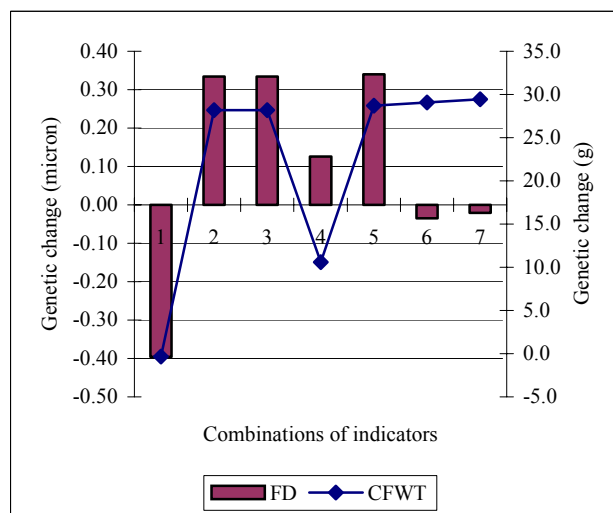
Selection for  $G_{FD}$  alone resulted in various rates of annual improvement depending upon the combination of selection criteria used (Figure 1). The response was  $-0.39$  micron/year when **FD** was the only selection criterion (combination 1). The response was much less, only  $-0.10$  micron/year when the easy to measure traits in combination 5 were used. Combination 7, using all traits, gave the best response of  $-0.42$  micron/year. Furthermore, there was no difference ( $P < 0.05$ ) in genetic response in **FD** by using combinations 1, 4, 6 and 7. Genetic response resulting from combination 2 differs from response by using combinations 1, 4, 6 and 7, but did not differ from combination 3 and 5 ( $P < 0.05$ ). The accuracies of prediction using all combinations were 0.55, 0.55, 0.10, 0.55, 0.11, 0.56 and 0.58 respectively.

Selection for  $G_{CFWT}$  alone achieved an increase of 28.2 g/year in **CFWT** when **CFWT** was the sole selection criterion (combination 2). Combination 7, using all traits, gave the best response of 29.5 g/year. (Figure 2). The accuracies of prediction for all combinations were .55, .53, .54, .20, .54, .55 and .55 respectively. Accuracy of estimating  $G_{CFWT}$  did not change much when

additional traits are considered together with the direct measurement. Genetic change in **CFWT** did not differ among the combinations ( $P < 0.05$ ).



**Figure 1.** Expected genetic response in **FD** (correlated response in **CFWT**) by selection on estimated breeding value for **FD** with estimates based on different combinations of selection criteria: 1- **FD** alone; 2- **CFWT**; 3- **CFWT** & **FL**; 4- **FD** & **FL**; 5- **CFWT** & **LWT**; 6- **CFWT**, **FD** & **FL**; 7- **CFWT**, **LWT**, **FD** & **FL**.



**Figure 2.** Expected genetic response in **CFWT** (correlated response in **FD**) by selection on estimated breeding value for **CFWT** with estimates based on different combinations of indicator traits: 1- **FD** alone; 2- **CFWT** alone; 3- **CFWT** & **FL**; 4- **FD** & **FL**; 5- **CFWT** & **LWT**; 6- **CFWT**, **FD** & **FL**; 7- **CFWT**, **LWT**, **FD** & **FL**.

Responses in aggregate merit and component traits resulting from direct selection for  $G_{FD}$  and for  $G_{CFWT}$  are presented in Table 2 along with the response to index selection using selection criteria combinations 5 & 7. In terms of **CFWT**, the two multi-trait indexes were not very different. However, the response to **FD** was sensitive to the indicator traits used in the index. A reduction of only

0.02 micron/year would be expected from an index using combination 5 whereas change of - 0.10 micron/year will result from combination 7. These results are in agreement with those previously published (Pattie et al. 1989b; Diaz et al. 1999).

Table 2. Expected annual responses from direct single trait selection of **CFWT** or **FD** and index selection for economic indexes of **CFWT** and **FD**

Objective Traits	INDEXES			
	CFWT (g)	FD (μ)	Economic index (\$) \$I = 10 \times \text{CFWT} - 100 \times \text{FD}	
Individual Traits	G <sub>CFWT</sub>	G <sub>FD</sub>	I <sub>5</sub>	I <sub>7</sub>
<b>CFWT (g)</b>	<b>28.2</b>	-0.34	<b>28.7</b>	<b>29.2</b>
<b>LWT (kg)</b>	1.1	-0.20	<b>0.14</b>	<b>0.15</b>
<b>FD (μ)</b>	0.3 <sup>a</sup>	<b>-0.40<sup>b</sup></b>	0.02 <sup>a</sup>	<b>-0.10<sup>b</sup></b>
<b>FL (mm)</b>	3.9	<b>-0.20</b>	1.4	<b>2.1</b>
\$Index	249 ± 37	36.6 ± .50	284 ± 37	302 ± 36
Accuracy	.55	.55	.54	.57

Note: Bold responses in a column reflect the traits are included in the selection criteria. <sup>a</sup> vs. <sup>b</sup> Numbers within a row with different superscripts are different (P < 0.05).

Selection for **FD** resulted in a substantial decrease in **FD**, but a negligible correlated response in **CFWT** (- 0.34 g/year). Direct single trait selection for **CFWT** promised substantial increase in **CFWT** but strategies to maintain **FD** are required if economic progress is to be achieved. These findings are consistent with previous studies. Diaz et al. (1999) pointed out that selecting for **CFWT** resulted in a substantial increase of cashmere weight up to 364 g, but the achieved correlated response in **FD**, +3.8 micron, implies that there would be almost a null probability of maintaining **FD** below 16.5 micron. In economic term, direct selection for **FD** would result in a loss \$265.4/year from cashmere income, being less disadvantageous than the economic returns expected from economic selection index using combination 7.

Using economic indexes based on indicator traits that include those that are both easy and difficult to measure can be expected to result in valuable economic progress. Such indexes may reduce the rate of improvement for individual traits compared to single trait selection but increase overall productivity and profitability.

### Implications

**CFWT** and **LWT** cannot be used to reliably predict **FD**. **FD**, a major determinant of cashmere value,

has no useful indirect indicators and its breeding values should be estimated from direct measurement even though it associated with extra costs. Economic selection indexes could be used to simultaneously improve genetically antagonistic traits- **CFWT** and **FD** in desired direction in integrated cashmere production enterprise.

### Literature Cited

- Baker, R. L., B. R. Southey, M. L. Biggam, and S. A. N. Newman. 1991. Genetic parameters for New Zealand Cashmere goats. *Proc. N.Z. Soc. Anim. Prod.* 51:423-427.
- Biggam, M. L., C. A. Morris, B. R. Southey, and R. L. Baker. 1993. Heritabilities and genetic correlations for live weight and fiber traits in New Zealand Cashmere goats. *Livest. Prod. Sci.* 33:91-104.
- Bishop, S. C., and A. J. F. Russel. 1996. The inheritance of fiber traits in a crossbred population of Cashmere goats. *J. Anim. Sci.* 63:429-436.
- Diaz, C., M. A. Toro, and R. Rekaya. 1999. Comparison of restricted selection strategies: An application to selection of cashmere goats. *Livest. Prod. Sci.* 60:89- 99.
- Gifford, D. R., R. W. Ponzoni, N. J. S. Ellis, F. C. R. Levinge, and M. L. Milne. 1990. Genetic parameters for production characteristics of Australian Cashmere goats. *Proc Aust. Assoc. Anim. Breed. Genet.* 8:461-465.
- Hazel, L. N. 1943. The genetic basis for constructing selection indexes. *Genet.* 28:476-490.
- Herrmann, S., and F. J. Worthmann. 1997. Opportunities for the simultaneous estimation of essential fleece parameters in raw cashmere fleeces. *Livest. Prod. Sci.* 48:1-12.
- Ning, M., L. Youngjin, S. Yaqin, L. Weimin, and L. Yujie. 1995. Estimates of genetic parameters of main traits in Liaoning cashmere goats. *Fine Fiber News.* 5:16-18.
- Pattie, W. A., and B. J. Restall. 1989a. The inheritance of cashmere in Australian goats. 1. Characteristics of Base Population and the effects of environmental factors. *Livest. Prod. Sci.* 21:157-172.
- Pattie, W. A., and B. J. Restall. 1989b. The inheritance of cashmere in Australian goats. 2. Genetic parameters and breeding values. *Livest. Prod. Sci.* 21:251-261.
- Ponzoni, R., and D. R. Gifford. 1990. Developing breeding objectives for Australian Cashmere goats. *J. Anim. Breed. Genet.* 107:351-370.
- Zagdsuren, Y., B. Mandakh, and G. Bolor-Erdene. 1997. Selection index for improvement of Mongolian Cashmere goat performance. *European fine fiber network, News.* 2: 18-19.
- Zhou, H. M., D. Allain, J. Q. Li, W. G. Zhang, and X. C. Yu. 2002. Effects of non-genetic factors on production traits of Inner Mongolia cashmere goats in China. *J. Anim. Breed. Genet.* 6:385-390.

## ESTIMATES OF GENETIC PARAMETERS OF FINAL WEIGHT AT SLAUGHTER, YIELD GRADE AND MARBLING SCORES IN BEEF CATTLE.

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**ABSTRACT:** Genetic parameters for final weight at slaughter (FSW), yield grade (YG), and marbling score (MS) were estimated. After 24 hr chill 500 carcasses of progeny differing in fractions of Charolais (C), Zebu (Z), Brangus (B), Hereford (H), Angus (A), and Holstein (HF) inheritance were evaluated. Measurements on rib eye area (REA), fat thickness on the ribeye area (FTREA), and percentage of kidney, pelvic, and heart fat (KPH%) were measured. Separate analysis for each trait was analyzed by using SAS (1989). The analytical model included, fixed main effects: of sire breed, sex, slaughter group, dam breed and interaction sire breed x dam. Random nested components of sires within sire breed, and dams within dam breed and residual. FSW values 530, 520, 518, 476, 475, 473, 470, 456, 448, 436, and 435 kg involved inheritance of CxB, CxZ, HFxHF, BxB, CxA, ZxB, BxA, HxB, HxZ, ZxZ and HxA respectively. The values in REA 34.62, 31.58, 31.60, 30.86, 29.50, 28.66, 28.60, 25.87, 25.85, 24.49, and 22.16 cm<sup>2</sup> corresponded to progeny involving CxZ, CxB, HxA, BxB, HFxHF, CxA, BxA, HxB, BxA, HxZ, and ZxZ respectively. Yield grade values 1.1, 1.2, 1.2, 1.3, 1.4, 1.4, 1.8, 1.8, 1.9, 2.0 and 2.1 corresponded to carcasses of progeny that involved CxZ, CxB, HxA, BxB, HFxHF, CxA, BxA, HxB, ZxA, HxZ and ZxZ inheritance respectively. Bos taurus inheritance had the most favorable effects on MS. The heritability estimates for FWS, YG, and MS were ( $h^2=.45\pm.05$ ,  $h^2=.47\pm.04$ , and  $h^2=.36\pm.06$ ) respectively.

Key words: Heritability, Crosses, Carcass traits.

### Introduction

Green et al., 1999 indicate that beef cattle producers are currently challenged to achieve simultaneous goals of cow adaptability to the production environment and carcass acceptability within a specific marketing. Cundiff and Gregory (1999) summarized that effectiveness of various crossbreeding systems in terms of heterosis utilization, use of breed complementarity and consistency of production, the most effective system at doing all three things, along with being the easiest to manage effectively, was composite breeding. The genetic

parameters, which are functions of (co) variance components, provide information about the genetic nature of traits, and are needed to predict direct and correlated responses (Van Vleck, 1987). Different reports have found strong negative ( $r_g=-0.73$  average for studies) genetic correlation between marbling and percent lean retail yield (Cundiff, 1987). An analysis of Angus field data showed there was essentially no genetic correlation between marbling and external fat thickness (Wilson, 1987).

### Materials and Methods

Results from evaluation of carcasses of steer and heifer progeny of the analyzed traits final live weight (FWS), area of longissimus at 12-th rib, fat thickness at 12<sup>th</sup> rib, percentage of kidney, pelvic, and heart fat, yield grade (YG) and marbling scores (MS). Cattle was slaughtered at a mean age of 440 d. Each cross or breed was stratified by weight and age to contribute to each group. The feeding period for the three groups was 211, 195, and 185 d, respectively. All cattle were slaughtered at a commercial slaughter facility, and carcasses were evaluated after 24-h chill.

Separate analysis for each trait was analyzed by least squares, SAS (1989). The analytical model included fixed main effects of: sire breed, sex, slaughter group, dam breed and interaction of sire breed x dam. Random nested components of sires within sire breed and dams within dam breed and residual.

### Results and Discussion

As shown in Table 1. Highest values 530, 520, and 518 kg in FWS corresponded to crosses involving CxB, CxZ, and HFxHF inheritance. Intermediate FWS 476, 475, 473, and 470 kg corresponded to crosses involving BxB, CxA, ZxB, and BxA inheritance. Lowest values 456, 448, 436, and 435 kg for FWS were to progeny involving HxB, HxZ, BxB, and HxA inheritance.

### Rib eye area

Table 1 also shown that the highest values in REA 34.62, 31.58, 31.60, and 30.86 cm<sup>2</sup>

corresponded to carcasses of crossbred progeny involving inheritance CxZ, CxB, HxA, and BxB, respectively. Intermediate values in REA 29.50, 28.66, and 28.60 cm<sup>2</sup> were to carcasses of progeny of HFxHF, CxA, and BxA in that order. Lowest values for the same trait 25.87, 25.85, 24.49, and 22.16 cm<sup>2</sup> involved inheritance of HxB, BxA, HxZ, and BxB respectively.

#### **Yield grade**

Highest values in yield gradeYG (1.1, 1.2, 1.2, 1.3, 1.3, 1.4, and 1.4) corresponded to crosses among CxZ, CxB, HxA, BxB, HFxHF, CxA, and BxA, respectively. Lowest values in yield grade YG (1.8, 1.9, 2.0, and 2.1) involved inheritance HxB, ZxA, HxZ, ZxB, and ZxZ, respectively (Table 1.)

#### **Marbling Scores**

Table 1 also shown MS values. Crosses involving higher fraction of Bos Taurus inheritance had the most favorable effects In MS 900, 800, 700, 700 and 700 on HxA, BxA, BxB, CxA, and HxB, respectively. Intermediate values in ms 600, and 400 were observed in carcasses of progeny involving CxZ, ZxA inheritance. Lowest values in MS 270, 300, and 300 involved inheritance of HFxHF, HxZ, and ZxZ, respectively. As Bos Taurus inheritance increased, MS generally decreased. Zebu had the most detrimental effect in MS in agreement with findings by Cole et al. (1963), Damon et al. (1960), Koch et al. (1982), Shackelford et al. (1991), and Wheeler et al. (1990) who found Zebu to have significantly ( $P < .05$ ) lower MS than Angus, Hereford or Angus-Hereford crosses.

#### **Heritability**

Estimates of heritability and their standard errors for each trait are shown in Table 3 ( $h^2 = 0.45 \pm .03$ ,  $h^2 = 0.47 \pm .04$ , and  $h^2 = 0.36 \pm .07$ ) for final weight at slaughter, yield grade, and marbling scores respectively. The estimated heritability value  $h^2 = 0.45 \pm .03$  for FWS is quit similar with the findings by Koots et al., 1994a, who summarized (n=52) studies to get a weighted mean value of heritability ( $h^2 = 0.41$ ). Estimates of heritability for yield grade ( $h^2 = 0.47 \pm .04$ ) is in agreement to the average value ( $h^2 = 0.45 \pm .05$ ) for this trait based in the estimated values of, Hakim et al., 1990, Van Vleck et al., 1992, and Green,

1999 who summarized (n=16) reports to estimate a weighted mean value of heritability ( $h^2 = 0.42$ ) for rib eye area. Estimates of

heritability ( $h^2 = 0.38$ ) for MS of (n=12) studies for Koots et al., (1994) are quite in agreement with the findings of ( $h^2 = 0.36 \pm .07$ ) for MS based in limited numbers at this study.

#### **Implications**

Significant genetic variation was found among crossbreds in final slaughter weight and economically important carcass traits as yield grade and marbling score. Those traits are moderately to high heritable. In cattle, greater production and use of F<sub>1</sub> from crosses of diverse biological types that optimize genetic potential for lean yield and objectively meat quality attributes, and match genetic potential to climatic and feed environments can increase uniformity of cattle in commercial production.

#### **Literature Cited**

- Cundiff, L.V., K.E. Gregory. What is systematic crossbreeding?. Proceedings of Cattlemens College, National Cattlemens Beef Association, Charlotte, NC. February 1999.
- Green, R.D. 1999. Breeding to satisfy customer requirements in the 21<sup>st</sup> century. Proceedings of Association for Advancement of Animal Breeding and Genetics, Mandurah, Western Australia.
- Green, R.D., T.G. Field, N.S. Hammett, and B. M. Ripley. 1999. Can Cow and Carcass Acceptability Both Be Achieved?. Beef Symposium American Society of Anim. Sci. WASAS.
- Hakim, A.F., L.D. Van Vleck, L.V. Cundiff, R.M. Koch, J.D. Crouse and K.G. Boldman. 1990. Estimates of genetic and phenotypic covariances among carcass and sensory characteristics of crossbred cattle with a multiple trait Animal model. J. Anim. Sci. 68(Suppl. 1): 238 (Abstr.).
- Koots, K.R., J.P. Gibson, C. Smith, and J. W. Wilton. 1994a. Analyses of published genetic parameter estimates for beef production traits. 1. Heritability. Anim. Breed. Abstr. 62:309-338.
- SAS. 1989. SAS/STAT User's Guide(Release 6.03). SAS Inst. Inc., Cary, N.C.

Van Veck, L.D., A.F. Hakim, L.V. Cundiff, R.M. Koch, J.D. Crouse and K.G. Boldman. 1992. Estimated breeding values for meat characteristics of crossbred beef cattle with an animal model. J. Anim. Sci. 70:363.

Wilson, L.L., J. R. McCurley, J. H. Ziegler, and J.L. Watkins. 1976. Genetic parameters of live and carcass characters from progeny of Polled Hereford sires and Angus-Holstein cows. J. Anim. Sci. 43:569-576.

**Table 1. Least squares means for weight at slaughter, rib eye area, yield grade, and marbling scores of 11 genetics groups of specific crosses: Bos taurus x Bos taurus, Bos indicus x Bos indicus, and Bos taurus x Bos indicus in different proportion.**

Genetic group Sire x Dam	Final slaughter weight (kg)	Rib- eye area cm <sup>2</sup>	Yield grade	Marbling scores
CxZ	520	34.62	1.1	600
CxB	530	31.58	1.2	700
H x A	435	31.60	1.2	900
B x B	476	30.86	1.3	700
HFx HF	518	29.50	1.3	270
CxA	475	28.66	1.4	700
BxA	470	28.66	1.4	800
HxB	456	25.85	1.8	700
ZxB	473	24.49	1.9	400
HxZ	448	24.49	2.0	300
ZxZ	436	22.16	2.1	300

CxZ= Charolais x Zebú, Cx B=Charolais x Brangus, HxA= Hereford x Angus, BxB= Brangus x Brangus, CxA=Charolais x Angus, BxA= Brangus x Angus, HxB= Hereford x Brangus, HFxHF=Holstein x Holstein, HxZ=Hereford x Zebú, and ZxZ= Zebú x Zebú.

Traces (TR)=300, Slight (SL)=400, Small (SM)=500, Modest (MT)=600, Moderate=(MD)=700, Slightly Abundant (SA) =800, and Moderately Abundant (MA)=900. For example, a marbling score of MT 30 is converted to a numerical score of 600+30=630.

**Table 2. Mean squares of carcass traits involving inheritance of Bos taurus x Bos taurus, Bos indicus x Bos indicus, and Bos taurus x Bos indicus in different proportion.**

Mean squares				
Effect	DF	Final slaughter weight (kg)	Yield grade	Marbling scores
Sex	1	60,912.20 **	13.93**	248.40**
Slaughter Group	2	35,054.20**	0.09	9.70
Sire breed	3	4,126.90*	4.97**	8.90
Sires:sirebreed	119	1,471.80**	0.61**	8.90
Dams breed	3	15,258.30**	3.11**	40.10**
Dams:				
Cow breed	119	1,201.60	0.43**	7.10
Sire x Dam	9	6,863.70	0.47	28.10**
Sex x Sire	6			
Sex x Dam	6			
Residual	241	989.70	0.45	71.00

\* Means significant differences (P<0.05)

\*\*Means highly significant differences (P<0.01).

**Table 3. Heritability estimates and their standard errors for final weight at slaughter (FSW), yield grade(YG), and marbling scores (MS) of crossbred beef cattle and a dairy beef breed.**

	<u>FWS</u>	<u>YG</u>	<u>MS</u>
$h^2$	0.45±.03	0.47±.04	0.36±.07

FSW= final slaughter weight; YG= yield grade; MS= marbling score.

## GENETIC PARAMETERS AND BREEDING VALUES FOR MILK YIELD OF HOLSTEIN SIRES IN A COMMERCIAL HOLSTEIN DAIRY HERD IN MEXICALI, MEXICO.

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**ABSTRACT:** 1,149 lactations of 722 Holstein cows daughters of 55 Holstein sires were analyzed by using least squares. The objective was to estimate genetic parameters and breeding values for milk yield. The cows were classified in three groups by season of parturition. The analytical model to estimate heritability included: the subclass (H) as years x season of parturition interaction as fixed effects; sire, cow within sire, and the residual as random. A second analytical model included: the subclass (H) as years x season of parturition interaction as fixed effects; cow and the residual as random) was used to estimate repeatability of milk yield by MIVQUE. The average milk yield 305 d 2X was  $8,437.79 \pm 2007.61$  kg, and  $10,649.31 \pm 2449.34$  kg to measure equivalent ME. The average values ( $438.70 \pm 25.30$ ,  $2.75 \pm 1.45$ ,  $59.17 \pm 27.06$ , and  $127.8 \pm 76.19$ ) corresponded to: parturition interval, services per conception, dry period, and open days), respectively. The estimates of heritability and repeatability values for milk yield, were ( $h^2=0.28$  and  $R=0.33$ ), respectively. The variance component for milk yield due to sire was (105,852.98), and the value of the phenotypic variance (1,491,139.08). The variance component for milk yield ME due to cow was (544, 955. 64), while the value of the phenotypic variance was (1,653, 948.33).

**Key Words:** Heritability, Repeatability, Breeding Values.

### Introduction

Sire evaluation can be structured as a process of prediction of future progeny of a sire produced by mating with specified dams and making their records in some specified environment (Henderson, 1972). Sewall Wright (1931) cited by Henderson (1972), suggested three types of prediction: (1) progeny of a particular mating, (2) future daughters in the same herd without include a new sample of dams, (3) daughters out of a random sample of dams of the breed. Henderson, (1966) suggested that to account for genetic trend and for different selection policies of AI studs and dairymen's choice of sires for natural service, to be evaluated be divided into groups and the evaluation be the sum of the

estimate of the group and the selection type evaluation of the deviation of the individual sire of this mean. Data used in sire evaluation research and application, are collected from herds in which culling and selection have been practiced. The objective of this study to estimate genetic parameters and breeding values for milk yield in a Holstein dairy herd located at north west of México.

### Materials and Methods

Data came from a commercial dairy herd at northwest of México. The analytical model included: the subclass H of year season of parturition interaction as fixed effects; sire and cow nested within a sire and the residual as random components. A second analytical model included: the subclass (H) as year x season of parturition interaction as fixed effects; cow and the residual as random components) was used to estimate repeatability of milk yield by MIVQUE. To estimate the variance components and to calculate the heritability and repeatability values for milk yield was used MIVQUE, Rao, (1971). The matrix model used for this purpose it is a mixed model of Searle cited by Goodnight, (1978) included:  $Y = X_0\beta_0 + (\sum_{i=1}^k X_i\beta_i) + e$  within it:  $Y =$  Vector of  $n$  observations,  $X_0$ ,  $X_1, \dots, X_k$  are  $n \times m$  known matrices,  $\beta_0$  vector of fixed effects. Assumption, vectors  $\beta_i (i=1 \dots k)$ , and  $(e'_s)$  are independent  $\sim N(Q, G_1 I_{m_i})$  where  $G^2 =$  variance,  $(e)$  is a  $n$  vector  $\sim N(0, Ge^2r)$ . Breeding values. The estimates of breeding values for milk yield was as follow:

1. Estimates the average milk yield in daughters of each sire, lactations were standardized 305d-2X, and milk yield projected to mature equivalent ME. Additionally, each record was corrected by year and season of parturition, as a deviation of the corrected mean of herd mates and the average milk yield of breed independent of age.

2. Estimates the weighting factors ( $\beta$ ) of each sire, were based in milk yield of its daughters, in comparison to herd mates, and by using the heritability index ( $h^2=0.28$ ) to milk yield estimated in this study. It was also considered the number of daughters of each sire, and the

environmental correlation among paternal half sibs ( $C^2=0.02$ ) proposed by Dimov, et al. (1995). The breeding values of cows were classified according to their own records and records of their paternal half sibs. 1. The procedure was as follow: estimates of the average milk yield in the herd per year, season of parturition according to the record of each cow. 2. Each record was deviated of the average milk yield of the herd. It was calculated the mean difference of each cow dividing the total difference of each cow by the number of their records. The weighting factors  $W_1$  and  $W_2$  of the individual averages of cows and daughters were also estimated. The predicted milk differences (PMD) values of the 55 sires used at the herd under study were taken of the sire's summaries of USA companies. The reason to use that source of information on (PMD) is due the fact those sires are tested more intensively in different herds, and also for higher number of daughters.

### Results and Discussion

Least squares means and their standard deviations for milk yield 305d-2X, milk yield to projected to mature equivalent (ME), parturition interval, services per conception, dry period, and open days are presented in (Table 1). As shown the average values ( $8,437.79 \pm 2007.61$  kg, and  $10,649.31 \pm 2449.34$  kg corresponded to milk yield 305d-2X and mature equivalent, respectively. The milk yield in the herd under study could be considered as reasonable high, if compared: to the national average milk yield (5570 kg) in México for Holstein and to the average milk yield for Holstein breed (7257 kg) 305d-2X for Holstein breed. The estimates of correlation ( $r_p=0.83$ ) corresponded to the projected milk yield to ME based in lactations 1 to 3. The average values ( $438.70 \pm 25.30$ ,  $2.75 \pm 1.45$ ,  $59.17 \pm 27.06$ , and  $127.8 \pm 76.19$ ) corresponded to: parturition interval, services per conception, dry period, and open days), respectively (Table 1). The average values of the reproductive traits can be considered as high values. Nevertheless, during the summer, four months (June to September, the average temperature is higher than  $40^\circ\text{C}$ , which compromises the productivity and the reproductive performance of dairy cattle under heat stress, due to seasonal variations, and the adaptability of the animals to environment, Thatcher, et al., 1984.

### Genetic parameters

The variance components for milk yield based in different number of lactations are presented in Table 2. As shown the effects of sires were highly significant ( $P<0.01$ ) for milk yield. The estimates of heritability for milk yield through the intraclass correlation among paternal half sibs, as four times the variance of sires divided by the total phenotypic variance was ( $h^2=0.28 \pm 0.03$ ) this value is quit similar to the estimates of heritability ( $h^2=0.29$ , and  $h^2=0.36$ ) of Hudson et al., 1981, and Dimov et al., 1995). However our estimates of heritability for milk yield was different to the estimates ( $h^2=0.44$ ) by Miztal et al., 1992).

### Breeding values

The estimates of breeding values expressed as the Predicted Difference for milk yield in this study were different to the reported values of Predicted Difference for this trait into sire's summaries. The genetic potential of a sire is relative and the validity of the estimates must be function of the population where it was estimated. Nevertheless, the publications of sire's summaries shown variations through time. It suggests being cautious to make predictions of the genetic potential of sires. The selection to other traits, the arbitrary genetic groups, the preferential treatment to some cows, and the techniques of measurement, still being a probable effect of reduction in accuracy of the genetic merit due to sire (Weigel et al., 1996; Wilder, 1988; Powell et al., 1964, and Samuelson, 1995).

Weigel et al., (1986) outstanding that the extensive international interchange of cattle, semen, and embryos during the last twenty five years, conducted to the development of more accuracy methods to compare the genetic merit of sires. Pearson et al., (1994) remarked the existence of limitations due to instable of regression equations used in the prediction of the genetic merit of sires. Additionally, the genetic evaluations of sires and variance components residuals must be of populations previously selected (Powell, et al., 1994). It is important that sires used to mate in a commercial herd represent A mix of sire families with the inclusion of some sire lines not strongly represented in the current herd females. It is important to consider that semen purchase is an investment with the payoff coming at least three years in the future.

### Implications

Results of this study suggest a reasonable high milk yield in the herd under study. Existing evidence suggesting that high production is positively correlated to later lactations. Most decisions on selecting sires must be made on basis of performance in first lactation of the daughters. Sires can be ranked on the daughters' first records with little loss in accuracy. First record gives essentially as reliable an estimate of cows, breeding values measured by the daughter's performance, as does an appropriately weighted combination of multiple records.

### Literature Cited

- Dimov, G., L. G. Albuquerque, J. F. Keown, L. D., Van Vleck and H. D. Norman. 1995. Variance of interaction effects of sire and herd for yield traits of Holsteins in California, New York, and Pennsylvania with an Animal Model. *J. Dairy Sci.* 78:939.
- Henderson, C. R. 1972. Sire evaluation and genetic trends. Proceedings of the Animal Breeding and Genetic Symposium in Honor of Dr. Jay L. Lush. Virginia Polytechnic Institute and State University Blacksburg, Virginia. American Society of Animal Science, American Dairy Science Association.
- Henderson, C.R and L.D., Van Vleck. 1964. Improvement in production of New York Holsteins due to artificial insemination. *J. Dairy Sci.* 44: 1328.
- Henderson, C. R. 1966. A sire evaluation method which accounts for unknown genetic and environmental trends, herd differences, season, age effects, differential culling. *Proc of Symp. on Estimating breeding values of Dairy Sires and Cows.* Washington, D. C.
- Hudson, G.F. S. and L.D. Van Vleck. 1981. Relationship between production and stayability in Holstein cattle. *J. Dairy Sci.* 64:2246.
- Miztal, I. T., Lawlor, T., Short, and M. Van Raden. 1992. Multiple trait estimation of variance components of yield and type traits using an Animal Model. *J. Dairy Sci.* 75:544.
- Powell, R. L., Wiggans, G. R., and P.M. Van Raden. 1994. Factors affecting calculation and use of conversion equations for genetic merit of dairy bulls. *J. Dairy Sci.* 77:2679.
- Samuelson, D. J., Pearson, R. E. 1995. Accuracy of predicting genetic merit from pedigree information for bulls entering stud sampling programs. *J. Dairy Sci.* 78: 2057.
- Thatcher, W. W., R. L. Badinga, R. L. Collier, H. H. Head, and C. J. Wilcox. 1984. Thermal stress effects on the bovine conceptus: early and late pregnancy. Reproduction of ruminants in zone tropicale. *Inst. Natl. Res. Agron Publ (Abstr.)*.
- Weigel, K. A. 1996. Impact of second country data of foreign bulls on international evaluations of dairy bulls. *J. Dairy Sci.* 77:900.

**Table 1. Least squares means and their standard deviations for milk yield 305 d 2X, milk yield to ME, parturition interval, and services per conception, dry period, and open days.**

Items	Mean	S. D. <sup>a</sup>
Milk yield 305 d 2x	8,437.79	2007.61
Milk yield ME	10,649.31	2449.34
Parturition interval	438.70	25.30
Services per conception	2.75	1.45
Dry period	59.17	27.06
Open days	127.8	76.19

<sup>a</sup>Standard Deviation

**Table 2. Variance components estimated by MIVQUE for milk yield in Holstein cows of different lactations.**

Source of Variation	Variance Components
SIRE	105,852.98
COW (SIRE)	311,116.23
RESIDUAL	1'074,369.87
TOTAL	1,491,339.08

**Table 3. Variance components estimated by MIVQUE for repeatability of milk yield projected to mature equivalent in Holstein cows.**

Source of Variation	Variance Components
COW	544,955.64
RESIDUAL	1'108,992.69
TOTAL	1'653,948.33

## ESTIMATES OF GENETIC PARAMETERS FOR BIRTH WEIGHT, WEANING WEIGHT AND CALVING DIFFICULTY IN CROSSBRED BEEF CATTLE.

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**ABSTRACT:** Genetic parameters for calving difficulty, weights at birth and weaning were evaluated by using least squares. Cows were F<sub>1</sub> involving inheritance of Charolais (C), Hereford (H), and Local strains (L) which were bred to Gelbvieh (G) bulls. These Gelbvieh sires were mated to 117 cows (98 of those) produced calves at 28 and 39 month of age. Mean birth weight was 33.4 kg from 28-month-old cows. Birth weight (BW) ranged from 31.5 kg in calves involving inheritance of local strains mated to Gelbvieh sires to 44.76 kg in crossbred Gelbvieh-sired calves from Charolais and Hereford females. Mean birth weight of the Gelbvieh sired calves from Charolais and Hereford cows 39 months of age was 39.07 kg. Birth weight ranged from 34.96 kg in calves from crossbred cows of local strains inheritance to 44.60 kg in crossbred Gelbvieh sired calves from Charolais and Hereford females. Calving difficulty was greater ( $P<0.05$ ) in Gelbvieh-sired calves from Charolais and Hereford 28-month-old cows (1.40; scale; 1= least calving difficulty, 7=most calving difficulty). Less calving difficulty was observed in females involving Local strains inheritance mated to Gelbvieh sires. Breed rank for birth weight was highly associated with age at puberty. Calf crop weaned (%) was higher ( $P<0.05$ ) in matings involving Charolais and Hereford females. Mean weaning weight at 200d (215.76 kg) was highest ( $P<0.05$ ) in crossbred Gelbvieh-sired calves from Charolais and Hereford females. Calves from females of Local strains inheritance resulted the lightest at weaning. Estimates of heritability values ( $h^2=0.30\pm0.03$ ,  $h^2=0.20\pm0.03$ , and  $h^2=0.05\pm0.04$ ) corresponded to birth weight and weaning weight, and calving difficulty, respectively. These results, which are based on limited numbers of observations, suggest an advantage by involving breeds of Continental inheritance in beef breeding plans for commercial cow-calf system under the specific conditions of local environment. Additionally, it is important to take in account the actual trends within biological types of beef and biological types for traits being considered in crossbreeding plans or purebreeding plans under an specific system of animal production (Arango, et al., 2004).

Key Words: Heritability, Birth weight, Weaning Weight, Calving Difficulty

### Introduction

Performance attributes of traits of greatest economic value for different breed or breed crosses are important in determining the potential value of alternative germplasm resources for profitable beef production. There are several types of crossbreeding programs. These have been discussed in detail (e.g. Bourdon, 1994; Kress, 1994; Cundiff and Gregory, 1999). Koots et al., (1994b) summarized published estimates of genetics and phenotypic correlations between a number of traits of interest. Dickerson(1970) suggested that all changes in a commercial cow-calf operation must be evaluated in terms of their effect on profitability of the whole enterprise.

### Materials and Methods

This study was conducted in a commercial cow-calf operation located at northwest of Baja California, México. Cows used involving inheritance of Charolais (C), Hereford (H), and dams of Local strains (L) which were bred by natural service to Gelbvieh (G) bulls to 98 cows to produce calves at 28 and 39 month of age.

### General management

Cows were maintained in an open rangeland grazing system of desert brush characterized by (*Simmonsia chinensis*), (*Cercidium microphyllum*, (*Olneya tesota*), (*Eriogonum phasciculatum*), and (*Fraxinum trifoliate*). Grasses are scarce under this vegetative rangelands system. Dams born in the same year were treated as a contemporary group for nearly all analyses. Calving was in (March, April, and May). At birth all calves were identified, dehorned (paste), and vaccinated against viral scours. Calves were weaned and weighed at approximately 200d.

### Data collection

The traits analyzed in this study were birth weight, weaning weight (adjusted to 200 d of age), and calving difficulty measured categorically. Calving difficulty was subjectively evaluated categorically using descriptive scores (i.e., 1= no difficulty, 2= little difficulty by hand, 3=little difficulty with jack, 4= slight difficulty with a calf jack, 5=moderate difficulty with jack, 6=major difficulty with calf jack, and 7= Caesarean birth presentation.

## Analyses of data

Separate analyses for each trait was analyzed by using least squares procedure SAS (1989). The analytical model included: dam breed, and sex of the calf as fixed main effects; sires and the residual as random components.

## Results and Discussion

Least squares means for Birth weight, Weaning weight, and Calving difficulty are given Table 1.

### Calves with 28 month old dams.

Mean birth weight was 35.84 kg. Birth weight ranged from 31.5 kg in calves involving inheritance of females of Local strains mated to Gelbvieh sires to 40.18 kg in crossbred Gelbvieh sired calves from Charolais and Hereford females. Calving difficulty was greater ( $P<0.05$ ) in Gelbvieh sired calves from Charolais and Hereford 28-month-old-cows. (1.40 scale; 1=least calving difficulty, 7=most calving difficulty). Less calving difficulty (1.00) was observed in females of Local strains mated to Gelbvieh sires. Breed rank for birth weight was highly associated with age at puberty. Mean weaning weight at 200d (177.50 kg). Highest weaning weight (215.76 kg) was observed in crossbred sired calves from Hereford x Charolais females. Calves from females of Local strains resulted the lightest as average (172.00 kg) at weaning.

### Calves with 39 month old dams.

Overall mean birth weight was 35.84kg. Birth weight ranged from 33.96 kg in calves involving inheritance of dams of Local strains mated to Gelbvieh sires, to 44.00 kg in crossbred Gelbvieh sired calves from Charolais and Hereford females. Calving difficulty (2.00) was Less calving difficulty (1.30) was observed in dams involving inheritance of Local strains mated to Gelbvieh sires. Mean weaning weight at adjusted to 200d (177.74 kg) was highest ( $P<0.05$ ) in crossbred Gelbvieh-sired calves from Charolais x Hereford females 28 month-old, and 186.12 kg mean weaning weight ( $P<0.05$ ) in calves from Charolais x Hereford cows 39 month-year old. Weaning weight ranged from 169.6 to 190.22 kg in crossbred Gelbvieh sired calves from Charolais x Hereford 28 month-old cows.

Weaning weight 200d ranged from 173 kg in calves involving inheritance of dams of Local strains mated to Gelbvieh sires to 199.75 kg in crossbred Gelbvieh sired calves from crossbred Gelbvieh sired calves from Charolais and Hereford 39-month-old-cows. Lightest calves at weaning resulted from cows of Local inheritance mated to Gelbvieh sires.

## Genetic parameters

Heritabilities ( $h^2$ ) values for the analyzed traits were estimated separately on the progenie of Gelbvieh sires by using the relationship among paternal half sibs as  $4[\sigma^2 \text{ sires}] / \sigma^2 P$  are shown in Table 2. The estimated values (direct) of heritability were ( $h^2=0.30\pm 0.03$ ,  $h^2=0.20\pm 0.04$ , and  $h^2=0.05 \pm 0.04$ ) for birth weight, weaning weight, and calving difficulty, respectively. Heritability estimates for birth weight and weaning weight are in agreement to the average values ( $h^2=0.31$  and  $h^2= 0.24$ ) for those traits in that order, summarized of ( $n=167$ ), and ( $n= 234$ ) number reports by (Koots et al.1994 and Green, 1999). Heritability estimates of calving difficulty ( $h^2=0.05\pm 0.04$ ) in this study is quit in agreement to the average weighted value of heritability ( $h^2=.07\pm .014$ ) for calving difficulty, summarized in ( $n=7$ ) reports by (Koots, et al., 1994). Based in ( $n=19$ ) and ( $n= 11$ ) reports of estimates of calving ease (direct and maternal) measured categorically (Koots et al., 1994, and Green 1999) estimated heritability values ( $h^2=0.07$  and  $h^2=0.09$ ) direct and maternal represented as weighted mean values. Greater calving difficulty is observed in male calves than female calves independent of sex of birth weight, indicating that anatomical differrences between sexes contribute to calving difficulty (Gregory et al., 1991). The authors also reported that effects of heterosis on calving difficuty were not consistent and generally not significant, i.e. effects of heterosis in birth weight are not reflected in in increased calving difficulty as a trait of the dam. Smith, (1976) found for Hereford and Angus cows mated artificially to Hereford, Angus, Jersey, South Devon, Limousin, Charolais and Simmental bulls that Charolais and Simmental crossbred calves were heaviest at birth. Selection for smaller birth weight may be the most effective criterion for improving calving ease. Not only because it is the best single indicator of calving difficulty but also because it can be measured accurately.

## Implications

Producers must consider birth weight, and calving difficulty in their breeding programs. These results show large differences in calving difficulty among dams breeds mated to Gelbvieh sires to produce calves at 28 and 39 month of age. Calves from Charolais dams, mated to Gelbvieh sires that had more calving difficulty were also significantly heavier at birth. Lowest birth weights involved calves from cows of Local strain. Calves from cows Charolais and Hereford produced the highest weights at weaning.

## References

- Arango, J.A., L.V. Cundiff, and L. Van Vleck .2004. Comparison of Angus, Charolais, Galloway, Hereford, Longhorn, Nelore, Piedontese, Salers, and Shorthorn breeds for weight, weight adjusted for condition score, height, and condition score of cows. J. Anim. Sci. 82:74-84.
- Bourdon, R. M. 1994. Breed roles in hitting a new target. Proceedings of Annual Mtg. Of Beef Improvement Federatio. Des Moines, IA, pp 115-122.
- Cundiff, L.V. and K.E. Gregory. 1999. What is systematic crossbreeding?. Proceedings of Cattlemens College, National Cattlemen's Beef Association, Charlotte, NC. February 1999.
- Dickerson, G. E. 1970. Efficiency of animal production- molding the biological components. J. Anim. Sci. 30-849.
- Green, R.D. 1999. Utilising new genetic improvement technologies to improve beef beef profitability and palatability. Proceedings of Association for the Advancement of Animal Breeding and Genetics. July 1999.
- Koots, K.P., J.P. Gibson, C. Smith, and J.W. Wilton. 1994b. Analyses of published genetic parameter estimates for beef production traits. phenotypic and genetic correlations. Anim. Breeding Abstr. 62:825.
- Kress, D.D. 1994. Crossbreeding with a New Target. Proceedings of Annual Mtg. of Beef Improvement Federation, Des Moines, IA, pp 83-92.
- SAS. 1990. SAS/STAT® User's Guide(6.03). SAS Inst. Inc., Cary, N.C.

Table 1. Least squares means for birth weight, weaning weight, and calving difficulty of dams of Local strain, Hereford and Charolais mated to Gelbvieh sires to produce calves at 28 and 39 month age.

	Age to calve 28 month			Age to calve 39 month		
	<u>L</u>	<u>H</u>	<u>HxC</u>	<u>L</u>	<u>H</u>	<u>HxC</u>
BIRTHW						
WEIGHT, kg	31.50	35.85	40.18	33.96	39.70	44.00
WEANING						
WEIGHT, 200d	169.60	172.70	190.22	173.00	185.60	199.75
CALVING						
DIFFICULTY	1.00	1.40	2.00	1.00	1.30	2.00

**Table 2. Estimates of heritability <sup>a</sup> ( $h^2$ ) values for birth weight, weaning weight and calving difficulty of dams of Local strain, Hereford, and Charolais mated naturally to Gelbvieh sires to produce calves at 28 and 39 month age.**

<b>BW</b>	<b>WW</b>	<b>CDC</b>
<b><math>h^2 = 0.30 \pm .03</math></b>	<b><math>h^2 = 0.20 \pm .04</math></b>	<b><math>h^2 = 0.05 \pm .04</math></b>

<sup>a</sup>  $h^2$  estimated through the intraclass correlation among paternal half sibs.

EFFECTS OF EARLY WEANING ON PRODUCTION EFFICIENCY IN A COW / CALF SYSTEM

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**ABSTRACT:** Over a 3-yr period 240 spring-calving cows (age  $5.9 \pm 0.04$  yrs, body weight (BW)  $605 \pm 8$  kg; body condition score (BCS)  $2.6 \pm 0.06$  (scale 1-5); calving date: May 1) were used to compare very early (VEW:  $72 \pm 12$  d) with early (EW:  $132 \pm 12$  d), and normal weaning (NW:  $192 \pm 12$  d). Heifers calves were backgrounded on pasture and steers split between pasture (VEWP, EWP) and drylot (VEWF, EWF) until NW. After NW all calves moved into drylot. Cow BCS and BW increased as weaning age decreased ( $P < 0.05$ ). Two out of the three years VEW and EW cows had significantly ( $P < 0.05$ ) greater BCS and BW than NW cows. Pregnancy rates, calving interval and calf birth weight were similar ( $P > .10$ ) for all treatments; but culling rates were lowest ( $P < .05$ ) for VEW. Heifer ADG from VEW to NW were lower ( $P < .05$ ) for VEW heifers ( $0.8$  kg/d) and marginally ( $P < .10$ ) lower for EW heifers ( $0.9$  kg/d) than for NW heifers ( $1.0$  kg/d). Through the backgrounding period differences in ADG for heifers were negligible ( $P > .10$ ). Pooled results for cyclicity (74% of VEW, 85% of EW and 95% of NW) strongly indicated that weaning treatment had an effect on sexual maturity. Steer ADG from VEW to NW were the least ( $P < 0.05$ ) for VEWP ( $0.9$  kg/d); intermediate for VEWF and EWP ( $1.1$  kg/d) and greatest for EWF and NW ( $1.2$  kg/d). ADG in the feedlot was not affected by treatment ( $P > .10$ ), however from birth to slaughter VEWP had lower ADG ( $P < 0.05$ ) than EWP, EWF and NW steers and VEWF steers were intermediate. Days on feed and carcass traits for quality and yield grades were not affected by weaning treatment. Carcass wt, ribeye area (REA) and back fat depth were lower for VEWP and VEWF ( $P < 0.05$ ) compared to the EWP, EWF and NW steers. When REA was expressed as a ratio to carcass wt, there were no differences ( $P > .10$ ) among treatments. Feed to gain was the least efficient for VEWP ( $6.7:1$ ); intermediate for VEWF and EWF ( $6.3$  and  $6.4:1$ ) and greatest for EWP and NW ( $6.0$  and  $6.1:1$ ). Net income for the system indicated that EW and backgrounding the calves on pasture until NW generated the most net income to the system

INTRODUCTION

Production efficiency is a function of input and output for the production unit, and both must be measured in order to assess effects of treatments on efficiency. The output portion of production efficiency in a cow-calf unit is a function of weaning weight, cow BW, BCS, and number of calves weaned (Dickerson 1970; Wiltbank 1994). Early

weaning has shown promise as a means of increasing calf growth (Peterson et al. 1987; Myers et al. 1999a). Green and Buric (1953) concluded that 90-d weaning did not adversely affect beef calves, and the main difference between 90- and 180-d weaned groups was in early post weaning rate of gain.

In cows, BW and BCS are the primary measures of nutritional status and have the greatest influence on reproductive potential and feeding flexibility over the winter months (Short et al. 1996). Production efficiency can be enhanced by optimizing the BW and BCS of cows through manipulation of the weaning period, and likewise calf performance can be enhanced and morbidity/mortality reduced. Several researchers in North America have evaluated cow and calf performance when weaning occurred before about 150 d of age (Peterson et al. 1987; Gill et al. 1993; Boadi and Price 1996a and 1996b; Purvis et al. 1996). Economic modeling of the cow/calf enterprise (Spren and Laughlin 1986) indicated that weaning calves at 6 mo of age resulted in the greatest present value for gross income. But there are limited published data that evaluate the long-term biological and economic implications of early weaning on cow and calf performance.

MATERIAL AND METHODS

This study was conducted at the University of Alberta Kinsella Research Station located in East Central Alberta. The study was conducted from March 30, 1998 to March 30, 2001 and involved 240 Kinsella Hybrid (Berg et al. 1986) spring-calving cows (BW:  $605 \pm 8$  kg; BCS:  $2.6 \pm .06$  at March 30, 1998), assigned to one of three weaning management groups based on BW, BCS and age. The three weaning treatments were:

- very early weaning (**VEW**:  $72 \pm 12$  d age;  $n=96$ )
- early weaning (**EW**:  $132 \pm 12$  d age;  $n=92$ )
- normal weaning (**NW**:  $193 \pm 12$  d age;  $n=50$ )

Cows remained in their assigned weaning treatments for the next three years unless they were culled from the herd for reproductive failure or dystocia, poor feet, BCS less than 1.5, low udder score or loss of calf.

After the normal weaning (November 9) each year, cows were managed as a group on range, until grazing ceased to be possible and then they were assigned to one of three winter management treatments. The three winter management treatments were Underfed (**UF**: 85% recommended NRC), Normally Fed (**NF**: 100% recommended NRC) and Overfed (**OF**: 115%

recommended NRC). The assignment of weaning and management groups was an independent 3 x 3 factorial design. During the spring and summer, all cows were managed as a single group and grazed cool-season native (*fesctuca halli*) and cool-season tame (*Bromus inermis*, *Poa pratensis*) grass pastures. Weaned cows stayed with the rest of the herd until winter-feeding began. The amount of hay, supplement, and inputs specifically associated with each weaning management group were recorded. Cows that fell below 1.5 body condition score (five point scale: 1 = emaciated, 5 = obese; Lowman et al. 1973) were removed from the trial as culls. All cows were weighed and body condition scored about one month prior to calving, at each weaning period and just prior to the winter feeding period.

Each year during the breeding season, all cows were managed in the same pastures and exposed to yearling Kinsella Hybrid bulls (Berg et al. 1986) for a 45-d breeding season beginning on July 9 (the day of very early weaning) and ending August 23 each year. Bull to cow ratio was 1 to 25. Calves were identified and weighed on a spring-loaded scale within 24 hr after calving. Cows were scored for ease of calving on a scale of 0 to 5 (0 = no assistance; 1 = slight assistance; 2 = puller used easily; 3 = puller used with difficulty; 4 = veterinarian required; and 5 = caesarean birth). Mammary systems were scored (1 = small ideal teats; 2 = ideal teats; 3 = large teats; 4 = very large (bottle) teats; 5 = pendulous udder; 6 = one or two blind teats; 7 = mastitis) within 24 hr after calving. Pregnancy rates were determined in November by rectal palpation. Cows that were removed from the experiment for any reason were not replaced.

Production costs associated with each weaning management treatment were documented for economic analysis. Amounts of hay, grain, protein supplement, and salt and mineral fed were logged and expensed to each group. Feed costs were determined from values reported by AFSC (2004) for the periods 1998, 1999, 2000 and 2001. Labor, overhead and operating costs associated with feeding were determined from data reported by Kaliel et al. (2004) in the Benchmarks for Alberta Producers: Aspen Parkland Region. Grazing costs were based on the opportunity value of an animal unit month (AUM) in the eastern central Alberta Aspen Parkland Region (AFSC 2004). Grazing costs were calculated based on cow lactational status and AUM value. Cow depreciation costs were determined using credit for cull cows and purchase-in cost of replacement bred heifers.

### Calves

Calves in this experiment were born into the three weaning treatments described above (VEW, EW and NW). All cows and calves were managed as a single herd on range until they were weaned. Prior to July 9 each year, calves were given access to a creep feeder containing a 20% dairy starter for two weeks. On July 9 each year, all the calves and cows were weighed and cows' body conditions scored. The VEW calves were removed and transported to a feedlot at the ranch headquarters and all the cows and calves not yet weaned were returned to native pasture.

Upon entering the feedlot the VEW calves had ad libitum access to second cut alfalfa / grass hay and 20% dairy starter for the first ten days, after which, they were allocated to their respective backgrounding treatments. The EW calves were weighed and treated in a similar fashion to VEW, except that they were not creep fed prior to weaning. At the time of NW (November 9) the remaining calves were weaned and, together with the VEW and EW calves, moved to the next stage of the study. The weaning procedure and dates were identical in all three years.

### Heifers

Each year the VEW heifers were held in a feedlot pen for the first ten days post weaning where they received ad libitum access to grass hay and 20% calf starter after which they were removed to a Smooth Brome / Bluegrass (*Bromus inermis* / *Poa pratensis*) pasture with access to oats creep feed (Table 1). They remained in the pasture until the last calves were weaned in November. Similarly, EW heifers were weaned into a feedlot pen after which they were moved onto grass pasture, similar to the VEW heifers, with access to oats creep feed until the NW calves were weaned in November. Once the NW treatment was weaned, the heifers from all treatments were gathered together, weighed and group fed a grass hay / oats ration (TDN 65%, CP 12%) to provide a target gain of 0.75 kg/d.

Heifers were weighed every 28 d and were evaluated for cyclicity beginning at the end of May. Blood samples were taken 10 d apart by jugular venipuncture collected into 10 ml heparinized evacuated glass tubes. The samples were centrifuged at 2500 rpm for 15 min at 4 °C and plasma samples were portioned into sterile plastic vials and stored at -20 °C for later radioimmunoassay. Plasma samples were assayed for progesterone using the Coat-A-Count® Progesterone kit (DPC, Los Angeles, CA). The criterion for identification of estrus was that plasma P<sub>4</sub> concentrations had to be above 1 ng/ml in two consecutive (i.e. 10 days apart) sampling periods (Boadi and Price 1996b). After the May blood sampling and weighing, heifers were removed from the experiment and either retained as replacements or sold.

Feed and labor costs associated with replacement heifer development were documented and used for later economic analysis. Grazing costs were based on the average cost of an AUM in East Central Alberta Aspen Parkland Region (AFSC 2004) adjusted for BW. All supplements, feed, bedding, labor and yardage costs associated with backgrounding the heifers were documented and used in the economic analysis. Four-year average market prices (1998-2001) for the feedstuffs were used to price the ration (AFSC 2004). Labor and yardage costs associated with feeding the heifers were charged according to data reported by Kaliel et al. (2004). Liveweight market prices used to value weaned and backgrounded heifers were average prices for the four year period 1998-2001 (Canfax 2004) in which the calves were weaned and marketed, and for specific weight ranges appropriate for each management group.

## Steers

Steer calves from the VEW and EW treatments were randomly allocated to one of two backgrounding systems. Half of the VEW steers (VEWP) were backgrounded from July to November on Brome / Bluegrass (*Bromus inermis* / *Poa pratensis*) pasture with access to oats creep feed; the other half (VEWF) were kept in drylot from July to November with ad libitum access to grass hay and oats. The EW steers were allocated to a similar backgrounding system: pasture with oat creep feed from September to November (EWP), or in the feedlot (EWF) from September to November with ad libitum access to grass hay and oats. When the NW calves were weaned (November 9), all steers were reweighed and randomly assigned within treatment to group feeding pens.

Steers were vaccinated with CattleMaster and Ultrabac 7/ Somubac® (Pfizer Inc, New York, NY), treated for parasites with Dectomax® (Pfizer Inc, New York, NY) and implanted with the growth promoter Component S (Elanco Animal Health, Indianapolis, IN). During the four-week adjustment period to full feed, steers were started on grass hay and fed an increasing amount of a finishing mixed diet consisting of 63% barley, 22% oats, 10% dehydrated alfalfa pellets, and 5% canola meal (TDN 81%, CP 13%). The amount of hay was gradually reduced until the finishing diet, which was offered ad libitum for the remainder of the feeding period, formed 100% of the consumed diet. After 90 d on feed the steers were re-implanted, this time with Component TBA (Elanco Animal Health, Indianapolis, IN). Steers were fed to an end point where the majority of the pen was visually assessed, by experienced feedlot personnel, to have 1 cm of back fat.

Steers were weighed at each weaning period and then at 28-d intervals for the remainder of the feeding period. Steer days on feed, feed to gain ratios, and the following carcass traits were recorded: hot carcass weight, fat depth between the 12th and 13th rib, quality grade, and dressing percent. Slaughter BW was taken just prior to the animals being loaded for delivery to the abattoir.

Production costs associated with each of the treatments including pasture costs, supplements, and feed and yardage were documented for economic evaluation. The economic analysis assessed performance each year based on market prices, weaning and finishing weight, receiving and finishing dry matter intake, and days on feed. Live weight market prices used to value weaned and finished steers were the four-year average prices for the 1998-2001 (Canfax 2004) time period in which the calves were weaned and marketed, and for specific weight ranges appropriate for each management group. Four-year average prices for feedstuffs (AFSC 2004) were used in ration costing. Ration costs were separated into backgrounding and finishing ration costs. Total feed cost for each period was based on dry matter intake, feed efficiency, days on feed, and ration cost per kg. Finished prices were determined from data reported by Canfax (2004). Gross income per steer, feed, yardage, veterinary, trucking, interest expense, and net income per steer were calculated.

## Pasture Quality

Each year four fistulated steers were used for the collection of pasture quality information for the cow/calf pastures and weaning pastures. Fistulated steers stayed with the herd or in the pasture being tested for a minimum of one week prior to sampling. The steers were penned in drylot at 1600 h the day before collections were to be made. Water was available in the pens, but the animals did not have access to feed. Collections were made the following morning at 0800 h after complete manual evacuation of rumen contents. Animals were allowed to graze for 20 – 30 minutes, and then gathered for sampling of the rumen extrusium. Collections were conducted throughout the grazing season over the three years using the same steers. Extrusium samples were dried to constant weight at 45°C and ground before analysis. Analysis of the feed and forage samples for DM, CP, ADF, NDF, TDN calculations, Ca and P was done in duplicate each year by Norwest labs (Lethbridge, Alberta).

Hay, grain and creep feed were sampled at two-week intervals and the samples compiled. Dry matter was based on the Malt Gravimetric Method (935.29A, 935.29C); Crude Protein was based on: Protein (crude) in Animal Feed, CuSO<sub>4</sub>/TiO<sub>2</sub> Mixed Catalyst Kjeldahl Method, (1990); ADF based on: Fiber (Acid Detergent) and Lignin in Animal Feed (1990); NDF based on: Neutral Detergent Fiber – Amylase Procedure; Minerals based on: Metals in Plants (1990).

## RESULTS AND DISCUSSIONS :

Cow BCS and BW increased as weaning age decreased ( $P<0.05$ ) over the three-year period. By March 2001 (end of the study) the BCS and BWs were: VEW cows 3.6 and 667 kg; EW cows 3.0 and 622 kg; and NW cows 2.8 and 608 kg. Two years out of the three VEW and EW cows had significantly ( $P<0.01$ ) greater BCS and BW than NW cows at the normal (November) weaning time (i.e. going into winter). Pregnancy rates were similar across weaning treatments; there was a year effect on pregnancy rates, but this was confounded by the age of the cows (older each year). Weaning treatment had no effect on calving intervals or calf birth weight, but culling rates were lowest for VEW cows and similar for the EW and NW treatments.

Growth rates from July through to November were significantly ( $P<0.05$ ) lower for VEW heifers (0.8 kg/d) and marginally ( $P<0.10$ ) lower for EW heifers (0.9 kg/d) than for NW heifers (1.0 kg/d). From November through to May differences in ADG were negligible by design, since heifers on all weaning treatments were limit fed (backgrounded) to gain at about 0.75 kg/d.

Pooled results for heifer cyclicity at the end of May (74% of VEW, 85% of EW and 95% of NW) strongly indicated that weaning treatment had an effect on sexual maturity. Steer growth rates from July to November were least ( $P<0.05$ ) for VEWP (0.9 kg/d); intermediate for VEWF and EWP (1.1 kg/d) and greatest for EWF and NW (1.2 kg/d). Average daily gain in the feedlot was not significantly affected by treatment ( $P>0.10$ ), however from

birth to slaughter VEWP had lower gains ( $P < 0.05$ ) than EWP, EWF and NW steers, and VEWf steers were intermediate.

Days on feed and carcass traits for quality and yield grades were not affected by weaning treatment. Carcass weights, rib eye area (REA) and back fat depth at the 12<sup>th</sup> rib were all lower for VEWP and VEWf ( $P < 0.05$ ) treatments compared to the EWP, EWF and NW treatments. When REA was expressed in a ratio with carcass weight, there were no significant differences ( $P > 0.10$ ) among weaning treatments. Feed / gain in the feedlot, from January through to slaughter, was highest (least efficient) for VEWP (6.7:1); intermediate for VEWf and EWF (6.3 and 6.4:1) and lowest for EWP and NW (6.0 and 6.1:1).

Differences in BW and BCS resulting from the weaning treatments strongly affected the annual cow production costs. The differences among treatments resulted from reduced grazing and winter feed costs, reduced cow depreciation and slightly improved weaning ratios of the VEW and EW treatments over the NW treatment. The annual cow expenses averaged over the three-year period were  $\$601.04 \pm 8.25$  for VEW,  $\$657.15 \pm 8.25$  for EW and  $\$707.06 \pm 8.25$  for the NW treatment. Weaning treatment not only had significant effects ( $P < 0.05$ ) on annual cow production costs but also on gross sale receipts. Selling calves at the time of weaning would have resulted in all treatments realizing a loss to the cow enterprise. Losses would have been greatest for VEW ( $-\$176.11 \pm 8.81$ ) followed by NW ( $-\$23.70 \pm 8.81$ ) and least for EW ( $-\$33.85 \pm 8.81$ ).

Feedlot phase net income, based on the opportunity value of the weaned steers, showed a positive net income in all treatments. Net income did however vary significantly ( $P < 0.05$ ) being greatest for the VEWP steers, followed by the VEWf and EWP, with the NW and EWF steers having the lowest net income. Opportunity value at the time of weaning and backgrounding expenses were significant ( $P < 0.05$ ) contributors to the profitability of the feedlot phase net income. Heifer development costs were also affected by weaning treatment. Even though feed costs and development costs were lowest for NW heifers, by the end of the backgrounding period overall net income was greatest ( $P < 0.05$ ) for VEW, intermediate for EW and least for NW heifers.

System analysis, combining the annual cow costs with realized net income from the finished steers and backgrounded heifers, indicated that EWP generated the greatest net revenue per cow ( $\$55.75 \pm 5.65$ ), followed by VEWP and EWF ( $\$9.29$  and  $\$13.61 \pm 5.65$ ), then by NW and VEWf ( $-\$30.67$  and  $-\$33.87 \pm 5.65$ ).

It is concluded from this study that the majority of the annual cow costs are incurred by the time the calf is 72 d of age. Weaning at a very young age ( $< 72$  days) results in reduced weaning weights and even allowing for a greater price per unit of weight, insufficient revenue would be generated to offset the annual cow costs. Using this information in on-farm decision-making regarding the most appropriate weaning age in any given year is complex and

needs to take into account year to year variations in factors such as supplementary feed and other management costs, and the biological type of the cattle.

#### IMPLICATIONS :

When to wean calves is a major management decision in a cow-calf enterprise. The decision affects both the production efficiency of the cow and that of the calf. Very early weaning of calves (72 d of age) will increase cow body weights and body condition, however the benefits to the cow must be balanced with proper nutrition and management of the calf. It is feasible that a low cost backgrounding system using high quality pastures and energy supplementation could maintain good calf gain. However, it is likely that very early-weaned calves would perform better if managed in a confinement feeding system where their nutrition could be optimized. Adoption of very early weaning would give the most benefit during periods of severe drought where forage resources are typically restricted and very expensive. By allowing the cows to go into winter in good to excellent body condition, a very early weaning strategy would save on expensive winter feed costs, particularly in a drought situation. It would also help increase the pregnancy rates of poorly conditioned first and second calvers.

To maintain good calf performance, backgrounding very early-weaned calves in a feedlot would be the best management decision. During more normal years delaying weaning by another 60 d to 130 d of age, would result in calves that perform as well as normally weaned calves but require less strict nutritional management. Depending upon the circumstances backgrounding on pasture or in the feedlot could work equally well. Weaning at 130 d would still improve cow BW and BCS compared to normal weaning and would also reduce the grazing pressure on the forage resource. Although the normally weaned cows in this study were lighter and had lower body conditions scores two years out of three, there were no long-term detrimental effects on pregnancy and weaning weights.

Provided that winter feed inputs were not restricted normally weaned cows compensated in BW and BCS by the following spring. Similarly, if calves are to be held until a year of age many of the negative aspects relating to slow initial growth rates immediately following early weaning, will be eliminated through compensatory gain. Even though weaning has a strong influence on the growth rates of both cows and calves, the costs of feed and yardage will have an even more profound influence on profitability. Biological type (body size, the propensity to fatten, milking potential, and growth rate) would also need to be taken into consideration. Most importantly when weaning is the management tool chosen, understanding how an economically beneficial change in one livestock enterprise can add costs to another must be fully evaluated.

Age of the calf at weaning influences not only cow weight, body condition and calf growth rates but also the economics of the production segments that follow. Understanding the costs of production of a cow calf

operation and how weaning age as a management tool shifts costs from one livestock enterprise to another is crucial, if weaning age is the approach taken to reduce costs of production.

#### LITERATURE CITED:

- AFSC. 2004. Forage and feed prices for Alberta producers.
- Berg, R.T., Goonewardene L.A, and Makarechian M. 1986. The University of Alberta beef breeding project - 1986 update. 65<sup>th</sup> Annual Feeders Day Report pp 3 – 6. University of Alberta, Agriculture and Forestry Bulletin.
- Boadi D and M.A. Price. 1996a. The effect of pre-calving feed restriction on reproductive performance of beef cows. *Can. J. Anim. Sci.* 76: 337 - 342
- Boadi D and M.A. Price. 1996b. The effects of catch up (compensatory) growth on reproductive performance of beef heifers. *Can. J. Anim. Sci.* 76: 523 - 29
- Canfax. 2004. Report on cattle prices – 1998 - 2001. Calgary, Ab.
- Dickerson, G. 1970. Efficiency of animal production – modeling and biological components . *J. Anim. Sci.* 30:849
- Environment Canada, 1998. Alberta Weather and Statistics.
- Fibre (Acid Detergent) and Lignin in Animal Feed. 1990. Method 973.18 in Official Methods of Analysis of the AOAC. 15<sup>th</sup> Edition. Pg. 82-83
- Gill, D. R., M. C. King, H. G. Dolezal, J. J. Martin, and C. A. Strasia. 1993. Starting age and background: Effects on feedlot performance steers. *Oklahoma Agric. Exp. Sta. Res. Rep. P-933.* pp197–203. Stillwater, OK.
- Green, W.W. and J. Buric. 1953. Comparative performance of beef calves weaned at 90 and 180 days of age. *J. Anim. Sci.* 12:561-572
- Grimes, J. F., and T. B. Turner. 1991. Early weaning of fall-born beef calves: I. Pre-weaning calf and cow performance. *J. Prod. Agric.* 4:464–468.
- Kaliel, D. Economic, 2004. Production and financial performance of Alberta cow/calf operations; Aspen Parkland Region – period 1998-2001. Alberta Agriculture, 2004.
- Lowman, B.G., N. Scott, S. Sommerville. 1973. Condition scoring of cattle. East of Scotland College of Agriculture, Bulletin. No. 6.
- Metals in Plants. 1990. 985.01. Official Methods of Analysis of the AOAC. 15<sup>th</sup> Edition
- Myers, S. E., D. B. Faulkner, F. A. Ireland, L. L. Berger, and D. F. Parrett. 1999a. Production systems comparing early weaning to normal weaning with or without creep feeding for beef steers. *J. Anim. Sci.* 77:300–310.
- Myers, S. E., D. B. Faulkner, F. A. Ireland, and D. F. Parrett. 1999b. Comparison of three weaning ages on cow-calf performance and steer carcass traits. *J. Anim. Sci.* 77:323–329.
- Peterson, G. A., T. B. Turner, K. M. Irvin, M. E. Davis, H. W. Newland, and W. R. Harvey. 1987. Cow and calf performance and economic considerations of early weaning of fall-born beef calves. *J. Anim. Sci.* 64:15–22.
- Purvis, H. T., C. R. Floyd, K. S. Lusby, and R. P. Wettemann. 1996. Effects of early weaning and body condition score (BCS) at calving performance of spring calving cows. *Oklahoma Agric. Exp. Sta. Res. Rep. P-951.* pp 126–132. Stillwater, OK.
- SAS. 1996. SAS User's Guide: Statistics (Version 6.08). SAS Inst .Inc., Cary, NC.
- Spreen, T. H., and D. H. Laughlin. 1986. Simulation of Beef Cattle Production Systems and Its Use in Economic Analysis. Westview Press, Boulder, CO.
- Wiltbank, J.N. 1994. Challenges for improving calf crop. In: M.J. Fields and R.S. Sand (Ed.) Factors affecting calf crop. p. 1 CRC Press, Boca Raton, FL.

# EVALUATION OF BEEF CATTLE OPERATIONS UTILIZING DIFFERENT SEASONS OF CALVING, WEANING STRATEGIES, POST-WEANING MANAGEMENT, AND RETAINED OWNERSHIP

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**ABSTRACT:** Production data from a 3-yr study conducted at Fort Keogh Livestock and Range Research Laboratory near Miles City, MT were utilized to evaluate impacts of season of calving (SOC), weaning strategy (W), post-weaning management of replacement heifers (PWM), and retained ownership of steer calves (RO) on enterprise profitability. The SOC evaluated were late winter (Feb), early spring (Apr), and late spring (Jun). The Feb and Apr calves were weaned at 6 and 8 mo of age; Jun calves were weaned at 4 and 6 mo of age. The PWM strategies included one treatment intended to allow heifers to grow at a constant rate from weaning to breeding and the second intended to minimize harvested feed inputs. The RO options included backgrounding in El Reno, OK and backgrounding in Miles City, MT utilizing two different diets. Production systems were modeled to characterize each possible combination of factors. Economic performance of each system was based on animal performance and variable input costs. Data were analyzed at each level of production (cow-calf and backgrounding) with system and year included in the model. There were no differences between systems utilizing different PWM. For cow-calf enterprises selling calves at weaning, Jun systems yielded higher ranch gross margin (RGM = gross revenue minus variable costs) than all other systems, and were higher than Apr early-weaned system ( $P < 0.05$ ). When steer calves were backgrounded after weaning, systems utilizing Jun calving yielded a higher gross margin than those utilizing Feb or Apr ( $P < 0.05$ ). There were no differences between backgrounding treatments within calving season. The SOC had larger impact than W, PWM, and RO on the enterprise profitability. Our results suggest that, in systems managed similarly to those modeled here, feed costs and time of marketing may have important effects on profitability.

Key Words: Beef Cattle, Calving Date, Marketing

## Introduction

Timing of calving season with respect to nutrient dynamics in forage and nutrient requirements of beef cows has a large effect on inputs and outputs from a rangeland-based cow-calf production system. Feed cost is one of the most important variables that influence profit in a production system and has been reported at approximately 70% of the total cost of raising beef cows (Peterson et al., 1987). Feed costs are highly related to weaning dates (May et al., 1999; Reisenauer, 2001),

where earlier calving seasons increase weaning weights, but also increase feed inputs (Grings et al., 2002). An optimal calving season carefully weighs desired outputs (weaning weights, prices received) against inputs (feed, labor) required to maintain animal performance. The objective of this study was to evaluate systems utilizing late winter, early spring, and late spring calving, and track economic performance of several management options and endpoint production within each calving season.

## Materials and Methods

This research evaluated the impact of season of calving (SOC), weaning strategy (W), post-weaning management of replacement heifers (PWM), and retained ownership of steer calves (RO) on enterprise profitability by utilizing data collected during a 3 yr study conducted at the Fort Keogh Livestock and Range Research Laboratory near Miles City, MT and modeling the economic progress of beef cattle production systems through backgrounding. Animal performance and feed input data were collected from three cow herds with differing calving seasons, late winter (Feb), early spring (Apr), and late spring (Jun) were observed from 1998-2001. Feb and Apr calves were weaned at 6- and 8-mo of age; Jun calves were weaned at 4- and 6-mo of age. Each calving season was managed separately and there were no differences in management for cows assigned to each weaning strategy within calving season except time of weaning. Two PWM treatments were applied to each SOC, continuous gain that was intended to allow heifers to grow at a constant rate from weaning to breeding, and stair-step gain that was intended to minimize harvested feed inputs. All steers were then placed into one of three RO options, (1) backgrounded in El Reno, OK on forage based diets, (2) backgrounded in Miles City, MT on a corn silage-hay based diet, and (3) backgrounded in Miles City, MT on a corn silage-hay-barley diet. A detailed description of the experimental design, diet compositions, and animal performance of all systems except RO1 and RO2 is offered in Grings et al. (2002), Grings et al. (2003), and Grings et al. (submitted).

## Economic Analyses

Production systems were developed to characterize each possible combination of factors ( $n=36$ ). Apr LW system was used as the base system as it best characterizes what 'traditionally' occurs in the study area. The fixed amount of land for the base system was set by using the forage intake per animal and applying that to a

500 cow herd. Each subsequent system deviated in the number of cows from the base system as the amount of grazing time differed between systems. Forage intake per animal was determined by using the MSU beef production system model (Tess and Kolstad, 2000a,b) that was parameterized to be consistent with the cattle performance for each specified management scenario. Economic progress of each system was based on animal performance and variable input costs. Point estimates of animal performance were used regardless of statistical significance. Monthly feeder cattle prices were based upon the Billings, MT cattle market. Actual prices for feed were used where available.

#### *Cow-calf*

The cow-calf systems were modeled to represent a system located in southwestern Montana approximately 217 km from Miles City, MT. It was assumed that all supplemental feed was purchased. Feed costs included winter feed costs, delivery, and interest as well as feed costs of PWM. All calves were sold at weaning from the ranch and culls were sold at auction in Miles City, MT for each system. Transportation costs and commission were added to cull animals that were sold via auction yard in Miles City, MT (217 km). Tables 1 and 2 present economic inputs including marketing, animal, and feed costs.

#### *Backgrounding*

In all systems, heifers were sold at weaning; data were available only for steers through the backgrounding phase. No morbidity or health cost information was collected during the study, therefore it was assumed constant across all systems and not accounted for in the model. Apr and Jun LW RO 1 system data for 2000 were excluded from this study because they were not shipped to El Reno until after the backgrounding phase. Steers were offered a pre-test diet before the application of the grower treatment, this period varied between herds and years and no data was collected for 1999. Therefore the cost of this diet was added to the grower portion.

Transportation costs were assigned for cattle traveling to location for backgrounding and steers were sold directly from the location. For systems backgrounded in MT, a 217 km haul was assumed for steers traveling to Miles City. For systems backgrounded in OK, the trip was 1910 km from Miles City to El Reno, OK. Feed costs are presented in Table 3. A pencil shrink of 2% was applied to steers at sale. No yardage fee was charged, opportunity cost on investment was 5%, and a 1% death loss was assumed for the entire phase. For steers backgrounded in treatments 1 and 2, the endpoint of the backgrounding phase was determined by date. For steers backgrounded in treatment 3, the endpoint of the backgrounding phase was determined by weight.

#### *Statistical Analysis*

Ranch gross margin (RGM = gross revenue minus variable costs) for cow-calf systems and cumulative gross margin (CGM = RGM plus gross revenue from backgrounding minus variable costs) for

systems utilized the backgrounding phase were analyzed with the GLM procedure in SAS (SAS Institute, Cary, NC). Each level of production was analyzed with a model that included system and year.

## **Results and Discussion**

Grings et al. (2002) reported that the proportion of heifers that reached puberty by the breeding season was greater for heifers fed to maintain a constant gain over those fed a diet intended to minimize the amount of harvested feed although heifers had reached similar weights by the breeding season. However, no differences were detected in CGM for systems utilizing the two PWM scenarios at any level of production. Therefore systems where PWM was the only difference were averaged together reducing the total number of systems to 18.

Grings et al. (2003) reported lighter weaning weights for Jun calves than for Feb and Apr calves. This is similar to the findings of Adams et al. (2001) and Smith et al. (2001) where weaning weight decreased as calving season advanced. However, our results show that for cow-calf enterprises selling calves at weaning, Jun systems yielded higher RGM than all other systems, and were statistically higher than Apr early-weaned system ( $P < 0.05$ ; Table 1). This is primarily due to the increase in feed costs for systems calving in Feb and Apr (Table 2). This is consistent with the results of May et al. (2000) and Adams et al. (1994), where later calving reduced feed costs during the winter feeding period. Armstrong et al. (1990) reported that as feed costs increased, net returns decreased regardless of resource constraints, management, or calving rates.

When steer calves were backgrounded after weaning, systems utilizing Jun calving yielded higher gross margin than those utilizing Feb or Apr ( $P < 0.05$ ; Figure 2). Although there were differences in feed and transportation costs (data not shown) between the backgrounding treatments, there were no differences in CGM between backgrounding treatments within calving season. This is primarily due to differences in the amount of value gained by steers in the backgrounding phase and timing of sale. Jun steers gained a large amount of weight compared with Feb and Apr steers (data not shown) in backgrounding treatments 1 and 2. In treatment 3, Jun steers were not sold on a time basis but on weight and were sold at a favorable time.

There were significant year effects for both the cow-calf and backgrounding phases ( $P < 0.05$ ). This was primarily due to environmental effects during the winter feeding period and the amount of feed needed to maintain animal performance for cow-calf systems as shown in Table 2. For systems that included backgrounding, most of the differences among years were due to differences in prices received and time of year steers were sold.

## **Implications**

Many cow-calf producers consider changes in calving season either to increase fall calf weights or to

more closely match nutrient requirements to the available forage quality. For producers in the Northern Great Plains, managing ranches similarly to these systems, June calving offers promise as a means to increase profit. The benefits of reducing input costs exceeded income lost due to the reduction in weaning weights through backgrounding.

### Literature Cited

- Adams, D.C., R.C. Clark, S.A. Coady, J.B. Lamb, and M.K. Nielsen. 1994. Extended grazing systems for improving economic returns from Nebraska sandhills cow/calf operations. *J. Range Manage.* 47:258-263.
- Adams, D.C., R.C. Clark, R. Sandberg, G. Carriker, T. Klopfenstein, and T. Milton. 2001. June versus March calving for the Nebraska sandhills: Production traits. 2001 Nebraska Beef Report. pp 8-9.
- Armstrong, S.L., J.W. Wilton, W.C. Pfeiffer, and L.R. Schaeffer. 1990. Influence of variations in biological and economical parameters on beef production net returns. *J. Anim. Sci.* 68:1857-1866.
- Grings, E.E., R.E. Short, T.W. Geary, and M.D. MacNeil. 2002. Heifer development within three seasons of calving. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 53:261-264.
- Grings, E.E., R.E. Short, and R.K. Heitschmidt. 2003. Effects of calving date and weaning age on cow and calf production in the Northern Great Plains. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 54:335-338.
- Grings, E.E., R.E. Short, and R.K. Heitschmidt. 2004. Post-weaning production of steers from varying calving and weaning strategies. *Proc. West. Sec. Amer. Soc. Anim. Sci.* Submitted.
- May, G.J., L.W. van Tassell, J.W. Waggoner, and M.A. Smith. 1999. Relative costs and feeding strategies associated with winter/spring calving. *J. Range Manage.* 52:560-568.
- Peterson, G.A., T.B. Turner, K. M. Irvin, M.E. Davis, H.W. Newland, and W.R. Harvey. 1987. Cow and calf performance and economic considerations of early weaning of fall-born beef calves. *J. Anim. Sci.* 64:15-22.
- Reisenauer, V.L., M.W. Tess, D.A. Griffith, and J.A. Patterson. 2001. Evaluation of calving seasons and marketing strategies in Northern Great Plains cow-calf enterprises. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 52:50-54.
- Smith, M.A, J.W. Waggoner, D.M. Perry, R.H. Hart, and G. Younglove. 2001. Late spring calving lowers cow wintering costs with minimal impact to calf performance. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 52:231-233.
- Tess, M.W. and B.W. Kolstad. 2000a. Simulation of cow-calf production systems in a range environment: I. Model development. *J. Anim. Sci.* 78:1159-1169.
- Tess, M.W. and B.W. Kolstad. 2000b. Simulation of cow-calf production systems in a range environment: II. Model evaluation. *J. Anim. Sci.* 78:1170-1180.

Table 1. General expenses for all cow-calf systems.

Item	Value
Marketing	
Brand inspection and checkoff, \$/animal	1.30
Pencil shrink on calves, %	2
Actual shrink on yearlings and cows, %	4
Commission, culls only, %	2.5
Annual expenses <sup>a</sup> , \$/animal	
Steer calves	11.56
Heifer calves	11.56
Yearling heifers	46.23
Two-year-old cows	50.57
Mature cows	51.18
Bulls	579.53
Interest on variable expenses, %	10

<sup>a</sup>Include vaccinations, property taxes, opportunity cost of investment (5% for yearlings and older), miscellaneous health treatments, ear tags, and depreciation (\$427, bulls only).

Table 2. Feed costs, morbidity, calf mortality, and calf weaned per cow calving by calving season for cow-calf systems.

	Feb	Apr	Jun
Feed, \$/animal			
1998-1999	149.23	97.72	87.93
1999-2000	75.71	106.87	0.00
2000-2001	216.13	278.73	122.00
Morbidity, %	6	2	2
Calf mortality, %	3.5	1.5	1.5
Calf weaned per cow calving, CWCC, %	96	98	98

Table 3. Yearly feed costs by calving season and weaning strategy for backgrounding systems.

Feed Costs	Feb		Apr		Jun	
\$/animal	EW	LW	EW	LW	EW	LW
Treatment 1						
1999	87.59	69.62	68.03	67.32	61.33	68.29
2000	161.06	150.21	107.09	n/a	109.20	n/a
2001	94.99	69.83	73.21	62.84	61.60	71.23
Treatment 2						
1999	203.80	176.16	147.76	131.73	124.57	118.02
2000	212.42	192.72	160.78	142.36	147.39	124.97
2001	153.77	125.12	164.78	128.66	128.87	119.47
Treatment 3						
1999	136.23	113.90	134.37	118.47	137.90	131.18
2000	143.34	125.32	149.84	157.31	157.36	156.10
2001	162.00	161.08	153.62	115.99	165.08	150.76

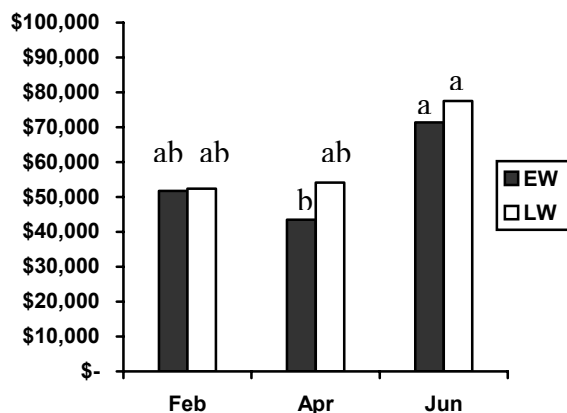


Figure 1. Average ranch gross margin for cow-calf systems utilizing three seasons of calving (Feb, Apr, Jun) and two weaning strategies (early and late weaning).

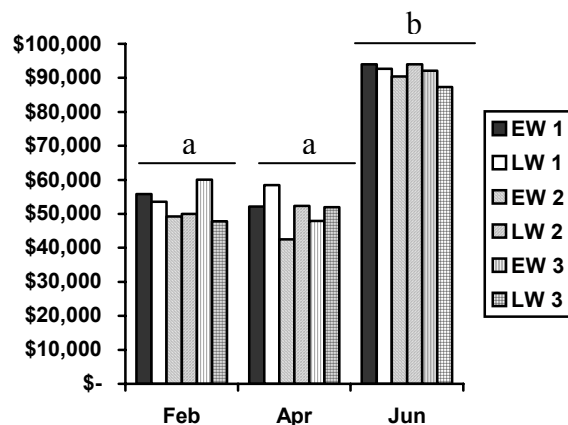


Figure 2. Average cumulative gross margin for backgrounding systems utilizing three backgrounding treatments (1, backgrounded in El Reno, OK; 2, backgrounded in Miles City, MT and fed similarly to those in El Reno; 3, backgrounded in Miles City, MT) within calving season and weaning strategies.

## POST-WEANING PRODUCTION OF STEERS FROM VARYING CALVING AND WEANING STRATEGIES

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**ABSTRACT:** The impact of varied calving and weaning times on post-weaning production of steer calves from the Northern Great Plains was evaluated in a 3-yr study. Steers (n = 215) born in one of three calving seasons [late winter (LW), early spring (ES), or late spring (LS)] were weaned at 4 (LS1), 6 (LW1, ES1, LS2), or 8 (LW2, ES2) mo of age after grazing with their dams on native range. Later weaned cow-calf pairs continued to graze native range until weaning. Steers were pen-fed a corn silage and alfalfa hay-based diet until the weaning group averaged 375 kg. They were then moved to an individual feeding facility and fed a higher energy diet. Steers were individually allotted to harvest dates based upon visual estimates of fat thickness. Data were analyzed as a completely random design with fat thickness as a covariate using mixed model procedures. Year and year by treatment were random effects. Non-orthogonal estimates were used to delineate treatment effects. Initial steer weights averaged  $216 \pm 12$  kg but were affected by calving season and age at weaning, with LS2 steers weighing  $24 \pm 10$  kg less ( $P < 0.05$ ) than the average of the LW1 and ES1, LW and ES steers weaned at 6 mo averaging  $26 \pm 8$  kg less ( $P < 0.01$ ) than those weaned at 8 mo of age, and LS1 weighing  $26 \pm 11$  kg less ( $P < 0.05$ ) than LS2. There were no treatment differences in ADG during the growing or finishing phases. Total days to harvest averaged  $311 \pm 15$  d and differed between LW and ES steers weaned at 6 versus 8 mo of age due to a  $37 \pm 12$  d difference ( $P < 0.01$ ) in time to reach harvest. Total days to harvest did not differ between LS steers weaned at 4 versus 6 mo of age. Steers averaged  $527 \pm 12$  kg at harvest and weights were  $23 \pm 10$  kg less ( $P < 0.01$ ) for ES than LW. Differences in production of steers among calving and weaning strategies may be related to differences in harvest weights and time on feed as affected by weaning weights.

**Key Words:** Calving Season, Age at Weaning, Growth, Beef Cattle

### Introduction

Calves from various calving seasons and weaning strategies may differ in weight at weaning in rangeland-based production systems (Grings et al. 2003). Profitability of post-weaning production of steer calves from these systems could then be influenced by the length of time in the feedlot and, potentially, carcass composition. This study was conducted to determine the post-weaning production characteristics of steers from various beef production systems in the Northern Great Plains.

### Materials and Methods

The 3-yr study was conducted at the Fort Keogh LARRL near Miles City, MT ( $46^{\circ} 22' N$   $105^{\circ} 5' W$ ). Steers (n = 215) were born in one of three calving seasons: late winter (**LW**; average = Feb 8), early spring (**ES**; average = Apr 5), or late spring (**LS**; average = May 31). Calving seasons were the result of 32-d breeding seasons with no overlap between seasons. Crossbred cows were bred by natural service to bulls from a composite herd ( $\frac{1}{2}$  Red Angus,  $\frac{1}{4}$  Tarentaise, and  $\frac{1}{4}$  Charolais). Each calving season had two weaning times: 6 (LW1, ES1) or 8 (LW2, ES2) mo of age for LW and ES steers or 4 (LS1) or 6 (LS2) mo of age for LS steers. Before weaning cow-calf pairs grazed native rangeland with supplemental feed as needed. Later weaned cow-calf pairs continued to graze native range until weaning. Calves received pre-weaning vaccines about 3 wks before weaning with boosters at weaning.

At weaning, steers were immediately transported to feeding facilities (within 5 miles) at weaning and received long-stemmed hay for a few days followed by a corn silage-based diet for about 3 wk. Steers were then weighed early in the morning about 24 hrs after feeding. Steers were sorted into three treatment groups per calving season with one pen per treatment-calving season combination. Results of only one post-weaning treatment will be reported here. Calves from the later weaning within a calving season were penned with the earlier weaned steers when they were placed on treatment. Steers did not receive any implants during their lifetime. Steers were pen-fed a corn silage-based diet (Table 1) until the weaning group averaged 375 kg. They were then moved to an individual feeding facility and trained to work electronic head gates. Once the group reached an average of 404 kg, they were shifted to a diet of higher energy concentration (Table 1). Steers were allotted to harvest dates based upon visual estimates of

<sup>1</sup> Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, Montana AES, or the authors and does not imply its approval to the exclusion of other products that may also be suitable. USDA, ARS, Northern Plains Area, is an equal opportunity/ affirmative action employer. All agency services are available without discrimination.

fat thickness. Steers were sent to a local abattoir for harvest and carcass characteristics were measured as in Grings et al. (2001).

Daily feed offered was recorded. Samples of the mixed diet were collected weekly and DM determined. Feed efficiency was calculated from weight gain while on the finishing diet and average daily DM intake.

Calving season and weaning assignments were assumed to create six treatments to create a completely random design. Data were analyzed using mixed model procedures (SAS, 1996) with year and year by treatment as random effects. Fat thickness was used as a covariate in the model for carcass characteristics to adjust all animals to an equal endpoint. Non-orthogonal estimates were used to delineate treatment effects. Estimates included comparisons of LW v ES calving season for both 6- and 8-mo weaning and for just the 6-mo weaning treatment, LS at 6 mo weaning v the average of LW and ES at 6-mo weaning, weaning at 6- v 8-mo for the average of the LW and ES calving seasons, 4- v 6-mo weaning ages for the LS calving season, and a linear effect of calving season for weaning in October.

## Results and Discussion

Initial steer weights averaged  $216 \pm 12$  kg but were affected by calving season (Table 2). The LS2 steers weighed 24 kg less ( $P < 0.05$ ) than the average of the LW1 and ES1. This lighter initial weight for the LS2 steers at an equivalent age to LW1 and ES1 is related to poorer forage quality during late fall grazing compared to late summer and early autumn forage conditions for growth of the LW and ES steers.

Steer age at weaning also affected initial weight (Table 2). Steers from the LW and ES herds weaned at 6 mo averaged 26 kg less ( $P < 0.01$ ) than those weaned at 8 mo of age. The LS1 steers weighed 26 kg less ( $P < 0.05$ ) than LS2 steers.

No treatment differences ( $P > 0.10$ ) in ADG during the growing or finishing phases (Table 2) were observed. Feed efficiency during the finishing period also did not differ ( $P > 0.10$ ) by treatment. Feed efficiency during the growing phase was not measured. Results are consistent with Lardy et al. (1999) who reported no effect of spring- versus summer-calving season on post-weaning ADG or feed efficiency.

Total days to harvest averaged  $311 \pm 15$  d. Age at weaning affected total days to harvest for LW and ES steers with those weaned at 6 mo of age requiring an extra 37 d ( $P < 0.01$ ) in the feedlot compared to those weaned at 8 mo of age. Total days to harvest are artificially elevated in this study because of the time required to adapt steers to electronic head gates between the growing and finishing periods.

Steers were harvested at an average of  $511 \pm 14$  d of age. Steers born in LS and weaned at 4 mo of age (LS1) were 35 d younger ( $P < 0.05$ ) at harvest

than LS calves weaned at 6 mo of age (Table 1). Additionally, age at October weaning exhibited a linear effect ( $P < 0.05$ ) on age at harvest. Several researchers have reported that steers weaned at about 3.5 to 4 mo of age reach harvest at younger ages than steers weaned at closer to 7 mo-of age when harvested at an equivalent fat thickness (Fluharty et al., 2000, Schoonmaker, 2002). However, Fluharty et al. (2000) and Myers et al. (1999) attributed this difference to weight gains between early and late weaning, which was not the case in this study. We previously reported an interaction between calving season and weaning times with a decline in rate of gain between early and late weaning as calving season became later (Grings et al., 2003).

Steers averaged  $527 \pm 12$  kg at harvest and weights were  $23 \pm 10$  kg less ( $P < 0.01$ ) for ES than LW steers (Table 3), even though animals were the same age at the start of the experiment. Relationships among treatments in weights at the start of the study did not carry over to harvest weights. This differs from results of Schoonmaker et al. (2002) who found age at feedlot entry to affect harvest weights, even when steers were harvested at equal fat thickness.

Hot carcass weight averaged  $304 \pm 6$  kg and weights were 18 kg less ( $P < 0.01$ ) for ES compared to LW (Table 3). There was a linear decrease ( $P < 0.01$ ) in hot carcass weight associated with decreasing age for steers weaned in October. In contrast to harvest weight, results for hot carcass weight are consistent with the results of Schoonmaker et al. (2002) who reported decreases in hot carcass weight for steers entering the feedlot at younger ages when harvested at a constant fat thickness endpoint.

Fat thickness did not differ ( $P > 0.10$ ) by treatment (Table 3), indicating that visual observations were adequate for determining time of harvest at equivalent fat thicknesses for this study.

Longissimus muscle area (LMA) averaged  $82.6 \pm 12$  cm<sup>2</sup>. A linear decrease in LMA as age decreased from 8- to 4-mo of age at weaning in October was observed (LW2, ES1, LS1; Table 3). Schoonmaker et al. (2002) reported decreased LMA in cattle weaned at 111 compared to 202 d of age but Myers et al. (1999) did not observe a similar effect for steers weaned at 90, 152 or 215 d of age. Breed type and diets varied among all of these studies.

Marbling score averaged  $417 \pm 28$  but differed ( $P < 0.05$ ) by 59 units between LW and ES steers (Table 3). The relationship was less and not significant ( $P > 0.10$ ) for LW1 compared to ES1. A linear effect of age at weaning in October also affected ( $P < 0.05$ ) marbling score. Other researchers have reported no effect of weaning age on marbling score (Myers et al., 1999, Schoonmaker et al., 2002).

When carcass characteristics for LS2 were compared to the average of LW1 and ES1 (all 6 mo of age at weaning), kidney, pelvic, and heart fat percentage and yield grade were both decreased ( $P < 0.05$ ) for LS2 (Table 3). Yield grade was also

decreased ( $P < 0.05$ ) by 0.13 for steers weaned at 6 versus 8 mo of age (LW1 + ES1 versus LW2 + ES2).

Quality grade averaged  $11.9 \pm 0.3$  or about Choice – for steers in this study (Table 3). Quality grade was greater ( $P < 0.05$ ) for LW than ES and there was a linear decrease ( $P < 0.05$ ) in quality grade for calves of younger ages at the October weaning.

### Implications

Season of birth can impact growth and carcass composition independent of age of steers at weaning when harvested at equivalent fat thickness. This may be related to environmental factors both during the feeding period and before weaning. Calving season should, therefore, be considered in effective feedlot management strategies along with weaning age and weight at feedlot entry.

### Literature Cited

- Fluharty, F. L., S. C. Loerch, T. B. Turner, S. J. Moeller, and G. D. Lowe. 2000. Effects of weaning age and diet on growth and carcass characteristics in steers. *J. Anim. Sci.* 78:1759-1767.
- Grings, E. E., R. E. Short, and R. K. Heitschmidt. 2003. Effects of calving date and weaning age on cow and calf production in the Northern Great Plains. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 54:335-338.
- Grings, E. E., R. E. Short, M. D. MacNeil, R. A. Roeder, and M. J. Roeder. 2001. Interactions in postweaning production of F1 cattle from Hereford, Limousin, or Piedmontese sires. *J. Anim. Sci.* 79:317-324.
- Lardy, G., D. Adams, R. Clark, and T. Klopfenstein. 1998. Performance of summer- and spring-born calves finished as calves or yearlings. *Nebraska Beef Report*. Pp. 3 – 5. Univ. Nebraska Coop Exten., Lincoln.
- Myers, S. E., D. B. Faulkner, F. A. Ireland, and D. F. Parrett. 1999. Comparison of three weaning ages on cow-calf performance and steer carcass traits. *J. Anim. Sci.* 77:323-329.
- SAS. 1996. SAS System for Mixed Models. SAS Institute Inc. Cary, NC.
- Schoonmaker, J. P., S. C. Loerch, F. L. Fluharty, H. N. Zerby, and T. B. Turner. 2002. Effect of age at feedlot entry on performance and carcass characteristics of bulls and steers. *J. Anim. Sci.* 80:2247-2254.

Table 1. Diets fed to steers during growing and finishing phases

Ingredient	Growing	Finishing
	-----% of DM-----	
Corn silage	62.5	16.5
Rolled Barley	19.4	39.5
Cracked corn	-	40.7
Alfalfa hay	14.8	-
Soybean meal	2.3	1.3
Limestone	0.3	1.4
Urea	0.5	0.3
Salt	0.1	0.2
Trace mineral mix	0.03	0.04
Vitamin A,D,E	0.04	0.04
Chemical		
DM, %	39.8	65.5
CP, % of DM	11.7	10.8
ADF, % of DM	25.7	12.3
Estimated NEm, Mcal/kg DM	1.65	1.97
Estimated NEg, Mcal/kg DM	1.03	1.31

Table 2. Means and treatment effects ( $\pm$  SE) for body weight gain, days on feed and feed efficiency during the growing and finishing periods of steers born in late winter (LW), early spring (ES), or late spring (LS) and weaned at two ages<sup>a</sup>

	Effect Estimates								
	Mean	ES - LW	ES1 - LW1	LS2 – ((LW1 + ES1)/2)	((LW2+ES2)/2) – ((LW1+ES1)/2)	LS2 - LS1	Oct linear <sup>b</sup>		
On test weight, kg	216 ± 12	-8 ± 8	-5 ±11	-24 ±10*	26 ± 8**	26 ± 11*	-82 ± 11**		
Grower ADG, kg/d	1.1 ± 0.06	0.01± 0.07	-0.02 ± 0.07	0.04± 0.06	0.06 ± 0.05	0.06 ± 0.07	-0.06 ± 0.07		
Days on growing diet	195 ± 24	14 ± 18	-15 ±26	35 ± 22	-27 ± 18	-40 ± 26	124 ± 26**		
Days on finishing diet	116 ± 9	-29 ± 8**	-37 ±11**	11 ± 10	2 ± 8	24 ± 11	-24 ± 11		
Total days to harvest	311 ± 15	-15 ± 12	-21 ±17	18 ± 14	-37 ± 12**	-21 ± 17	71 ± 17**		
Age at harvest, d	511 ± 14	-10 ± 11	-12 ±15	17 ± 13	17 ± 11	35 ± 15*	-39 ± 15*		
Gain to feed, finishing phase, g/kg DM	0.15± 0.01	-0.01± 0.01	-0.01 ± 0.01	-0.02± 0.01	-0.01 ± 0.01	-0.02 ± 0.01	0.004± 0.007		

<sup>a</sup> Weaning ages for LW and ES = 6 and 8 mo of age and for LS = 4 and 6 mo of age

<sup>b</sup> Linear effect of weaning in October at 8-, 6-, or 4-mo of age for LW, ES, LS, respectively.

\*,\*\* Effect significant at  $P < 0.05$  or  $0.01$ , respectively.

Table 3. Means and treatment effects ( $\pm$  SE) for carcass characteristics of steers born in late winter (LW), early spring (ES) or late spring (LS) and weaned at two ages<sup>a</sup>

	Effect Estimates								Oct linear <sup>b</sup>
	Mean	ES - LW	ES1 - LW1	LS2 – ((LW1 + ES1)/2)	((LW2+ES2)/2) – ((LW1+ES1)/2)	LS2 - LS1			
Harvest weight, kg	527 ± 12	-23 ± 10*	-40 ± 14*	-10 ± 12	-6 ± 10	5 ± 13	-13 ± 14		
Hot Carcass weight, kg	304 ± 6	-18 ± 4**	-27 ± 6**	-7 ± 5	-1 ± 4	7 ± 6	-17 ± 6**		
Fat thickness, cm	0.9± 0.1	-0.1 ± 0.1	-0.1 ± 0.1	-0.2 ± 0.1	-0.1 ± 0.1	0.1 ± 0.1	-0.1 ± 0.1		
Longissimus muscle area, cm <sup>2</sup>	82.2± 1.7	-2.0 ± 1.2	-2.2 ± 1.7	0.3 ± 1.5	1.7 ± 1.2	1.9 ± 1.7	-4.2 ± 1.7*		
Marbling score <sup>c</sup>	417 ± 28	-59 ± 20*	-47 ± 28	-26 ± 24	-18 ± 20	42 ± 2.8	-85 ± 28*		
Kidney , pelvic, heart fat, %	2.4± 0.1	0.04± 0.04	0.03± 0.12	-0.28± 0.1*	-0.15± 0.08	-0.10± 0.10	-0.0001± 0.12		
Yield grade	1.4± 0.08	-0.04± 0.05	-0.12± 0.08	-0.15± 0.07*	-0.13± 0.05*	-0.05± 0.08	0.05 ± 0.08		
Quality grade <sup>d</sup>	11.9± 0.3	-0.5 ± 0.2*	-0.2 ± 0.3	-0.3 ± 0.2	-0.2 ± 0.2	0.4 ± 0.3	-0.8 ± 0.3*		

<sup>a</sup> Weaning ages for LW and ES = 6 and 8 mo of age and for LS = 4 and 6 mo of age

<sup>b</sup> Linear effect of weaning in October at 8-, 6-, or 4-mo of age for LW, ES, LS, respectively.

\*,\*\* Effect significant at  $P < 0.05$  or  $0.01$ , respectively.

<sup>c</sup> Practically devoid = 100-199, traces = 200 to 299, slight = 300 to 399, small = 400 to 499, modest = 500 to 599, moderate = 600 to 699.

<sup>d</sup> Prime+ = 17 , Prime = 16 , Prime - = 15, Choice + = 14, Choice = 13, Choice - = 12, select = 11 standard = 10

## EFFECTS OF DELAYED CASTRATION OF BRITISH CROSS-BRED BEEF CATTLE ON WEIGHT GAIN, CARCASS TRAITS, AND CONSUMER ACCEPTABILITY

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**ABSTRACT:** The objective of this research was to determine the effect of time of castration on ADG, carcass characteristics and consumer preference. Sixty-five bull calves were randomly assigned to three treatments: early castrates (E-106 kg), mid castrates (W-243 kg) and late castrates (L-365 kg). All calves were treated the same relative to processing but at time of castration they received a single implant. Castration was performed using the Callicrate Smart Bander™ bloodless castration method. Calves were penned together and fed a balanced ration throughout the weaning, back grounding and finishing stages and ADG obtained using initial and final weights. Carcass data was also collected. Results indicated there were no differences between treatments for ADG ( $P=.89$ ), backfat ( $P=.23$ ), ending live weight ( $P=.73$ ), hot carcass weight ( $P=.69$ ), or dressing percentage ( $P=.82$ ). Ribeye area and cutability were higher for the L treatment ( $P=.06$  and  $P=.009$  respectively), and marbling score and yield grade were lower for L ( $P=.001$  and  $P=.009$  respectively). Consumer taste panels showed older (30-40 years average age) panelists who ate beef regularly identified the E treatment as more tender ( $P=.035$ ), juicy ( $P=.005$ ), and flavorful ( $P=.02$ ) than W or L. Overall acceptability was significantly better also for E over W and L ( $P=.05$ ). The younger (20-30 years average age) panelists who ate beef less than once a month rated the early castrates as less juicy than W or L treatments ( $P<0.01$ ), but did not detect any other differences for tenderness ( $P=.64$ ), flavor ( $P=.73$ ) or overall ( $P=.49$ ). Warner-Bratzler shear force measurements indicated a trend of decreased tenderness for the late castrate treatment ( $P=.40$ ).

Key words: Castration, Carcass, Preference

### Introduction

Delayed castration of beef calves has been suggested as a means to improve live animal performance (ZoBell et al., 1993). Typically, castration occurs at birth or late spring which prevents bullish carcass characteristics. Young bulls, however, have been documented to be very efficient producers of lean meat (Field, 1971, Klastrup et al., 1984, Seideman et al., 1982).

Despite excellent production traits, bulls can be aggressive in confinement and may produce high yielding, lower quality carcasses which lack acceptance

in the market place. Research indicates that bull carcasses compared to steers have less marbling, lower USDA quality grades, darker lean color and lower tenderness (Seideman et al. 1982). In addition, meat packers penalize bull carcasses with discounted prices. If castration management could produce accelerated gain without the negative bull carcass traits, delaying castration may be an alternative option for livestock producers.

One study showed that delayed castration did not result in behavioral or management problems, however, it did not produce a commercially useful increase in growth rate (Gazzola et al. 2002). Berry et al. (2001) and Brazle (1992) showed that lightweight, newly-received steer calves had improved performance over lightweight, newly-received bulls banded or surgically castrated. Steers gained faster during the 33-day receiving trial than bulls castrated by surgically or banding. In another study, Brazle (1992) found yearling steers gained faster than yearling castrated bulls. Other research showed that steers that were early castrated/implanted had weaning weights similar to those of bull calves, both of which weighed more than the early castrated/no implant contemporaries. Yet, 28 days after weaning, the early castrated/implanted steers weighed more than either of the early castrate/no implant or late castrated steers (Marston et al. 2003). ZoBell et al. (1993) showed both surgical and banded castrates had severe reduction on average daily gains when compared to intact bulls. However, banded bulls performance was higher than surgically castrated bulls. No differences in carcass traits were identified in this study.

The objective of this research was to determine the effect of time of castration on ADG, carcass characteristics and consumer preference.

### Materials and Methods

Sixty-five bull calves were randomly assigned to three treatments: Early (E) castrated prior to 90 days of age (106 kg), Weaned (W) castrated at 225 days of age (243 kg), and Late (L) castrated at 380 days of age (365 kg). All calves were implanted with Ralgro® at castration. Calves were weighed October 15 and received the following injections: Clostridium Chauvoei-Septicum-Novyi-Sordellii-Perfringens Types C & D-Haemophilus Somnus Bacterin-Toxoid (Pfizer Animal Health, New York, NY); Bovine Rhinotracheitis-Virus Diarrhea-Parainfluenza3-

Respiratory Syncytial Virus Vaccine Pasteurella Haemolytica Toxoid (Fort Dodge Animal Health, Overland Park, KS)); and Nasalgen\*IP (Schering-Plough Animal Health, Kenilworth, NJ). At time of castration calves were implanted with Ralgro® and given an injection with Bar Vac CD/T (Clostridium Perfringens Types C&D-Tetanus Toxoid (Boehringer Ingelheim, St. Joseph, MO). Calves were castrated using the Callicrate Smart Bander (No-Bull Enterprises LLC, P.O. Box 748, St. Francis, KS 67756).

The trial started on June 1 when the E calves were castrated. All cow-calf pairs were then pastured as a group on mountain meadows and weaned October 15. The treatment groups were fed together during the entire feeding phase (Oct 15 to slaughter). During the background feeding phase, the calves were fed 2.5% of their body weight (DMB) a diet consisting of 19% alfalfa hay, 27% oat/triticale hay, 36% wheat midds, and 18% rolled corn (DMB). The finishing diet consisted of 73% rolled corn., 8.2% corn silage, 9.4% alfalfa hay, 4.7% whole cottonseed, and 4.7% calf supplement with free choice trace mineralized salt (DMB).

The age of the dams ranged from 3 to 8 years of age. The calves were weighed individually at the beginning, 4 times throughout the 424 day period, and at the end of the trial.

The steers were harvested at a commercial slaughter plant and processed with accepted meat standards. Calves were sent to slaughter based on days on feed and weight. Individual carcasses were measured for rib eye area (REA), marbling score (MS), yield (CY) and quality grade (QG), back fat thickness (BF) and carcass weight (CW).

Five carcasses from each treatment were randomly selected and one ribeye from each carcass was removed for taste panels and tenderness studies (n=60). The ribeyes were aged for 10 days similar to a commercial situation. Two untrained consumer taste panel evaluations were completed. Panel #1 was completed during a break at a professional conference of Utah State University Extension employees which were working professionals. Panel #2 was completed at the Utah State University consumer taste panel laboratory using college students. Steaks were cooked to medium well done.

Frying procedures consisted of: Frozen steaks (-20° C) were tempered over-night at 2° C until meat temperature was about 0° C. Tempered steaks were placed on the preheated grill (163° C) for 2.5 min, and then flipped at 2.5 min intervals until internal temperature reached 74 ± 2° C (medium well done). The grill (Hotpoint electric grill; General Electric Model HG4, Chicago Heights, IL) consisted of a steel griddle plate, 600 x 500 x 25 mm with 2 temperature control devices, each connected to the heating elements mounted below the plate, under a ventilation hood at air velocity of 5.0m/sec. Internal

temperature during cooking was measured using a VERSATUFF 396 digital thermometer with micro-needle probe (Atkins Technical, Inc., Gainesville, FL). Treatments were given a three digit code and panelists rated the samples on a 1-9 scale for tenderness, juiciness, flavor and overall quality. The Rating scale was as follows: 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely. Panelists also indicated their age and how often they eat steak.

Warner-Bratzler (G. R. Electric Manufacturing Co., Manhattan, KS) shear force was measured as an indicator of tenderness of cooked steaks. Three steaks were randomly selected from each treatment (E, W, or L treatments) for shear measurements. After cooking, steaks were cooled 30 min at room temperature. Then 1-cm diameter cores (5 per steak) were taken parallel to the muscle fiber axis, so that shearing was perpendicular to the fiber axis.

Statistical analysis for the analysis of ADG and carcass traits were performed using the MIXED procedure of SAS (SAS Institute, Cary, NC) using a completely randomized design with a repeated measures treatment structure with animal as the experimental unit. Statistics for consumer taste panel evaluation of steaks from E, W and L steers were performed using Statistica™ (Statsoft Inc., Tulsa, OK). Treatment means were calculated by one-way ANOVA and significant differences between means determined by calculation of Fisher's LSD values, when appropriate.

## Results and Discussion

There were no differences in live animal performance between treatments throughout the trial ( $p \leq 0.05$ ) (Table1).

At harvest, dressing percent and hot carcass weights were not different between treatments (Table 2). Marbling scores decreased significantly in the later castrates. Eighty-seven percent of treatment E graded choice or better and 75% and 47.6% of treatment W and L, respectively, graded choice or better. Kidney, pelvic heart fat (KPH) and back fat (BF) were similar for all treatments. Delaying castration increased ribeye area (REA) and cutability. Yield grade is determined largely by REA and BF. Delayed castrates showed significantly lower yield grades reflecting increased red meat yield.

Consumer taste panel results were mixed. The demographics of the consumer groups influence results. Taste panel #1 was made of participants who were older, 30-40 years of age, and ate beef steak regularly, 1-3 times a month. Taste panel #2 panelists were younger, 20-30 years and ate beef steak less than once a month. The 62 panelists of taste panel #1 identified the early castrates as significantly more tender, juicy, and flavorful than either of the delayed castrate treatments. Overall acceptability was

rated higher for early castrates and was different from the other treatments ( $P<0.05$ ) (Table 3). The 77 participants of panel #2 indicated that the early castrates were less juicy than the other treatments ( $P<0.05$ ) (Table 4). No differences were detected in flavor, tenderness or overall acceptability between panelists.

Early, W and L castrates had Warner-Bratzler shear measurements of 3.80, 4.04 and 4.21 respectively ( $P>0.05$ ). Shear force measurements above 6.0 indicate unacceptable tenderness and an increased likelihood of consumer dissatisfaction.

### Conclusions

These results indicated there was no advantage in ADG or ending live weight when calves were left intact. There was, however, significant differences for late castrated calves on various carcass characteristics including increased REA and decreased MS. This may offer a marketing potential for producers desiring to market a leaner carcass. Consumer panels showed that improved tenderness, juiciness, flavor and overall acceptability could be identified in the early castrated treatment. However, there were not any unacceptable steaks in either treatment. This data suggests that in order for producers to produce a quality consumer product, it is recommended that calves be castrated early unless they are managed differently.

### References

Berry, B. A., W. T. Choat, D. R. Gill, C. R. Krehbiel, R.

A. Smith and R. L. Ball. 2001. Effect of Castration on health and performance of newly received stressed feedlot calves. <http://www.ansi.okstate.edu/research/2001rr/21/21.htm>.

Brannang. 1966. Annals of Agriculture, Sweden, Vol. 32:329-415.

Brazle F. K. 1992. Effect of castration method on stocker health and gain. Cattleman's Day Report of Progress 651. P.80-2. Kansas State University Ag Exp. St.

Field, R.A. 1971. Effects of castration on meat quality and quantity. J. Anim. Sci. 32:849.

Gazzola C., M. R. Jeffery, D. H. White, R. A. Hill, and D. J. Reid. 2002. Effect of delayed castration on growth rate, behavior and serum insulin-like growth factor-1 concentration of beef cattle on tropical pasture. British Society of Animal Science 75:41-47.

Klastrup, S. et al. 1984. Effects of castration and electrical stimulation on beef carcass quality and palatability characteristics. J. Anim. Sci. 58:75-84.

Marston T.T., D. A. Llewellyn, L. C. Hollis and J. W. Homm, 2003. Effects of castration age and a growth implant during suckling on weaning and preconditioning weights. Cattleman's Day 2003.

Seideman, S.C. et al. 1982. Utilization of the intact male for red meat production: A Review. J. Anim. Sci. 55:826.

ZoBell, D.R., L. A. Goonewardene, and K. Ziegler. 1993. Evaluation of the bloodless castration procedure for feedlot bulls, Can. J. Anim. Sci. 73:967-970.

Table 1. The effect of age at castration of beef males on ADG ( $\text{kg}\cdot\text{d}^{-1}$ ).

Period	Early (E)	Weaning (W)	Late (L)	SEM	P
0-136d	1.00	1.05	1.07	0.08	0.40
136-252d	0.77	0.69	0.67	0.08	0.18
252-291d	0.99	1.00	1.02	0.08	0.32
291-385d	1.23	1.25	1.16	0.09	0.32
385-424d	1.45	1.43	1.58	0.09	0.07
Overall	1.09	1.08	1.10	0.04	0.89

<sup>a,b</sup> Different superscripts in the same row are significantly different between means ( $P<0.05$ ).

Table 2. The effect of delaying castration of beef males on carcass weights and carcass characteristics.

Variable	Early (E)	Weaning (W)	Late (L)	SEM	P
Live wt. (kg)	577.9	568.7	565.8	15.3	0.73
Dressing %	61.4	60.7	61.2	0.98	0.82
Hot wt (kg)	355	344.9	349.9	11.7	0.69
Marbling score	5.89 <sup>a</sup>	5.73 <sup>a</sup>	4.99 <sup>b</sup>	0.24	0.001
KPH (%)	2.40	2.38	2.38	0.15	0.98
REA (sq cm)	81.2 <sup>a</sup>	82.8 <sup>a,b</sup>	87.1 <sup>b</sup>	2.45	0.06
Back fat (cm)	1.25	1.07	1.09	0.13	0.23
Cutability (%)	49.4 <sup>a</sup>	50.3 <sup>a,b</sup>	50.6 <sup>b</sup>	0.35	0.01
Yield grade	3.15 <sup>a</sup>	2.80 <sup>a,b</sup>	2.67 <sup>b</sup>	0.15	0.01

<sup>a,b</sup> Different superscripts in the same row are significantly different between means ( $P<0.05$ ).

Table 3. Consumer Taste Panel #1. The effect of delaying castration of beef males on tenderness, juiciness, flavor and overall acceptability.

Variable	Early (E)	Weaning (W)	Late (L)	SEM	P
Tenderness	7.48 <sup>a</sup>	6.95 <sup>b</sup>	6.90 <sup>b</sup>	0.20	.04
Juiciness	7.50 <sup>a</sup>	6.96 <sup>b</sup>	6.75 <sup>b</sup>	0.20	.01
Flavor	7.00 <sup>a</sup>	6.24 <sup>b</sup>	6.35 <sup>b</sup>	0.20	.02
Overall	7.37 <sup>a</sup>	6.56 <sup>b</sup>	6.73 <sup>b</sup>	0.20	.05

<sup>a,b</sup> Different superscripts in the same row are significantly different between means (P<0.05).

Table 4. Consumer Taste Panel #2. The effect of delaying castration of beef males on tenderness, juiciness, flavor and overall acceptability.

Variable	Early (E)	Weaning (W)	Late (L)	SEM	P
Tenderness	6.64	6.84	6.62	0.20	0.64
Juiciness	6.24 <sup>a</sup>	6.98 <sup>b</sup>	6.67 <sup>b</sup>	0.20	0.01
Flavor	6.70	6.66	6.53	0.20	0.73
Overall	6.50	6.76	6.66	0.20	0.49

<sup>a,b</sup> Different superscripts in the same row are significantly different between means (P<0.05).

## COMPUTER MATCHING OF DIGITAL IMAGES OF RETINAL VASCULAR PATTERNS OF SHEEP FOR ANIMAL VERIFICATION

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**ABSTRACT:** The accuracy and repeatability of a matching process using digital images of the retinal vascular pattern (RVP) of sheep as a component of an animal verification system were evaluated. In sheep and other ruminants the central artery and vein enter the back of the eye along the optic nerve and divide to supply the retinal surface. The configuration of this vascular bed develops and is completed during fetal growth and remains constant throughout the life of the animal. The number and position of these vascular branches can serve as a biomarker to identify individual animals. Hardware and software were developed to capture digital images of the RVP and convert the images into a quantifiable format. A single technician collected digital RVP images from both eyes of 108 ewes and the information stored in a database. To evaluate the ability to match each animal to the correct profile in the database, the ewes were subsequently randomized and RVP images were collected from only one eye, by the same technician. The process of matching the 1-eye records to the original data resulted in 100% of the animals being correctly matched and identified. Of the 108 1-eye records, 106 were initially matched using the current generation of matching software. The two remaining records were subsequently matched to their respective 2-eye profile by visual observation. To evaluate repeatability of the matching process, nine of the 108 ewes were randomly selected and RVP images were collected from a single eye at three separate times. In all cases (100%) the ewes were correctly identified by comparison to the database, using the current software. These results confirm that hardware and software developed to collect and process digital RVP images from sheep can be accurately used for animal identity verification.

**Key Words:** Animal Identification, Retinal Vascular Pattern, Source Verification

### Introduction

Retention of individual identification records for livestock throughout their lifespan is increasingly important to the livestock industry and may, in fact, be mandated in the future. In addition to routine bookkeeping, individual identification is critical for selection of superior breeding animals and value-based marketing of animals for slaughter. Individual identification procedures coupled with the ability to track

animal movement (i.e. such as through GPS methods) may also be essential to identify sources of disease and confirmation of “non-exposure” to diseased animals because of site limits.

A collaborative project was conducted to evaluate the ability of a retinal-imaging device to identify individual animals and track their movements through GPS methods. This experiment was conducted to provide estimates of our current ability to individually identify ewes based on previously collected retinal images.

### Materials and Methods

Individually identified ewes (n = 108) greater than one year of age were selected from the University of Wyoming flock. Retinal vascular images were collected by a single technician from both eyes of all ewes using a retinal imaging device (Optibrand Ltd, LLC; Fig. 1) and were stored in a database along with each animal's identification code. Subsequently, the ewes were mixed, numbered 1 to 108 and a single image was collected from one eye of each ewe by the same technician without knowledge of individual ewe identification. The single image was compared to the database to determine animal identity using Optibrand software. Actual ewe identification was not provided to the technician until completion of the study.

To assess repeatability of the matching process, nine of the original 108 ewes were randomly selected and an image was collected from one eye of each ewe animal at three subsequent times without knowledge of their true identity. At each time, code numbers (i.e. 1-9) for image collection were based on their order through the chute.

Values evaluated at the conclusion of the experiment included; number and proportion of ewes correctly identified, incorrectly identified, and unable to be identified. In addition, times required for the collection of images were recorded throughout the experiment.

### Results and Discussion

Alliances within the sheep industry have, and are being formed to enhance the industry's ability to compete on a global scale. One approach to capitalizing on production and carcass traits of superior animals is

value-based marketing. Unique aspects of western range sheep operations, however, pose special concerns that require solutions for the long-term success of such a value-based marketing alliance. For example, most of the ewes in this region are maintained in large groups, many are herded on private and public lands, and multiple sires are used at breeding. Environmental conditions often dictate times of lambing and, once weaned, lambs are placed in large feeding groups prior to slaughter. Hence, continued improvement in those carcass traits that warrant “premium payments” is hampered by the inability to retain the identity of individual lambs from birth through the slaughter facility. In addition, the ability to positively identify individual animals throughout their productive life is necessary to allow differential compensation to be paid for product quality and encourage “good management practices” (i.e. antibiotic treatments and withdrawal intervals, injection sites, etc.).

Common methods of identification such as ear tags or paint brands are often lost, become unreadable, and can create confusion when animals from different producers are combined because of non-conformity in the use of accompanying codes. More elaborate methods of identification such as DNA testing do exist, but are currently cost prohibitive. Vascular patterns of the retina are individually unique (De Schaepdrijver et al., 1989), much akin to the uniqueness of fingerprints, and may offer an alternative permanent method of identification. Based on this likelihood, Optibrand Ltd., LLC developed the OptiReader™ Device that captures and stores the image of an animal’s retina (Fig. 2). At the time of image capture, a GPS date, time and location stamp is also stored. An accompanying software package has also been developed that allows for data storage and retrieval (Whittier et al., 2003).

Of the 108 ewes with retinal images from both eyes recorded in the database, 106 subsequently scanned in the single eye scanning session were matched and correctly identified using current Optibrand software. The remaining two animals were correctly identified by visually comparing the remaining single eye images to images remaining in the database. In addition, the nine ewes were correctly identified at each of the subsequent three times using the software providing a repeatability estimate of 100%. The mean image collection time for the 370 images was  $15.4 \pm 0.8$  s (range 1.7 to 96.7 s).

In conclusion, retinal vascular imaging is a reliable method that can be used for positive animal identification.

### Implications

The retinal scanning methodology provides the ability to positively identify animals throughout their productive life and following slaughter. This technology also has the potential of revolutionizing record keeping because the software has the potential of storing all production data for individual animals in association with their identification.

### Literature Cited

- De Schaepdrijver, L., L.P. Simoons, H. Lauwers, and J.P. DeGeest. 1989. Retinal vascular patterns in domestic animals. *Res. Vet. Sci.* 47:34-42.
- Whittier, J.C., J.A. Shadduck, and B.L. Golden. 2003. Secure identification, source verification of livestock – The value of retinal images and GPS. In: *Precision Livestock Farming*. S Cox (Ed.). Wageningen Acad. Pub. The Netherlands. Pp. 167-172.



Figure 1. The retinal-imaging device

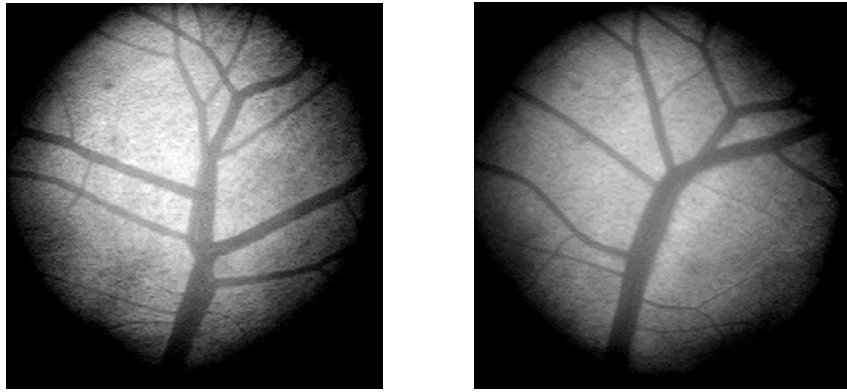


Figure 2. Images of the vascular patterns of the retinas of two eyes. Note the branching and outline of the blood vessels are unique to each eye.

# EFFECT OF SUPPLEMENTING RUMINALLY UNDEGRADABLE FIBER TO FEEDLOT STEERS ON FECAL NUTRIENT FRACTIONS AND FECAL AMMONIA EMISSIONS.

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**ABSTRACT:** Four beef steers were used in a 4 x 4 Latin square to evaluate effects of estimated ruminally undegradable fiber (RUF) on fecal nutrient fractions and NH<sub>3</sub> volatilization. Treatments were: corn control (CORN), barley (BAR), corn with corn bran (CB), and corn with confection sunflower hulls (CSH). Estimates of RUF were based on in vitro fiber digestibilities. Diets were formulated to provide RUF equal to BAR. Diets were fed as a TMR, including 7.5% grass hay, grain and fiber source. Steers were adapted to diets for 9 d followed by 5 d of collection, including total fecal collections. Twice per period, feces (3.5 cm deep, evenly packed) was placed in chambers for NH<sub>3</sub> collection. A 0.1 N HCl trap captured NH<sub>3</sub> for analysis. Feed, orts and fecal samples were analyzed for DM, ash, CP, ADF, NDF, and P, in addition, feces were analyzed for total C, and soluble N. Treatment affected ( $P \leq 0.05$ ), fecal OM, ADF, NDF, P, total C, and C:N. Confection sunflower hull addition tended to lower fecal total N ( $P = 0.06$ ) and soluble N ( $P = 0.06$ ). Fecal C:N were CORN, 19.01; BAR, 18.03; CB, 20.67; and CSH, 22.44. These results suggest fecal N produced from CB and CSH diets is more likely to be organically bound. Barley produced feces higher in total N and soluble N (similar to CORN) and lower OM and C:N, indicating that N was in more volatile forms. Treatment did not affect volatilization of NH<sub>3</sub> ( $P = 0.3$ ). Feces with higher C:N has potential to capture more N. Feces analyzed in this study was free of urinary N, perhaps explaining lack of an effect on NH<sub>3</sub> volatilization.

Key Words: Fiber, Ammonia, Steer

## Introduction

The majority (80 to 90%) of N consumed by feedlot cattle is excreted (Giger-Reverdin et al., 1991), much of this N is subject to volatilization loss. Volatilized NH<sub>3</sub> returns to the earth via rainfall, dry deposition and direct absorption (CAST, 2002) and can enrich terrestrial and aquatic systems. Excessive N can cause loss of species diversity, acidify soils and water bodies, contribute to surface water eutrophication, contaminate groundwater, and increase loss of N<sub>2</sub>O to atmosphere.

It may be possible to lower NH<sub>3</sub> emissions from feedlot cattle through dietary manipulation. Erickson et al. (2002) partially replaced corn with corn bran in feedlot diets and reported higher manure C:N and OM, increasing ability to capture N. Other studies have shown diet type can shift excretion of N from volatile urinary forms to more stable forms of fecal N (Bierman et al., 1999; Giger-

Reverdin et al., 1991) through stimulation of hindgut fermentation.

The large intestine plays a very important role in digestion of structural carbohydrates. Ulyatt et al. (1975) report that 5 to 30% of digestible cellulose is fermented in the large intestine, and more hemicellulose. Hindgut fermentation can alter excretion of N by increasing production of microbial protein and increasing fecal OM.

Ruminally undegradable fiber (**RUF**) may have potential to alter N excretion and lower NH<sub>3</sub> loss. Barley is a commonly used high fiber feed grain. Corn bran and confection sunflower hulls are also high in fiber. These feedstuffs may have potential to increase hindgut fermentation or increase fecal C:N and OM.

Partially replacing corn with sunflower hulls results in decreased digestibility of DM and ADF (Park et al., 1982). Park et al. (1997) reported sunflower hulls could safely be fed to cattle up to 30% DMI.

The objective of this study was to evaluate effects of estimated RUF from barley, corn bran and confection sunflower hulls on fecal nutrient fractions and NH<sub>3</sub> volatilization.

## Materials and Methods

### Animals and Housing

Four Angus cross steers (324 ± 8 kg) were randomly assigned to dietary treatments in a 4 x 4 Latin square. All animals were cared for in accordance with protocols which were approved by the NDSU Institutional Animal Care and Use Committee. Steers were penned individually during diet adaptation then placed in metabolism stalls for collection periods.

### Dietary Treatments

Treatments included: corn control (**CORN**), barley (**BAR**), corn with corn bran (**CB**), and corn with confection sunflower hulls (**CSH**). Diets were formulated to provide a minimum of 12% CP with adequate DIP (NRC, 1996). Actual nutrient composition of the diets after laboratory analysis is shown in Table 1. The basal diet was a total mixed ration (TMR) composed of a 92.5% corn or barley grain based concentrate and 7.5% grass hay (DM basis; Table 1). Ruminal degradability estimates (Escue et al., 2004) were used to formulate CB and CSH to provide the equal estimates of fiber to the hindgut as BAR. Confection sunflower hulls were ground to increase palatability; corn and barley were coarse rolled. Diets were fed once per day for ad libitum intake, targeting 10% feed refusal. Water was available freely throughout the study.

### *Sample Collection and Analysis*

Experimental period was 15 d in length, with 8 d for diet adaptation and 5 d for sample collection. Total fecal collections were achieved utilizing chutes. Chutes were attached to steers using harnesses, similar to those used for fecal bags. The open end of chutes were placed inside plastic garbage bags and secured to fecal pans, ensuring capture of all fecal material. Total fecal output was weighed daily and sub-sampled at 10% for lab analyses. On d 1 and d 4 of collection, feces were reserved for chamber analysis. Fecal samples were stored at 20° C until collection period was completed, then mixed in a rotary mixer, sub-sampled again. Orts were weighed and sub-sampled daily. Samples of TMR were taken at mixing, and TMR ingredients were also sampled. Feed, Orts, and fecal samples were dried at 55° C for 48 h, and analyzed for ash, CP, ADF, NDF, and P. Feces were also analyzed for total C, and soluble N.

### *Chamber Design and Measurements*

Feces were placed in chambers (3.5 cm depth, evenly packed) for NH<sub>3</sub> emission collection over 72 h. Chambers were modeled after McGinn et al. (2002). Calibrated intake and outlet fans drew air into and out of the chambers at the same rate, minimizing pressure gradient from ambient air to chamber air. Air from the chambers was subsampled by pumping (Brailsford and Company, Inc, model TD3LS7, Rye, NY) through an acid trap of 0.1 N HCl to capture NH<sub>3</sub>. Acid was changed at 24 h intervals and analyzed for ammonium using phenol-hypochlorite Bethelot reaction (Broderick and Kang, 1980).

### *Statistics*

Fecal composition data was analyzed by the GLM procedure of SAS (SAS Inst., Cary, NC), model contained effects for animal, period, and treatment. When significant ( $P < 0.10$ ), means were separated by LSD.

Ammonia emissions data was analyzed by the GLM and mixed procedures of SAS. Mixed model contained effects for period, treatment, day of emissions collection (**day**), and treatment x day; random variable was animal and repeated variable was day.

## **Results and Discussion**

Mean daily DMI were  $11.2 \pm 1.8$ ,  $9.8 \pm 1.3$ ,  $10.9 \pm 1.2$ , and  $9.9 \pm 1.3$  kg/d for CORN, BAR, CB, and CSH, respectively.

Treatment affected ( $P < 0.05$ ) fecal ash, OM, ADF, NDF, P, total C, and C:N. Treatment tended to effect total N ( $P = 0.06$ ), and soluble N ( $P = 0.08$ ) (Table 2).

Fecal ADF and NDF values were lowest ( $P < 0.01$ ) for CORN. Fecal N was higher ( $P = 0.06$ ) for CORN than CSH, and soluble N was similar to BAR but higher ( $P = 0.08$ ) than CSH. Fecal C:N from CORN was similar ( $P > 0.10$ ) to BAR. Fecal ash from CORN was similar ( $P > 0.10$ ) to CB. OM and C values were similar ( $P > 0.10$ ) to CB.

Barley used in this study was 7% ADF and 22% NDF (DM basis). Escue et al. (2004) reported extent of digestion for barley as 87% and 64% for NDF. Estimates

barley RUF were 51% for ADF and 87% for NDF (Escue et al., 2004). These estimates indicate that the majority of barley fiber should be available for hindgut fermentation.

Barley RUF did not perform equally to RUF from CB and CSH. Fecal ADF and NDF were different ( $P < 0.01$ ) than all other treatments. Fecal N was similar ( $P > 0.10$ ) to CB, and soluble N was similar ( $P > 0.10$ ) to CORN. Fecal C was higher ( $P = 0.05$ ) than all other treatments, and C:N was similar ( $P > 0.10$ ) to CORN. Fecal ash was higher ( $P < 0.01$ ) than CSH, and BAR fecal OM was lower ( $P < 0.01$ ) than all other treatments. Fecal P was also highest ( $P < 0.01$ ) from BAR.

Corn bran used in this study was 16% ADF and 65% NDF (DM basis). Escue et al. (2004) reported extent of digestion for corn bran as 95% for both ADF and NDF. Estimates of corn bran RUF were 67% for ADF and 72% for NDF (Escue et al., 2004). These estimates indicate that corn bran has much potential to stimulate hindgut fermentation, and should deliver more RUF to the hindgut than barley.

Addition of corn bran RUF produced fecal NDF values similar ( $P > 0.10$ ) to CSH. Fecal ADF from CB was different ( $P < 0.01$ ) than all other treatments. Fecal N and soluble N were similar ( $P > 0.10$ ) to other treatments. Fecal C:N was numerically raised by CB, however values were statistically similar to other treatments ( $P > 0.10$ ). Fecal C was similar ( $P > 0.10$ ) to CORN and CSH. Fecal ash, OM, and P from CB were similar ( $P > 0.10$ ) to other treatments as well.

These results tended to confirm our hypothesis that corn bran addition would increase hindgut fermentation, potentially increasing microbial utilization of N before excretion and shifting fecal N towards organic forms. Perhaps a greater dietary corn bran inclusion level would cause more significant difference in fecal N fractions.

Confection sunflower hulls used in this study were 67% ADF and 86% NDF (DM basis). Escue et al. (2004) reported extent of digestion for confection sunflower hulls to be 15% for ADF and 10% for NDF. Estimates of confection sunflower hull RUF were 91% for ADF and 95% for NDF (Escue et al., 2004). These estimates indicate that confection sunflower hull fiber should bypass both rumen and hindgut fermentation, increasing the OM and C:N of feces.

Addition of confection sunflower hulls caused the highest ( $P < 0.01$ ) fecal ADF. Fecal NDF was similar ( $P > 0.10$ ) to CB. Fecal N was lower ( $P = 0.06$ ) than CORN but similar to BAR and CB. Soluble N was lower ( $P = 0.08$ ) than CORN and BAR, but similar to CB. Fecal C:N from CSH was higher ( $P = 0.04$ ) than CORN and BAR, but similar to CB. Fecal C and OM were similar ( $P > 0.10$ ) to CORN and CB. Fecal ash and P were lower ( $P < 0.01$ ) than BAR.

This indicates that RUF from CSH may shift fecal N towards organically bound forms. In agreement with Park et al. (1982), addition of confection sunflower hulls lowered ADF digestion, resulting in the highest fecal ADF across diets. This finding confirms our hypothesis that more confection sunflower hull RUF would leave the animal intact, increasing fecal C:N over the corn control.

Treatment did not affect volatilization of  $\text{NH}_3$  ( $P = 0.30$ ). Feces with higher C:N has potential to capture more N. The added RUF treatments (CB and CSH) raised C:N over corn and barley treatments. Manure  $\text{NH}_3$  primarily arises from urinary urea. Feces analyzed in this study were free of urinary N, perhaps explaining lack of an effect on  $\text{NH}_3$  volatilization.

### Implications

Dietary addition of digestible fiber, which bypasses the rumen, may show promise in reducing  $\text{NH}_3$  volatilization from feedlot cattle. Results of this study indicate that confection sunflower hulls may reduce fecal N fractions and increase fecal C:N when compared to corn-based finishing diets. Corn bran may also be used, however more research must be done to determine optimum dietary inclusion levels.

### Literature Cited

- Bierman, S., G. E. Erickson, T. J. Klopfenstein, R. A. Stock, and D. H. Shain. 1999. Evaluation of nitrogen and organic matter balance in the feedlot as affected by level and source of dietary fiber. *J. Anim. Sci.* 77:1645-1653.
- Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63:64-75.
- CAST. 2002. Animal diet modification to decrease the potential for nitrogen and phosphorus pollution. Issue Paper No. 21. Council for Agricultural Science and Technology, Ames, Iowa.
- Erickson, G. E., T. J. Klopfenstein, and T. Milton. 2002. Corn bran level in finishing diets and N losses from open-dirt pens. Pages 54-57 in Nebraska Beef Report. Cooperative Extension, Univ. Nebraska, Lincoln.
- Escue, S. G., M. L. Bauer, G. P. Lardy, and S. A. Soto-Navarro. 2004. Influence of substrate and buffer pH on fiber digestion kinetics in vitro. Presented at the Midwestern Section ASAS and Midwest Branch ADSA 2004 Meeting, Des Moines, IA. (Abstr. 255).
- Giger-Reverdin, S., D. Sauvant, J. Hervieu, and M. Dorleans. 1991. Fecal and urinary nitrogen losses as influenced by the diet carbohydrate and protein fractions in goats. Pages 358-360 in Proc. 6th Int. Symp. Protein Met. And Nut., Herning, Denmark.
- McGinn, S. M., K. M. Koenig, and T. Coates. 2002. Effect of diet on odorant emissions from cattle manure. *Can. J. Anim. Sci.* 82:435-444.
- NRC. 1996. Nutrient Requirements of Beef Cattle. 7<sup>th</sup> rev. ed. Natl. Acad. Press, Washington, DC.
- Park, C. S., D. O. Erickson, G. R. Fisher, and C. N. Haugse. 1982. Effects of sunflower hulls on digestibility and performance by growing dairy heifers fed varying amounts of protein and fiber. *J. Dairy Sci.* 65:52-58.
- Park, C. S., G. D. Marx, Y. S. Moon, D. Wiesenborn, K. C. Chang, and V. L. Hofman. 1997. Alternative uses of sunflower. Pages 765-807 in Sunflower Technol. and Prod. Agron. Monograph no. 35. Am. Soc. Agron., Crop Sci. Soc. Am, Soil Sci. Soc Am, Madison, Wisconsin.
- Ulyatt, M. J., D. W. Dellow, C. S. W. Reid, and T. Bauchop. 1975. Structure and function of the large intestine of ruminants. Pages 119-133 in Digestion and Metabolism in the Ruminant: Proc. 4<sup>th</sup> Int. Symp. Ruminant Physiology. I. W. McDonald, and A. C. I. Warner, eds. The University of New England Publishing Unit, Armidale, NSW, Australia.

Table 1. Total mixed ration composition and laboratory analysis of nutrient composition.

Ingredient	Treatment <sup>1</sup>			
	CORN	BAR	CB	CSH
<i>TMR composition (% DM)</i>				
Barley	-	83.0	-	-
Corn	81.0	-	65.0	69.0
Corn bran	-	-	16.8	-
Confection sunflower hulls	-	-	-	10.6
Grass hay	7.5	7.5	7.5	7.5
De-sugared molasses	5.0	5.0	5.0	5.0
<i>Supplement</i>				
Finely ground corn	0.56	0.57	0.11	0.40
Soybean meal	2.93	1.71	2.56	4.56
Limestone	1.88	1.84	1.77	1.78
Urea	0.75	-	0.75	0.75
Dicalcium phosphate	-	-	0.13	0.03
Salt	0.25	0.25	0.25	0.25
Mineral premix <sup>2</sup>	0.06	0.06	0.06	0.06
Vitamin E premix <sup>3</sup>	0.02	0.02	0.02	0.02
Vitamin A and D premix <sup>4</sup>	0.02	0.02	0.02	0.02
Monensin premix <sup>5</sup>	0.02	0.02	0.02	0.02
Tylosin premix <sup>6</sup>	0.01	0.01	0.01	0.01
<i>Laboratory Analysis (% DM)</i>				
Ash	4.55	5.84	4.47	5.24
CP	13.41	12.47	13.37	13.08
ADF	5.47	8.61	7.65	12.16
NDF	14.08	23.19	23.44	22.11
Fat	2.64	1.73	2.55	2.57
Ca	0.66	0.89	0.71	0.92
P	0.28	0.34	0.27	0.26

<sup>1</sup>Treatments included: corn control (CORN), barley (BAR), corn with corn bran (CB), and corn with confection sunflower hulls (CSH).

<sup>2</sup>Contains 30 g Cu, 45 g Fe, 180 g Zn, 128 g Mn, 2.78 g I, and 0.56 g Co per kg

<sup>3</sup>Contains 9.1 kIU/kg

<sup>4</sup>Contains 48,000 kIU vitamin A and 4,268 kIU vitamin E per kg

<sup>5</sup>Formulated to be fed at 27.5mg/kg

<sup>6</sup>Formulated to be fed at 11mg/kg

Table 2. Treatment effects on fecal fractions (% DM).

Item	Treatment <sup>1</sup>				SE
	C	B	CB	CSH	
Ash	7.92 <sup>ab</sup>	11.14 <sup>b</sup>	7.74 <sup>ab</sup>	7.11 <sup>a</sup>	0.412
OM	92.08 <sup>b</sup>	88.86 <sup>a</sup>	92.26 <sup>b</sup>	92.89 <sup>b</sup>	0.412
ADF	13.07 <sup>a</sup>	24.38 <sup>c</sup>	18.02 <sup>b</sup>	27.97 <sup>d</sup>	1.129
NDF	29.54 <sup>a</sup>	49.69 <sup>c</sup>	44.91 <sup>b</sup>	46.44 <sup>b</sup>	1.698
P	0.71 <sup>ab</sup>	0.98 <sup>b</sup>	0.57 <sup>ab</sup>	0.54 <sup>a</sup>	0.054
C	47.70 <sup>b</sup>	45.33 <sup>a</sup>	47.73 <sup>b</sup>	47.80 <sup>b</sup>	0.572
C:N	19.01 <sup>a</sup>	18.03 <sup>a</sup>	20.67 <sup>ab</sup>	22.44 <sup>b</sup>	0.910
N	2.53 <sup>f</sup>	2.52 <sup>ef</sup>	2.31 <sup>ef</sup>	2.15 <sup>e</sup>	0.093
Soluble N	2.37 <sup>f</sup>	2.26 <sup>f</sup>	1.82 <sup>ef</sup>	1.62 <sup>e</sup>	0.193

<sup>1</sup>Treatments included: corn control (CORN), barley (BAR), corn with corn bran (CB), and corn with confection sunflower hulls (CSH).

<sup>a, b, c, d</sup> Means in same row with different superscripts differ ( $P < 0.05$ ).

<sup>e, f, g</sup> Means in same row with different superscripts tend to differ ( $P < 0.08$ ).

# EFFECTS OF SUPPLYING WATER WITH VARYING LEVELS OF TOTAL DISSOLVED SOLIDS AND SULFATES TO STEERS DURING THE GROWING PERIOD ON SUBSEQUENT FINISHING PERFORMANCE

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**ABSTRACT:** Previous results have shown that water with elevated total dissolved solids (TDS) and sulfates was detrimental to performance of growing steers. The objective of this study was to determine the effects of water quality during the growing period on subsequent finishing performance. In yr 1, 78 steers (374 kg) were assigned to one of eight pens (2-4 pens/treatment) based on water supplied during the 84-d growing period. Water TDS and sulfates during growing were: 1) 1,019 and 404; 2) 4,835 and 3,087; and 3) 6,191 and 3,947 mg/L of TDS and sulfates, respectively. In yr 2, 75 steers (381 kg) that were previously supplied water during a 104-d growing period averaging: 1) 1,226 and 441; 2) 2,933 and 1,725; 3) 4,720 and 2,919; and 4) 7,268 and 4,654 mg/L of TDS and sulfates respectively, were received and fed in one pen. Both yr, all steers were fed a common finishing diet and had access to rural water. In yr 1, steers receiving treatment 1 had higher ( $P < 0.10$ ) ADG and DMI compared to 2 and 3 during the previous growing period. During the initial 28-d of finishing, 2 and 3 had higher ( $P < 0.10$ ) ADG than 1. Steer DMI was not different ( $P = 0.19$ ) between treatments during the first 28-d. Over the entire 126-d trial, ADG, DMI and carcass characteristics were not different due to treatment ( $P > 0.10$ ). In yr 2, there was a quadratic decline in ADG with increasing TDS ( $P < 0.05$ ) during the previous growing phase, resulting in treatment 4 have lower initial weight ( $P < 0.05$ ) compared to 1, 2, and 3. During the first 28-d of finishing, ADG was higher ( $P < 0.10$ ) for 2 and 3 compared to 1, with 4 being intermediate. Over the 133-d trial, ADG of 2 and 3 was greater ( $P < 0.10$ ) than 1, with 4 being intermediate, resulting in 4 having lower carcass weight ( $P < 0.05$ ) compared to 1, 2 and 3. Other carcass traits were not significantly different due to treatment. Steers receiving water during the growing period with 5000 mg/L TDS and 3000 mg/L sulfates or less were able to compensate for lost growing performance during the finishing period.

Key words: Finishing steers, Sulfates, Water quality

## Introduction

Surface and subsurface water available to cattle in South Dakota is often high in total dissolved solids (TDS) and sulfates. Ingestion of high sulfate water causes increased ruminal  $H_2S$  generation (Loneragan et al., 1997) and can result in sulfur-associated polioencephalomalacia (McAllister et al., 1997; Gould, 1998). Previous research at South Dakota State University has demonstrated that supplying water

containing high concentrations of TDS and sulfates to growing steers not only increased the incidences in polioencephalomalacia, but also reduced DMI, water intake, ADG and efficiency of gain (Patterson et al., 2002; 2003).

In a review, Smith (1998) stated that subclinical disease events may be more economically important than clinical events. The reduction in ADG of cattle that suffer from respiratory diseases during the early phases of growth can persist through finishing. The respiratory morbidity not only depresses finishing phase performance but also can reduce carcass weight, fat deposition and longissimus muscle area (Gardner et al., 1999). Loneragan et al. (2001) observed that high sulfate water supplied to feedlot steers at a subclinical level reduced performance. However, it is not know if the depression in growing phase performance due to poor water quality will have a lasting effect on performance until cattle are harvested. Therefore, the objective of this study was to determine if supplying water with high concentrations of TDS and sulfates during the growing period had any subsequent effect on finishing performance and carcass characteristics.

## Materials and Methods

In this study, steers that were backgrounded at the South Dakota State University Cottonwood Range and Livestock Research Station, near Philip, SD on two previous water quality studies (Patterson et al., 2002; 2003) were shipped to the Southeast South Dakota Experiment Farm, Beresford, SD to determine the effect of water quality during the growing phase on subsequent finishing phase performance. In yr 1, 78 steers (374 kg) were assigned to one of eight pens (2-4 pens/treatment) based on water supplied during the 84-d growing period (Patterson et al., 2002). Water TDS and sulfates during growing were: 1) 1,019 and 404; 2) 4,835 and 3,087; and 3) 6,191 and 3,947 mg/L of TDS and sulfates, respectively. In yr 2, 75 steers (381 kg) that were previously supplied water during a 104-d growing period averaging: 1) 1,226 and 441; 2) 2,933 and 1,725; 3) 4,720 and 2,919; and 4) 7,268 and 4,654 mg/L of TDS and sulfates, respectively (Patterson et al., 2003), were received and fed in one pen.

In both years, upon arrival, steers had *ad libitum* access to long-stem, grass hay and rural water. The following morning, steers were vaccinated against viral (BOVI-K; Pfizer Animal Health, Exton, PA) and bacterial agents (ULTRABAC 7; Pfizer Animal Health) and treated for internal and external parasites (ivermectin;

PROMECTIN B POUR-ON; Vedco, Inc., St. Joseph, MO). All steers were adapted to a common finishing diet (Table 1) using four step-up diets over 21-d, and they had *ad libitum* access to rural water. Diets were mixed once daily and fed at 0800. Bunks were managed to be slick just prior to feed delivery and any feed refusal was weighed and recorded. Complete mixed diets and feed ingredients were sampled weekly and frozen immediately. Samples were later dried at 57°C, ground through a Wiley mill, equipped with a 1 mm screen, and analyzed for DM (Georing and Van Soest, 1970), CP (macro-Kjeldahl N; AOAC, 1984), and NDF (Van Soest et al., 1991).

Table 1. Composition of diet fed to steers during finishing (Year 1 and 2)

Ingredient	% of diet DM
Cracked corn	79.00
Alfalfa hay	10.00
Molasses	3.50
Supplement	
Ground Corn	3.20
Dicalcium phosphate	0.24
Limestone	0.83
Corn oil	0.13
Potassium Chloride	0.18
Soybean meal, 44% CP	1.56
Urea	0.80
Trace mineralized salt <sup>a</sup>	0.51
Rumensin 80 <sup>b</sup>	0.10
Tylan 40 <sup>c</sup>	0.02
Vitamin A <sup>d</sup>	0.01

<sup>a</sup>Composition (%): Na, > 37.0; Zn, > 0.35; Fe, > 0.2; Mn, > 0.2; Cu, > 0.03; I, > 0.007; Co, > 0.005.

<sup>b</sup>Contained 176 g of monensin per kg.

<sup>c</sup>Contained 88 g of tylosin per kg.

<sup>d</sup>Contained 30,000 IU vitamin A per gram.

Steer weights were taken in the morning prior to feeding at the beginning and end of the trial and every 28-d. Steers were implanted with 120 mg trenbolone acetate and 24 mg estradiol-17 $\beta$  (REVALOR-S, Intervet Inc., Millsboro, DE) on d 28. At the end of the trials (126 and 133 d for yr 1 and yr 2, respectively), steers were processed at a commercial processing plant (Caldwell Packing Company, Windom, MN) and carcass data was recorded after a 48-h chill.

In yr 1, data were analyzed as a completely randomized design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. The model contained steer on-trial weight, off-trial weight, ADG, DMI, feed efficiency, and carcass data as the dependant variables and growing phase treatment as the independent variable. In yr 2, since all steers were fed in one pen, data were analyzed as a completely randomized design using GLM procedures of SAS with animal as the experimental unit. The model contained steer on-trial weight, off-trial weight, ADG, and carcass data as the dependent variables and growing phase treatment and as the independent variable. Treatment

effects for all data were considered different at a significance level of  $P < 0.10$ .

## Results and Discussion

In yr 1, steers receiving treatment 1 (1,019 mg/L TDS and 404 mg/L sulfates) had higher ( $P < 0.10$ ) ADG and DMI compared to steers receiving 2 (4,835 mg/L TDS and 3,087 mg/L sulfates) and 3 (6,191 mg/L TDS and 3,947 mg/L sulfates) during the previous growing period (Patterson et al., 2002). This resulted in steers on treatment 1 have higher ( $P < 0.05$ ) final weights than steers on treatments 2 and 3 after the growing period. Once all steers were shipped from the Cottonwood Station to the Southeast Research Farm and allowed access to long-stem hay, the initial weights were numerically higher for steers that previously received treatment 1 compared to steers that received treatments 2 and 3, but was not determined to be significantly different. This lack of significance was primarily due to an increased in variation of body weights of the steers within treatment. During the initial 28-d of finishing, steers from treatments 2 and 3 had higher ( $P < 0.10$ ) ADG than 1 (Table 2). Steer DMI was not different ( $P = 0.19$ ) between treatments during the first 28-d. Over the entire 126-d trial, ADG, DMI and efficiency of gain were not different due to treatment ( $P > 0.40$ ). This resulted in there being no difference ( $P > 0.70$ ) in final weight and hot carcass weight due to treatment. In addition, there were no differences ( $P > 0.20$ ) in dressing percentage, longissimus muscle area, 12<sup>th</sup> rib fat thickness, kidney, pelvic and heart fat, USDA yield grade, or marbling score.

In yr 2, there was a quadratic decline in ADG with increasing water TDS and sulfate concentrations ( $P < 0.05$ ) during the previous 104-d growing phase (Patterson et al. 2003), resulting in steers that previously received treatment 4 (7,268 mg/L TDS and 4,654 mg/L sulfates) having lower initial finishing phase weight ( $P < 0.05$ ) compared to steers that received treatments 1 (1,226 mg/L TDS and 441 mg/L sulfates), 2 (2,933 mg/L TDS and 1,725 mg/L sulfates), and 3 (4,720 mg/L TDS and 2,919 mg/L sulfates). During the first 28-d of finishing, ADG was higher ( $P < 0.10$ ) for 2 and 3 compared to 1, with 4 being intermediate (Table 3). Over the 133-d trial, ADG of 2 and 3 was greater ( $P < 0.10$ ) than 1, with 4 being intermediate. This resulted in 4 having lighter final weight and carcass weight ( $P < 0.05$ ) compared to 1, 2 and 3. However, dressing percentage, longissimus muscle area, 12<sup>th</sup> rib fat thickness, kidney pelvic and heart fat, USDA yield grade and marbling score were not different ( $P > 0.20$ ) due to treatment.

Results of these trials suggest that steers that received water with intermediate concentrations of TDS and sulfates during the growing phase were able to compensate for lost growth performance during the initial part of the finishing period in which they received rural water. Additionally, this loss in performance in the early stages of growth did not have lasting effects on carcass characteristics. Steers that received water above 7,000 mg/L TDS and 4,500 mg/L sulfates during the growing phase had adequate finishing phase gains compared to the

control steers, but they did not compensate for the lost body weight that occurred during the growing phase. Other than the reduction in carcass weight, supplying water with high concentrations of TDS and sulfate did not adversely affect any of the other carcass parameters measured. Loneragan et al. (2001) observed that the reduction in steer performance was the greatest during the early stages of growth when water that contained sulfates greater than 583 mg/L was supplied during the finishing

period. Steers in the study of Loneragan et al. (2001) appeared to adapt to high sulfate water during the latter stages of trial, but this compensation was not enough to overcome the loss in body weight that occurred early in the finishing period. Besides the reduction in hot carcass weight, Loneragan et al. (2001) reported that dressing percentage and predicted yield grade decreased linearly with increasing water sulfate concentration.

Table 2. Influence of water with varying concentrations of total dissolved solids (TDS) and sulfates offered to steers during the growing phase on subsequent finishing performance (Year 1)

Treatment	1	2	3	
TDS/sulfate, mg/L <sup>a</sup>	1,019/404	4,835/3,087	6,191/3,947	SEM
<i>n</i>	2	2	4	
Initial wt., kg	383	366	373	8
d 0-27				
ADG, kg/d	1.32 <sup>b</sup>	1.54 <sup>c</sup>	1.53 <sup>c</sup>	0.11
DMI, kg/d	8.85	8.53	8.94	0.14
Gain:Feed	0.150	0.182	0.172	0.011
Final wt, lb	591	585	590	13
Trial (d 0-126)				
ADG, kg/d	1.65	1.70	1.72	0.09
DMI, kg/d	9.84	9.75	10.16	0.27
Gain:Feed	0.168	0.175	0.169	0.004
Hot carcass wt., lb	361	357	363	14
Dressing, %	61.2	61.1	61.6	0.5
Fat thickness, cm	1.35	1.09	1.32	0.13
KPH, %	1.93	1.74	1.89	0.11
Longissimus muscle area, cm <sup>2</sup>	82.2	83.9	81.2	2.1
USDA Yield Grade	2.79	2.41	2.81	0.20
Marbling <sup>d</sup>	577	600	567	24

<sup>a</sup>Average total dissolved solids and sulfates in water supplied to steers during the 84-d growing phase (Patterson et al., 2002).

<sup>b,c</sup>Means with different superscripts differ ( $P < 0.10$ ).

<sup>d</sup>Slight<sup>0</sup>=400; Small<sup>0</sup>=500; Modest<sup>0</sup>=600.

Table 3. Influence of water with varying concentrations of total dissolved solids (TDS) and sulfates offered to steers during the growing phase on subsequent finishing performance (Year 2)

Treatment	1	2	3	4	
TDS/sulfates, mg/L <sup>a</sup>	1,226/441	2,933/1,725	4,720/2,919	7,268/4,654	SEM
Initial wt., kg	387 <sup>b</sup>	387 <sup>b</sup>	383 <sup>b</sup>	357 <sup>c</sup>	5.9
d 0-29 ADG, kg/d	1.86 <sup>d</sup>	2.13 <sup>c</sup>	2.12 <sup>c</sup>	1.88 <sup>de</sup>	0.14
Final wt, kg	650 <sup>b</sup>	668 <sup>b</sup>	666 <sup>b</sup>	618 <sup>c</sup>	14
Trial ADG, kg/d	1.97 <sup>d</sup>	2.12 <sup>c</sup>	2.12 <sup>c</sup>	1.96 <sup>d</sup>	0.07
Hot carcass wt., kg	386 <sup>b</sup>	392 <sup>b</sup>	393 <sup>b</sup>	364 <sup>c</sup>	8
Dressing, %	59.5	58.8	59.1	58.8	0.4
Fat thickness, cm	1.55	1.55	1.57	1.32	0.13
KPH, %	2.36	2.17	2.19	2.16	0.08
Longissimus muscle area, cm <sup>2</sup>	82.1	83.4	85.0	79.3	2.2
USDA Yield Grade	3.66	3.61	3.56	3.34	0.12
Marbling <sup>g</sup>	568	607	595	582	22

<sup>a</sup>Average total dissolved solids and sulfates in water supplied to steers during the 104-d growing phase (Patterson et al., 2003).

<sup>b,c</sup>Means with different superscripts differ ( $P < 0.05$ ).

<sup>d,e</sup>Means with different superscripts differ ( $P < 0.10$ ).

<sup>f</sup>Slight<sup>0</sup>=400; Small<sup>0</sup>=500; Modest<sup>0</sup>=600.

## Implications

Water quality continues to be a concern in the Upper Great Plains and Western United States. Water with high concentrations of total dissolved solids; especially those that contain high concentrations of sulfates, can have negative effects on growing phase performance of cattle. However, in the current research, steers receiving water during the growing period with 5000 mg/L TDS and 3000 mg/L sulfates or less were able to compensate for lost growing performance during the finishing period. Water extremely high in total dissolved solids and sulfates provided to younger cattle may cause reductions in the final weight of the cattle.

## Literature Cited

- AOAC. 1984. Official Methods of Analysis. 14<sup>th</sup> ed. Association of Official Analytical Chemists, Arlington, VA.
- Gardner, B. A., H. G. Dolezal, L. K. Bryant, F. N. Owens, and R. A. Smith. Health of finishing steers: Effects on performance, carcass traits, and meat tenderness. *J. Anim. Sci.* 77:3168-3175.
- Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). Agric Handbook No. 379. ARS, USDA, Washington, DC.
- Gould, D. H. 1998. Polioencephalomalacia. *J. Anim. Sci.* 76:309-314.
- Loneragan, G. H., D. H. Gould, J. J. Wagner, F. B. Garry, and M. A. Thoren. 1997. The effect of varying water sulfate content on H<sub>2</sub>S generation and health of feedlot cattle. *J. Anim. Sci.* 75(Suppl. 1):272(Abstr.).
- Loneragan, G. H., J. J. Wagner, D. H. Gould, F. B. Garry, and M. A. Thoren. 2001. Effects of water sulfate concentration on performance, water intake, and carcass characteristics of feedlot steers. *J. Anim. Sci.* 79:2941-2948.
- McAllister, M. M., D. H. Gould, M. F. Raisbeck, B. A. Cummings, and G. H. Loneragan. 1997. Evaluation of ruminal sulfide concentration and seasonal outbreaks of polioencephalomalacia in beef cattle in a feedlot. *J. Am. Vet. Med. Assoc.* 211:1275-1279.
- Patterson, H. H., P. S. Johnson, T. R. Patterson, D. B. Young, and R. Haigh. 2002. Effects of water quality on animal health and performance. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 53:217-220.
- Patterson, H. H., P. S. Johnson, and W. B. Epperson. 2003. Effects of total dissolved solids and sulfates in drinking water for growing steers. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 54:378-380.
- Smith, R. A. 1998. Impact of disease on feedlot performance: A review. *J. Anim. Sci.* 76:272-274.
- Van Soest, P. J., J. B. Roberson, and B. A. Lewis. 1991. Symposium: Carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. *J. Dairy Sci.* 74:3583-3597.

**EFFECT OF FEEDING YUCCA SCHIDIGERA (DK POWDER) TO THE SOW  
ON PIGLET BLOOD OXYGENATION AND SURVIVAL**

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**ABSTRACT:** The effect of prepartum and postpartum feeding of yucca powder (*Yucca schidigera*) to sows on neonatal mortality, preweaning mortality, 21 day piglet growth rate and feed intake, and blood oxygen of sows and neonates, and neonate rectal temperature were determined. Dried yucca powder was fed to sows at 120 g per tonne from day 107 of gestation to day 21 of lactation. Parturition was induced on day 113 of gestation. There were 9 sows on the control diet and 10 on the dried yucca (DK powder) treatment. Number and percentage of stillbirths were 0.78 and 7.2% in the control group, and 0.5 and 4.7% for the DK Powder group. The greatest incidence of stillbirth (71.4%) occurred in the last third of the birth order in control sows, and was reduced to 40% in DK Powder-fed sows. Blood oxygenation (blood oxygen saturation) was similar in both groups of sows and piglets either at birth in the umbilical vein or at one hour post-partum in the umbilical artery. Piglets from DK powder-fed sows had a higher ( $p<0.01$ ) rectal temperature at 24 hours after birth compared to piglets from control sows. This effect was particularly prominent with the smaller birth weight piglets. The effect of yucca powder on body temperature is a likely cause of increased neonatal survival in piglets from DK Powder-fed sows. In summary, piglets from sows fed yucca powder exhibited improved thermoregulatory abilities after birth and tended to have a lower incidence of stillbirth and pre-weaning mortality. The mechanism does not appear to involve improved blood oxygenation.

**Keywords:** Swine, *Yucca schidigera*, stillbirths, blood oxygenation

### **Introduction**

The *Yucca schidigera* plant and its extracts have a long history of safe use as a food material for livestock. Yucca powder has been successfully added to animal feeds to enhance animal performance, bind ammonia, stimulate the immune system or inhibit urease (for review see Cheeke, 2000). The precise mode of action of yucca extract is still a matter of debate but there is increasing evidence that its main active compound, saponin, is implicated in its dietary effects and medicinal properties. Yucca contains a high percentage of naturally occurring steroidal saponins, a

complex glycoside molecule well-known for its broad spectrum of biological actions (Cheeke, 2000). Dried yucca powder contains about 12% total saponin, while many commercial yucca extract products contain about 6% saponin (personal communication, Paul Hiley, Desert King International). Search for new saponin applications is therefore of considerable economic and biological interest. Beneficial effects of adding yucca extract to the sow diet during pregnancy and lactation have been described (Cline et al, 1996). Piglets born from such sows exhibited higher blood oxygen saturation levels at birth, the incidence of stillbirth was reduced and postnatal survival was improved. This effect was particularly noticeable in primiparous sows and was associated with an increase in feed consumption. Similarly, Isley et al. (2003) reported a reduction in stillbirths in litters from sows fed yucca extract. Considering the potential impact of such practice to reduce the high level of perinatal mortality in pig production, the present experiment was performed to examine the effects of feeding Yucca product to primiparous sows, using the source of *Yucca schidigera* produced by Desert King International (DK Powder). The DK powder consists of finely ground dried yucca stems.

### **Materials and Methods**

Sows were fed a gestation and a lactation diet (Table 1) containing either 0 (Control) or 120 g per tonne of DK Powder<sup>1</sup> from day 107 of gestation through day 21 of lactation. In both groups, feed consumption was limited to 2.6 kg/d during gestation and was increased progressively by 0.5 kg/d after farrowing up to 6.5 kg/d. Farrowing was induced with an i.m. injection of a prostaglandin analogue (IC 80996) on day 113 of gestation to ensure farrowing on day 114. All farrowings were attended and piglets were provided with straw bedding and two infrared lamps of 250W on each side of the sow in order to minimise heat loss. Litter size was equalized to 910 piglets two days after farrowing in order to standardize milk production of the sow; runt piglets (birth weight lower than 800g) were not used in the experiment.

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<sup>1</sup> DK Yucca Powder obtained from Desert King International, 7024 Manya Circle, San Diego, CA 92154.

Sows were weighed at the beginning of the treatment, before and after farrowing, and at weaning at 21 days. Daily feed consumption was recorded during the whole period. In each group, three sows were catheterized in the carotid artery two weeks before farrowing, to allow blood sampling on the day of farrowing and at the end of the treatment. Blood was sampled with an heparinized blood gas syringe and subsequently analyzed for the content and oxygen saturation (HbO<sub>2</sub>) of hemoglobin, as well as the partial pressure of oxygen (pO<sub>2</sub>) and the oxygen content of whole blood, as previously described (Herpin et al, 1996).

The piglets were ear notched at birth. As each piglet was born, the exact time of birth, birth order, distribution of stillbirth by birth order and the total duration of farrowing were recorded. Two blood samples were successively collected at birth and at one hour of life on ten piglets per litter, using heparinized blood gas syringes. At birth, blood was collected from the umbilical vein (which supplies the piglets with the oxygenated blood coming from the sow) to have an estimate of piglet oxygenation during the farrowing process. At one hour of life, blood was collected from the umbilical artery, to determine the actual blood oxygen content of the piglets. Blood analysis were conducted as described for the sows. Body weight and rectal temperature were recorded at one hour, one day, seven days and 21 days of life as previously described (Herpin et al, 1996). Mortality rates and their causes were recorded daily between birth and weaning at 21 days.

Means  $\pm$  SE were calculated in each group for sow characteristics and compared by Student's t test, with sow being the experimental unit. Analysis of variance (GLM procedure of SAS) was used to test the effects of treatment and litter on piglet characteristics. Chi-square analysis (FREQ procedure of SAS) was performed to analyze the difference in survival rate between control and DK powder piglets. Regressions were computed using the REG procedure of SAS (1990) to determine the relationships between rectal temperature and birth weight in each group.

## Results and Discussion

The experiment was designed to exclude any difference in feed consumption between control and DK Powder fed sows, in order to analyze the actual effect of Yucca extracts independently from any change in feed intake. After one or two days of adaptation, sows consumed the DK Powder diet without any problem. As expected (Table 2), feed consumption was similar in both groups, averaging 5.6 kg per day, and sows lost about 10.5 kg during lactation in both groups. Present weight changes during lactation are high but similar to previous data on primiparous sows (Dourmad et al, 1998). Piglets' birth weight, postnatal growth and consumption of dry food the last week before weaning were also similar in both groups (Table 2). These data suggests that under adequate nutritional (feed intake close to ad libitum) and breeding (ten

piglets per litter) conditions, feeding DK Powder to the sow had no beneficial effects on the postnatal growth of the piglets.

Number and percentage of stillbirths per litter averaged 0.78 and 7.2% in the control group, respectively, compared to corresponding values of 0.5 and 4.7% for DK Powder animals (Table 3). In addition, distribution of stillbirths by birth order was recorded. The greatest incidence of stillbirth (71.4%) occurred in the last third of the birth order in control sows as shown previously (Zaleski and Hacker, 1993; Tuchscherer et al., 2000), this percentage being reduced to 40% in DK Powder fed sows. Pre-weaning mortality averaged 8.2% and 6.6% in control and DK powder piglets, respectively, corresponding values being 15.5% and 11.3% for total mortality. As usual, number of dead piglets was highly variable between litters, and it is clear that these data will have to be confirmed on a larger number of litters. However, the actual trend for a reduced mortality rate in sows fed DK powder confirms recent field trials with the same Yucca powder (Desert King International field trials) and results obtained with sows fed yucca extract (Cline et al, 1996; Isley et al., 2003). Further, it is interesting that 40% of the sows fed DK Powder presented no piglet mortality before weaning, compared to only 11% in the control group (Table 3). In addition, present results ruled out the possibility for these potential beneficial effects to be associated with an increased feed intake of the sow. Inability to observe significant changes in piglet mortality probably comes firstly from the reduced number of sows used in this study – although justified by our main objective, i.e., provide precise physiological data on sow and piglets oxygen levels – and secondly from the quite low mortality rate recorded in the control sows - 7.2% of stillborns and 8.2% of pre-weaning mortality. Indeed, in France, total pre-weaning losses including stillbirths are higher and have remained constant around 18-19% throughout the last 20 years. During this period, the incidence of stillbirths has dramatically increased in parallel to litter size, being as high as 10-15% in herds using hyperprolific breeds (Herpin and Le Dividich, 1998). Therefore, it is likely that more benefits could be expected if Yucca products are fed to such sows.

In an attempt to analyse the effect of the treatment on piglet and sow oxygenation, blood samples were collected at various times. Indeed, previous studies have suggested that feeding Yucca extracts to the sow prior to the start of farrowing increased the blood oxygen supply to the fetus (blood oxygen saturation) during birth thus lowering the incidence of stillbirth and reducing pre-weaning mortality (Cline et al, 1996). Our data do not support this statement. Blood oxygen content was strictly similar in both groups either at birth in the umbilical vein (oxygenated blood supply by the sow) or at one hour of life in the umbilical artery (Table 4). However, it is interesting to notice that the averaged oxygen saturation of hemoglobin found in our study, i.e. 74-77%, was already close to the value found for yucca extract-fed animals (Cline et al, 1996).

The control piglets from this other experiment exhibited much lower values (68%). In other words, beneficial effects of *Yucca* extracts are only evident in sub-optimal oxygenated conditions. As shown previously (Herpin et al, 1998), oxygen content of piglet blood increases after birth in both groups due to a rise in both the partial pressure of oxygen and the oxygen saturation of hemoglobin. The only difference induced by the treatment was a slight increase in blood hemoglobin content. Although the actual physiological meaning of such a small difference remains to be addressed, its beneficial effect on blood oxygen carrying capacities, occurrence of anemia and oxidative metabolism cannot be excluded.

Measurements of arterial blood oxygen content of the sows (Table 5) do not give further information on this aspect. Oxygen content is similar in both groups after farrowing and 21 days later, despite a lower partial pressure of oxygen in DK Powder fed sows after farrowing. No significant differences were found in blood hemoglobin contents, although values tended to be lower for DK Powder fed sows at the end of the treatment. However, additional measurements performed on blood samples collected by venous puncture on all the sows, confirmed that blood hemoglobin contents were similar in both groups ( $10.6 \pm 0.35$  vs  $10.8 \pm 0.2$  g/dL for control and DK Powder, respectively).

Finally, as neonatal survival relies on the ability of piglets to thermoregulate early after birth, and piglets that died before weaning have a much lower rectal temperature 24 hours after birth (Herpin et al, 1996), we have followed the postnatal changes in rectal temperature in both groups. No differences were observed at one hour, seven days and 21 days of life, but DK Powder piglets presented a slightly but significantly higher rectal temperature at 24 hours of life (Table 3). Moreover, rectal temperature increased ( $P < 0.01$ ) linearly with body weight (Figure 1) and this relationship was different ( $P < 0.05$ ) between the two groups. In fact, the slope of the regression line relating rectal temperature to birth weight was three times higher for control than DK Powder piglets. In other words, the effect of the treatment on the ability of the piglets to maintain rectal temperature at 24 hours of life is more marked on light piglets.

Since light piglets are less viable at birth (Zaleski and Hacker, 1993), have reduced thermoregulatory abilities (Herpin et al, 2000) and are less prone to adapt to extra-uterine life, these beneficial effects of *Yucca* extracts on temperature regulation will probably increase their chances of survival. Indeed, in relation with this result, Wang and Lee (2000) reported recently that acute systemic injection of saponins from ginseng was able to increase cold tolerance of young rats.

In conclusion, our results do not support the idea that feeding *Yucca* extracts to primiparous sows during the peri-parturient period may increase blood oxygen supply to the fetus. However, DK powder piglets exhibited better thermoregulatory abilities after birth and tended to have a lower incidence of stillbirth and pre-weaning mortality, as

previously shown by others. Although the enhanced thermoregulatory abilities of light DK Powder piglets may increase their chances of survival, the actual biological mechanisms responsible for the mild beneficial effects of *Yucca* extracts still remains unexplained. As saponins, the main active ingredients in *Yucca schidigera*, are probably involved various hypothesis can be proposed. Indeed, development of immunity is of utmost importance for the newborn pig and purified saponins are known to play an important role in stimulating the immune system (Chavali et al, 1987). Alternatively, the oxygen radical scavenging activities of some saponins (Okubo and Yoshiki, 1996) might also be beneficial to light and small for gestational age piglets, because work performed on premature infants has shown that they have limited antioxidant defence mechanisms (Kelly, 1993). Further studies are necessary to clarify these points.

Finally, it is pertinent to note that yucca contains other constituents besides saponins which might play a role in responses to yucca powder. These include stilbenes, resveratrol and phenolics (Oleszek et al., 2001). These substances are present in the dried yucca powder; their occurrence in yucca extracts is not documented. This might account for differences in responses between yucca powder and yucca extracts.

#### Acknowledgements

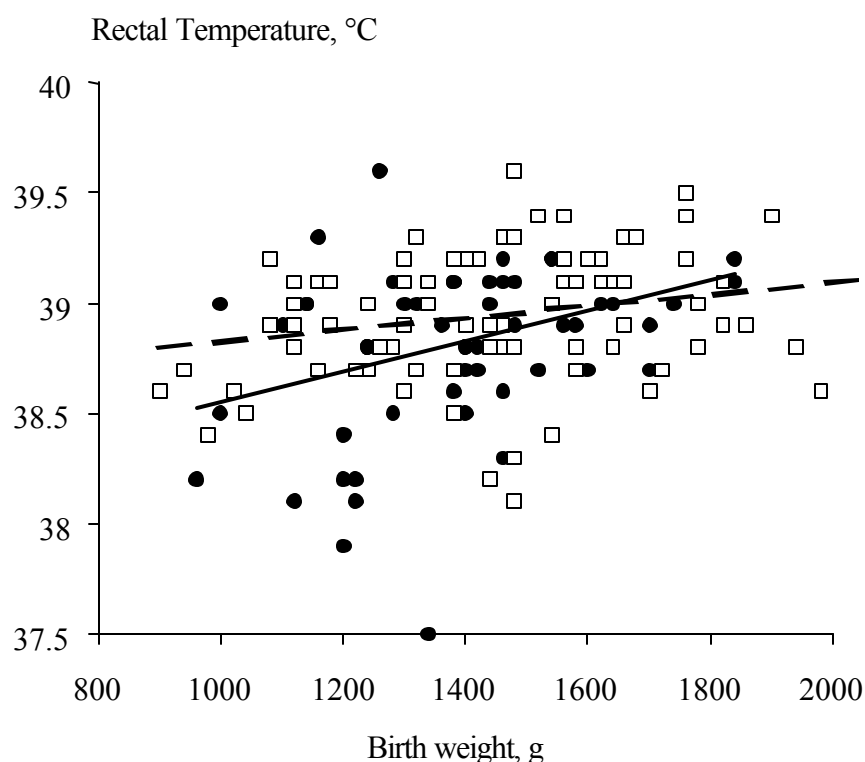
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#### References

- Chavali, S.R., Campbell, J.B., 1987. Immunomodulatory effects of orally-administered saponins and nonspecific resistance against rabies infection. *Int. Arch. Allergy Appl. Immunol.* 84(2), 129-134.
- Cline, J.L., Fisher, B.A., Trottier, N.L., Walker, R.D., Easter, R.A., 1996. Effect of feeding MICRO-AID on stillbirths, preweaning mortality, blood oxygen values of piglets and blood urea nitrogen in sows. *J. Anim. Sci.* 74 (suppl. 1), 189.
- Dourmad, J.Y., Noblet, J., Etienne, M., 1998. Effect of protein and lysine supply on performance, nitrogen balance, and body composition changes of sows during lactation. *J. Anim. Sci.* 76, 542-550.
- Herpin, P., Le Dividich, J., Hulin, J.C., Fillaut, M., De Marco, F., Bertin, R., 1996. Effects of the level of asphyxia during delivery on viability at birth and early postnatal vitality of newborn pigs. *J. Anim. Sci.* 74, 2067-2075.
- Herpin, P., Le Dividich, J., 1998. Conséquences de l'augmentation de la prolificité des truies sur la survie et la croissance du porcelet. *INRA Prod. Anim.* 11, 253-255.

- Herpin, P., Wosiak, F., Le Dividich, J., Bertin, R., 1998. Effect of acute asphyxia at birth on subsequent heat production capacity in newborn pigs. *Res. Vet. Sci.*, 66, 45-49.
- Herpin, P., Hulin, J.C., 2000. Effect of birth weight on thermoregulatory abilities of Chinese (Meishan) and European (Large White x Piétrain) newborn piglets. In: A. Chwalibog and K. Jakobsen (Eds). *Proceedings of the 15<sup>th</sup> Symposium on Energy Metabolism in Animals*. 10-16 September 2000, Snekersten, Denmark.
- Kelly, F.J., 1993. Free radical disorders of preterm infants. *Br. Med. Bull.* 49(3), 668-678.
- Okubo, K., Yoshiki, Y., 1996. Oxygen-radical-scavenging activity of DDMP-conjugated saponins and physiological role in leguminous plant. *Adv. Exp. Med. Biol.* (405), 141-154.
- Oleszek, W., M. Sitek, A. Stochmal, S. Piacente, C. Pizza, and P. Cheeke. 2001. Resveratrol and other phenolics from the bark of *Yucca schidigera* Roetzl. *J. Agric. Food Chem.* 49:747-752.
- SAS, 1990. *SAS/STAT User's Guide: Statistics*. SAS Inst. Inc., Cary, NC.
- Zaleski, H.M., Hacker, R.R., 1993. Variables related to the progress of parturition and probability of stillbirth in swine. *Can. Vet. J.* 34, 109-113.
- Tuchscherer, M., B. Puppe, A. Tuchscherer and U. Tiemann. 2000. Early identification of neonates at risk: Traits of newborn piglets with respect to survival. *Theriogenology* 54:371-388.
- Wang, L.C., Lee, T.F., 2000. Effect of ginseng saponins on cold tolerance in young and elderly rats. *Planta Med.* 66, 144-147.

Figure 1. Relation between rectal temperature and birth weight in Control ( ) and DK Powder (---□---) piglets at one day of age.



Rectal temperature (y, °C) was significantly related to birth weight (x, g) in both groups.  
Control group:  $y = 0.00073x + 37.76$  ( $n = 70$ ,  $r = 0.39$ ,  $P < 0.001$ ).  
DK Powder group:  $y = 0.00027x + 38.56$  ( $n = 88$ ,  $r = 0.23$ ,  $P < 0.05$ ).

Table 1. Composition and nutritional value of the diets

Diet	During gestation	During lactation
<i>Ingredient (%)</i>		
Barley	33.0	25.0
Wheat	22.15	22.8
Corn	10.0	12.0
Wheat bran	15.0	10.0
Soybean meal 48	9.0	21.0
Fat	2.0	2.0
Sugar beet pulp	5.0	0.0
Molasses	0.0	3.0
L-Lysine, HCL	0.0	0.05
Vitamin/mineral mixture	3.85	4.15
Total	100	100
<i>Chemical composition (%)</i>		
Dry matter	88.2	87.3
Protein	15.2	18.3
Fat	3.4	3.15
Gross energy (kJ/kg DM)	18.3	18.3

In the experimental diet, DK Yucca Powder was introduced on the basis of 120 g/tonne.

Table 2. Effect of DK Yucca Powder on food consumption and growth

Treatment	Control	DK Yucca Powder
<i>Sows</i>	n=9	n=10
Weight after farrowing (kg)	209 ± 5	210 ± 5
Weight at weaning at 21d (kg)	198 ± 6	199 ± 7
Weight loss during lactation (kg)	- 10.9 ± 2.1	- 10.5 ± 3.8
Food intake during lactation (kg)	111 ± 3	112 ± 3
<i>Piglets<sup>1</sup></i>	n=97	n=106
Birth weight (g)	1396 ± 22	1428 ± 26
Body weight gain 0-7d (g)	177 ± 5	178 ± 5
Body weight gain 0-21d (g)	242 ± 6	240 ± 5
Consumption of dry food the last week (g/litter)	116 ± 34	112 ± 44

<sup>1</sup> Litter size was adjusted to 9-10 piglets two days after farrowing.

Mean ± SE

Table 3. Effect of DK Yucca Powder on piglet body temperature and preweaning mortality

Treatment	Control	DK Yucca Powder
Number of litters	9	10
Number of pigs/litter	10.8 $\pm$ 0.7	10.6 $\pm$ 0.7
Stillbirths		
Per litter	0.78 $\pm$ 0.32	0.50 $\pm$ 0.22
% of total pigs born	7.2% (7/97)	4.7% (5/106)
in the last third of the birth order, %	71.4% (5/7)	40% (2/5)
Postnatal mortality,%	8.2% (8/97)	6.6% (7/106)
Total mortality,%	15.5% (15/97)	11.3% (12/106)
Number of litters with no mortality	1	4
Rectal temperature at 1d, °C	38.8 $\pm$ 0.05	39.0 $\pm$ 0.03 **

Means  $\pm$  SE. Rectal temperature at 1d was recorded from 70 control and 89 DK Powder piglets .

Statistical significance : \*\*, P < 0.01.

Table 4. Effect of DK Yucca Powder on blood oxygen levels of the piglets

Treatment	Control	DK Yucca Powder
<i>At birth</i>		
pO <sub>2</sub> (mm Hg)	37.5 $\pm$ 0.96	36.6 $\pm$ 0.97
Hemoglobin (g/dl)	9.30 $\pm$ 0.14	9.59 $\pm$ 0.12 *
HbO <sub>2</sub> (%)	77.4 $\pm$ 1.7	74.8 $\pm$ 1.44
O <sub>2</sub> content (ml/dl)	10.31 $\pm$ 0.29	10.27 $\pm$ 0.24
<i>At 1h of life</i>		
pO <sub>2</sub> (mm Hg)	50.7 $\pm$ 1.7	49.9 $\pm$ 1.8
Hemoglobin (g/dl)	9.58 $\pm$ 0.13	9.82 $\pm$ 0.14
HbO <sub>2</sub> (%)	90.4 $\pm$ 1.1	88.0 $\pm$ 1.4
O <sub>2</sub> content (ml/dl)	12.38 $\pm$ 0.25	12.37 $\pm$ 0.27

Mean  $\pm$  SE. Blood was sampled from the umbilical vein at birth and from the umbilical artery at 1h of life. Blood samples were not available from all the piglets because of technical problems (see Material and Methods) : at birth, blood was taken from 62 control and 75 DK Yucca Powder piglets ; at 1h of life, from 49 control and 62 DK Powder piglets.

pO<sub>2</sub>: partial pressure of O<sub>2</sub>; HbO<sub>2</sub>: O<sub>2</sub> saturation of hemoglobin.

Statistical significance : \*, P < 0.1.

Table 5. Effect of DK Yucca Powder on arterial blood oxygen levels of the sows

Treatment	Control	DK Yucca Powder
<i>After farrowing</i>		
pO <sub>2</sub> (mm Hg)	114.1 $\pm$ 0.8	99.0 $\pm$ 5.1 **
Hemoglobin (g/dl)	10.9 $\pm$ 0.5	11.1 $\pm$ 0.4
HbO <sub>2</sub> (%)	98.4 $\pm$ 0.2	96.7 $\pm$ 0.8
O <sub>2</sub> content (ml/dl)	15.3 $\pm$ 0.9	15.2 $\pm$ 0.3
<i>At 21d (end of treatment)</i>		
pO <sub>2</sub> (mm Hg)	100.9 $\pm$ 5.8	107.8 $\pm$ 3.8
Hemoglobin (g/dl)	11.2 $\pm$ 0.8	10.5 $\pm$ 0.6
HbO <sub>2</sub> (%)	96.7 $\pm$ 0.8	97.5 $\pm$ 0.3
O <sub>2</sub> content (ml/dl)	15.2 $\pm$ 1.1	14.4 $\pm$ 0.9

Means  $\pm$  SE (n=3). Blood was sampled from the carotid artery using an indwelling catheter.

Statistical significance : \*\*, P < 0.05.

# EFFECT OF SELECTING FOR SCRAPIE RESISTANCE AT CONDON 171 ON RAM PERFORMANCE AND CARCASS QUALITY

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**ABSTRACT:** In the US, sheep possessing alleles for the prion protein with glutamine (Q) or histidine (H), both reported as Q, at codon 171 are highly susceptible to scrapie. Incidence of scrapie infection is rare when animals possess at least one allele for arginine (R) at codon 171. The objective of this study was to determine if ram performance or carcass yield differed among genotypes (QQ, QR, or RR) at codon 171. Data collected during two yr at the Wyoming Rambouillet (n = 180) and terminal sire (Suffolk and Hampshire; n = 124) tests were used to determine if genotype was associated with test performance. Performance indexes were calculated. The Rambouillet test index was based on ADG, wool fiber diameter, and 365 d adjusted wool fiber length and clean fleece weight. The terminal sire test index was based on estimated rib-eye area, ADG, and BW. Indexes from both tests were analyzed and included yr as well as breed for the terminal sire test. Rambouillet test performance did not differ ( $P = 0.23$ ) by genotype. Performance index in the terminal sire test was, however, associated ( $P = 0.01$ ) with genotype. Rams genotyped QR were higher ( $P = 0.003$ ) indexing than QQ rams but not different ( $P = 0.32$ ) from RR rams. When individual components of the index were analyzed, ADG differed ( $P = 0.002$ ) by genotype with QR rams gaining better ( $P \leq 0.03$ ) than QQ or RR rams. Initial BW did not differ ( $P = 0.18$ ) among genotypes. To determine if genotype is associated with carcass yield, carcass measurements from lambs (n = 155) with known genotype were analyzed with parental breed and experiment included in the model. Hot carcass weight ( $P = 0.51$ ), adjusted 12<sup>th</sup> rib fat depth ( $P = 0.73$ ), rib-eye area ( $P = 0.62$ ), and yield grade ( $P = 0.62$ ) did not differ by lamb genotype. In conclusion, carcass yield and Rambouillet ram test performance did not differ by genotype at codon 171. Although ram performance in the terminal sire test differed by genotype, higher indexing rams were QR at codon 171 and would be considered resistant to most strains of scrapie.

Key Words: Scrapie, Genotype, Performance

## Introduction

Scrapie, a fatal neurological disease of sheep and goats, was introduced into the United States in 1947 and is now endemic in many states (Wineland et al., 1998). The American Sheep Industry estimates losses attributable to scrapie at \$20 to \$25 million annually (NIAA, 2004a). Scrapie is a member of a heterogeneous group of prion diseases called transmissible spongiform encephalopathies,

which includes bovine spongiform encephalopathy (BSE). Although scrapie has not been transmitted to humans from sheep or goats, the apparent transmission of BSE to humans in the United Kingdom resulted in a call for eradication of all transmissible spongiform encephalopathies in food producing animals (Hueston et al., 2000; Johnson et al., 2004).

There is no cure or treatment for scrapie. To date, however, scrapie (whether associated with clinical disease or with accumulation of prion protein in absence of clinical signs) has been detected only in sheep in the United States with alleles for the prion protein with glutamine (Q) or histidine (H) at codon 171, both reported as Q (O'Rourke et al., 1996, 2000). The susceptibility of lysine (K) to scrapie at codon 171 is unknown and is treated as Q for regulatory purposes (NIAA, 2004b). Incidence of scrapie infection is rare when animals possess at least one allele for arginine (R) at 171. Scrapie resistance of a flock can be improved by selectively breeding scrapie resistant animals (Smit et al., 2002; NIAA, 2004b). However, improving the scrapie resistant status of a flock may be detrimental to production if there are any deleterious effects of selecting for scrapie resistance on production traits or carcass quality. Therefore, the objective of the present study was to determine if ram performance during standardized testing or carcass quality differed among genotypes (QQ, QR, or RR) at codon 171.

## Materials and Methods

Ram performance data collected during two consecutive yr of the Wyoming Rambouillet (n = 180) and terminal sire tests (Suffolk and Hampshire; n = 124) were utilized for this study. Rams were genotyped at a USDA Animal Plant Health Inspection Service (APHIS) approved commercial laboratory (GeneCheck, Ft. Collins, CO). Distinctions between glutamine (Q), histidine (H), or lysine (K) at codon 171 were not made (Debbie et al., 1997). These variants are considered equivalent for regulatory purposes and are reported as Q (NIAA, 2004b).

Performance indexes were calculated for both tests. The Rambouillet performance index was calculated as follows:

**Index** = 60 (average daily gain in pounds) + 4.0 (365 d adjusted staple length in inches up to 5.5 inches) + 4.0 (365 d adjusted clean wool in pounds) + wool fiber diameter and variability points<sup>1</sup>

The terminal sire performance index was calculated as follows with rib-eye measurements estimated by ultrasound (Pie Classic Medical, Tequesta, FL):

**Index** = (estimated rib-eye area / Final BW \* ADG)\*100

Indexes were analyzed by GLM methods of SAS (Ver. 8.1, SAS Institute, Cary, NC). For the terminal sire test, ram breed and test yr were included in the model. The model for the Rambouillet test only included year. Differences among means were separated using PDIF procedures of SAS using only probabilities associated with pre-planned comparisons.

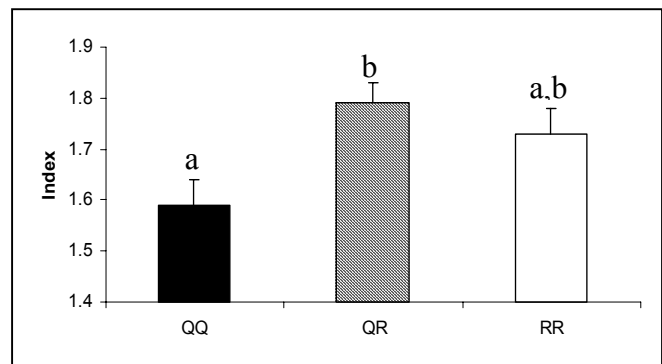
In lamb carcass evaluation, carcass quality is primarily determined by yield characteristics. To determine if carcass yield differed among genotypes at codon 171, data collected from 155 lamb carcasses with known genotype were analyzed. Carcass measurements including hot carcass weight, adjusted fat depth, rib-eye area, and yield grade were performed by trained personnel following standard industry procedures (USDA, 1992; Boggs et al., 1998). All lambs were produced from the same commercial western white-faced ewe flock with known sires. These data were compiled from three different experiments and were analyzed by GLM methods of SAS with sire breed and experiment included in the statistical model.

## Results and Discussion

For the eradication of scrapie through the selection of genetically resistant breeding stock to be acceptable, real or perceived production advantages possessed by scrapie susceptible animals must be identified. Since scrapie has historically been thought to primarily affect black-faced breeds (Wineland et al., 1998), a relationship between scrapie susceptibility and lean meat production may be postulated.

In the present study, Rambouillet ram performance index was not associated ( $P = 0.23$ ) with genotype at codon 171 (index scores of 113.5, 115.9, 117.5 [ $\pm 1.6$ ] for QQ, QR, and RR, respectively; data not shown). Suffolk and Hampshire rams in the terminal sire test did, however, differ ( $P = 0.01$ ) by genotype at codon 171. Rams genotyped QR were higher ( $P = 0.003$ ) indexing than QQ rams but not different ( $P = 0.32$ ) from RR rams (Figure 1). The performance index is comprised of BW, estimated rib-eye area and ADG. Although beginning test BW (119.7  $\pm$  3.1) did not differ ( $P = 0.18$ ) among genotypes, all measures included in the index (final BW, rib-eye area, ADG) differed ( $P \leq 0.04$ ) among genotypes (Table 1). In all

measures, QR rams out-performed rams genotyped QQ at codon 171. Ram breed was not significant ( $P \geq 0.12$ ) for any measure of performance. Alexander et al. (2003) reported Suffolk, but not Hampshire, ewes genotyped QR weaned more kg of lamb than ewes genotyped QQ. Suffolk sheep with genotype QR are considered to be scrapie resistant (O'Rourke et al., 1996). However, this resistance may not be absolute as there have been reported cases of QR171 animals with scrapie (Smit et al., 2002). Scrapie development in QR animals is rare (Smit et al., 2002), with a longer onset than in QQ animals, and may be limited to scrapie strains that affect the prion protein at codon 136. Nonetheless, only codon 171 is currently utilized in the recommendations by the National Institute for Animal Agriculture for improving flock scrapie resistance through genetic selection (NIAA, 2004b).



**Figure 1.** Performance index for rams genotyped at codon 171 in the Wyoming terminal sire (Suffolk and Hampshire;  $n = 124$ ) test. Columns with differing subscripts differ ( $P = 0.003$ ). Animals with at least one arginine (R) at codon 171 are considered resistant to most strains of scrapie (NIAA, 2004b).

Carcass measurements are a final measure of lamb production and performance. In this study, no measure of carcass yield was associated with lamb genotype at codon 171. Hot carcass weight ( $P = 0.51$ ), adjusted fat depth ( $P = 0.73$ ), and rib-eye area ( $P = 0.62$ ) did not differ among genotypes (Table 2).

## Implications

Producers can minimize the risk of scrapie infection by selecting for scrapie resistance as prescribed by the national scrapie eradication program (NIAA, 2004b) without detrimentally affecting flock production.

## Literature Cited

- Alexander, B. M., R. H. Stobart, W. C. Russell, K. I. O'Rourke, and G. E. Moss. 2003. Differences in production traits between scrapie resistant and scrapie susceptible ewes. *Proc. West. Sect. Am. Soc. Anim. Sci.* 54: 101-103.
- Boggs, D. L., R. A. Merkel, and M. E. Doumit. 1998. Lamb Carcass Evaluation and Grading. Chapter 14 in *Livestock and Carcasses: An Integrated Approach*

<sup>1</sup> Diameter points are calculated as (22.0 – actual fiber diameter in microns)\*3 with maximum of  $\pm 9$  points. Fiber variability points are calculated as (22.0 – actual coefficient of variation)\* 1.25 with a maximum of  $\pm 5$  points.

- to Evaluation, Grading, and Selection. 5<sup>th</sup> ed. Kendall/Hunt Publishing Co. Debuque, IA, USA.
- Debbie, P., K. Young, L. Pooler, C. Lamp, P. Marietta, and R. Wagner. 1997. Allele identification using immobilized mismatch binding protein: detection and identification of antibiotic-resistant bacteria and determination of sheep susceptibility to scrapie. *Nucleic Acids Res.* 25:4825-4829.
- Hueston, W. D., J. L. Voss, J. Bartz, E. D. Belay, L. A. Detwiler, J. M. Miller, D. Olander, M. E. Potter, R. Rubenstein, L. B. Schonberger, A. Vicari, and E. S. Williams. 2000. Transmissible Spongiform Encephalopathies in the United States. Council for Agricultural Science and Technology. CAST Task Force Report No. 136.
- Johnson, R.T., H.J. Alter, D.O. Cliver, L.D. Cowan. R.Y. Dodd, F.A. Murphy, M.B.A. Oldstone, D. Relman, R.P. Roos, D.M. Taylor, R.B. Wickner, and R.G. Will. 2004. Prion Diseases: An Overview. Chapter 2 in *Advancing Prion Science: Guidance for the National Prion Research Program*. Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science. R. Erdtmann and L.B. Sivitz, ed. Medical Follow-up Agency, Institute of Medicine of the National Academies. The National Academies Press. Washington, D.C., USA.
- NIAA (National Institute for Animal Agriculture). 2004a. What you as a producer need to know about the new scrapie eradication program. Available at: <http://www.animalagriculture.org/scrapie/NIAA%20Producer%20kit.pdf>. Accessed 3/29/04.
- NIAA (National Institute for Animal Agriculture). 2004b. A guide to the national scrapie eradication program for veterinarians. Available at: <http://www.animalagriculture.org/scrapie/NIAA%20Producer%20kit.pdf>. Accessed 3/29/04.
- O'Rourke, K. I., R. P. Melco, and J. R. Mickelson. 1996. Allelic frequencies of an ovine scrapie susceptibility gene. *Anim. Biotech.* 7:155-162.
- O'Rourke, K. I., T. V. Baszler, T. E. Besser, J. M. Miller, R. C. Cutlip, G. A. H. Wells, S. J. Ryder, S. M. Parish, A. N. Hamir, N. E. Cockett, A. Jenny, and D. P. Knowles. 2000. Preclinical diagnosis of scrapie by immunohistochemistry of third eyelid lymphoid tissue. *J. Clin. Microbiol.* 38:3254-3259.
- Smit, M. A., N. E. Cockett, J. E. Beever, T. L. Shay, and S. L. Eng. 2002. Scrapie in sheep: A transmissible spongiform encephalopathy. *Sheep and Goat Res.* 17:21-32.
- USDA. 1992. Official United States Standards for Grades of Lamb, Yearling Mutton, and Mutton Carcasses. Agric. Marketing Serv., USDA, Washington, DC.
- Wineland, N. E., L. Detwiler, D. Salman. 1998. Epidemiological analysis of reported scrapie in sheep in the United States: 1,117 cases (1947-1992). *J. Amer. Vet. Med. Assoc.* 212:713-718.

**Table 1.** Ram performance values for rams genotyped at codon 171 from the Wyoming terminal sire (Suffolk and Hampshire; n = 124) test that comprise the performance index (Figure 1). Animals with at least one arginine (R) at codon 171 are considered resistant to most strains of scrapie (NIAA, 2004b). Within a row, means without a common subscript letter differ ( $P < 0.05$ )

	QQ	QR	RR	SEM	P value
Final BW, kg	83.5 <sup>a</sup>	88.2 <sup>b</sup>	83.4 <sup>a</sup>	1.5	0.02
Loin-Eye Area, cm	19.6 <sup>a</sup>	21.2 <sup>b</sup>	20.5 <sup>a,b</sup>	0.5	0.04
ADG, kg	0.44 <sup>a</sup>	0.48 <sup>b</sup>	0.45 <sup>a</sup>	0.01	0.002

**Table 2.** Carcass data from lambs genotyped at codon 171. Animals with at least one arginine (R) at codon 171 are considered resistant to most strains of scrapie (NIAA, 2004b)

	QQ	QR	RR	SEM	P value
Carcass Wt, kg	30.5	30.2	29.4	1.5	0.51
Fat, cm	0.53	0.53	0.50	0.02	0.73
Loin-eye area, cm	15.5	16.2	15.6	0.1	0.62
Yield Grade	2.7	2.6	2.5	0.1	0.62

## COOLING DRY COWS DURING SUMMER REDUCES STRESS AND IMPROVES POSTPARTUM MILK YIELD

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**ABSTRACT:** In order to determine some dry period physiological responses, and some postpartum production responses, to a summer cooling system, 15 multiparous Holstein cows were assigned to one of two groups approximately 60 days prior to their projected calving date: one group with spray and fans (n=7), and a second group without a cooling system (n=8). The study was conducted at the experimental dairy unit of the Instituto de Ciencias Agrícolas (UABC) located in the Mexicali Valley in Baja California (Mexico). The cooling system operated from 10:00 to 18:00 h daily. Rectal temperatures and respiration rates were measured on two days each week at 10:00, 14:00 and 18:00 h during the dry period. Calf birth weight was recorded and milk yield was measured weekly during the first 8 weeks of lactation. Response variables were analyzed using repeated measurements within a completely randomized block design using analysis of variance. The highest and lowest ambient temperatures observed during the experimental period were 48°C and 19°C. Cooled cows had lower ( $P<0.01$ ) respiration rates and rectal temperatures at 10:00 h ( $53.6$  vs.  $61.5 \pm 1.3$  breaths/min;  $38.7$  vs.  $38.9 \pm 0.07$  °C); at 14:00 h ( $56.0$  vs.  $72.3 \pm 1.3$  breaths/min;  $38.9$  vs.  $39.2 \pm 0.08$  °C), and at 18:00 h ( $60.8$  vs.  $70.3 \pm 1.7$  breaths/min;  $39.1$  vs.  $39.5 \pm 0.1$  °C) than non-cooled cows. Calf birth weight tended ( $P=0.18$ ) to be higher in cooled cows ( $37.9 \pm 2.6$  vs.  $31.8 \pm 3.4$  kg for cooled and non-cooled cows). Milk yield for the full 8 week period was higher ( $P<0.01$ ) in the cooled group compared with the non-cooled group ( $24.9 \pm 1.3$  vs.  $19.4 \pm 1.1$  kg). Cooling dry cows using fans with water spray reduced heat stress under these very hot conditions, and resulted in higher productivity during the subsequent lactation.

**Keywords:** Heat stress, dairy cows, prepartum period, milk production, Mexico.

### Introduction

Hot climates depress feed intake, milk production, and reproductive performance in dairy cattle. The depression in productive and reproductive parameters is becoming a worldwide problem and brings about serious economic losses to the dairy industry. This situation is particularly common in the arid southwest of United States as well as the northwestern region of Mexico, where temperatures may reach 50 °C and dairy cows experience heat stress for prolonged periods (Correa et al., 2000). However, most

of the research done regard the effect of heat stress on dairy cattle is directed to the lactating cow. Studies on the effect of heat stress during the prepartum dry period on production performance have been insufficient. Hot environmental temperatures during the last trimester of gestation have shown to retard placental and fetal growth, resulting in the lower birth weight of calves and higher fetal deaths in sheep (Bell, 1989; Flamenbaum et al., 1995). Wolfenson et al. (1988) demonstrated that calf birth weight increased an average of 2.6 kg for calves born to cows assigned prepartum to a shade structure equipped with fans and sprinklers for cooling, compared with those assigned only to a shade structure. When calf birth weight was associated with milk production, larger calf birth weights were positively related to higher subsequent milk production (Collier et al., 1982; Moore et al., 1992). Prolonged hyperthermia during late gestation results also in hormonal alterations that may affect mammary development, lactogenesis, and milk yield. Collier et al. (1982) found that reduced thyroxine concentrations during pregnancy in nonshaded cows altered the metabolic state of the dam at parturition and potentially decreased mammary development prior to the initiation of lactation.

In summary, it is clear that heat stress during late gestation period induces altered endocrine function that may result in negative effects on subsequent lactational performance. Consequently, strategies to keep cows cool and comfortable prepartum should be a high priority for dairy producers in hot climates zones. The objective of the present study is to determine the effect of a cooling system during the dry period, on some prepartum physiological constants as well as postpartum performance of Holstein dairy cows under hot stressful conditions.

### Material and Methods

This trial was conducted in the facilities of the Experimental Dairy Unit of the Instituto de Ciencias Agrícolas which is dependent on the Universidad Autónoma de Baja California. It is located at the Ejido Nuevo León about 42 km Southeast from Mexicali, the capital city of the state Baja California which is located adjacent to Calexico (CA). Climatic conditions of this zone are dry and extreme with maximum average temperatures during summer up to 50°C. Annual average precipitation is 85 mm and the site is 2 m below sea level

(Garcia, 1985). Fifteen multiparous Holstein cows with expected calving dates between August and October 2001 were used. Cows were housed in two open adjacent corrals with shades in the central area of the corral. All dry cows were managed identically and were fed *ad libitum* with the same total mixed ration twice a day, which included alfalfa hay, wheat straw, wheat grain, wheat bran, and a vitamin/mineral premix. Fresh water was available at all times during the experiment. After calving, all cows were moved to the same pen, which was provided with shades but had no fans or misters, and fed a ration appropriate for cows in early lactation. Cows were milked twice daily at 05:00 and 17:00 h. Cows were paired based on body condition score, and one of each pair was assigned to one of the treatments: 1) Control, no cooling system during the dry period (i.e., 60 d prepartum), and 2) Treated, a cooling system during the same period. The cooling system was installed under the shades for the treated cows and consisted of two fans of 30" diameter and a mist ring of 26" diameter circle with 6 mist heads. Each fan motor was of ½ HP (115/230 volt, single three-phase motor with pressure of 17.58 cm<sup>3</sup> of water). Water delivery of the system was 3 gal/h and they operated from 10:00 to 18:00 h daily. Climatic variables were recorded hourly at the Climatic Experimental Station, located 300 m from the trial site. The collected variables were maximum and minimum temperatures, maximum and minimum relative humidity, and solar radiation. With these variables, the Temperature-Humidity Index was obtained using the following formula proposed by Hahn (1999):

$$THI = (0.81 \times MAT) + REHUM (MAT - 14.4) + 46.4$$

Where:

THI = is the Temperature-Humidity Index,

MAT = is the maximum temperature,

REHUM = is the average relative humidity.

Rectal temperatures (RT) and respiration rates (RR) were recorded twice a week (Tuesday and Friday), three times a day (10:00, 14:00, and 18:00 h). RT was measured using electronic thermometers (Model No. 15-600-000, Mabis HealthCare Inc.), and RR was measured by visual observation to count the breaths per minute. Calf birth weights were recorded and milk yield was recorded weekly until week 8. Data on rectal temperatures, respiration rates, and milk production were analyzed by using repeated measures ANOVA by the GLM procedures of SAS (SAS, 1998) on weekly basis. The linear model included the main effects of cow, treatment (treated vs untreated), body condition (as blocking variable), and the residual error term. Calf birth weight was analyzed by using a completely randomized block design with the same statistical package. All statistical analyses were performed with SAS (1998) software package (version 6.12).

## Results and Discussion

The maximum ambient temperature registered during the experimental period was 49 °C and the minimum 19 °C. Figure 1 shows the maximum and minimum temperature-humidity index during the 15 weeks of the study. The maximum THI was 95 and the minimum 63. Heat stress for dairy cattle starts at THI of 72, so most of the experimental period cows were in a heat stress considered from moderate to severe (Armstrong, 1994).

During the dry period, when cows were cooled or not cooled, there were significant treatment differences ( $P < .01$ ) in RR and RT at 10:00, 14:00, and 18:00 h (Table 1). The results obtained in these physiological variables suggest that non-cooled cows were more heat stressed than cows under the cooling system. However, calf birth weights (Table 1) were only numerically ( $P = .18$ ) higher (37.9 vs. 31.8 kg) in cooled cows. Cooled cows had higher ( $P < .01$ ) milk production (24.9 vs. 19.4 kg) during the full 8 week experimental period (Table 1). The results obtained agree with those obtained from Wolfenson et al. (1988) and Moore et al. (1992). Those authors found advantages with the use of cooling systems during the dry period. The use of a cooling system during the dry period represents an alternative to reduce the stress and to improve postpartum milk production.

## Literature Cited

- Armstrong, D.V. 1994. Heat stress interaction with shade and cooling. *J. Dairy Sci.* 77:2044.
- Bell, A. W. 1989. Chronic heat stress and prenatal development in sheep. I. Conceptus growth and maternal plasma hormones and metabolites. *J. Anim. Sci.* 73:2804.
- Collier, R., S. G. Doelger, H. Head, W. W. Thatcher, and C. J. Wilcox. 1982. Effect of heat stress during on maternal hormone concentrations, calf birth weight and postpartum milk yield in Holstein cows. *J. Anim. Sci.* 54:309.
- Correa, C. A., L. Avendaño, R. Villanueva, D.V. Armstrong, J.F. Smith, y S.K. Denise. 2002. Efecto de un sistema de enfriamiento en la productividad de vacas lecheras bajo estrés calórico. *Agrociencia* 36:531-535.
- Flamenbaum, I., D. Wolfenson, P. L. Kunz, M. Maman, and A. Berman. 1995. Interactions between body condition at calving and cooling of dairy cows during lactation in summer. *J. Dairy Sci.* 78: 2221.
- García, E. 1985. Modificaciones al Sistema de Clasificación Climática de Köppen (para adaptarlo a las condiciones de la República Mexicana). Instituto de Geografía. Universidad

Hahn, G.L. 1999. Dynamic responses of cattle to thermal  
heat loads. J. Dairy Sci. Vol. 82(Suppl. 2):10.

Wolfenson, D., I. Flamenbaum, and A. Berman. 1988.  
Dry period heat stress relief effects on prepartum  
progesterone, calf birth weight, and milk  
production. J. Dairy Sci. 71:809.

Moore, R. B., J.W. Fuquay, and W.J. Drapala. 1992.  
Effects of late gestation heat stress on

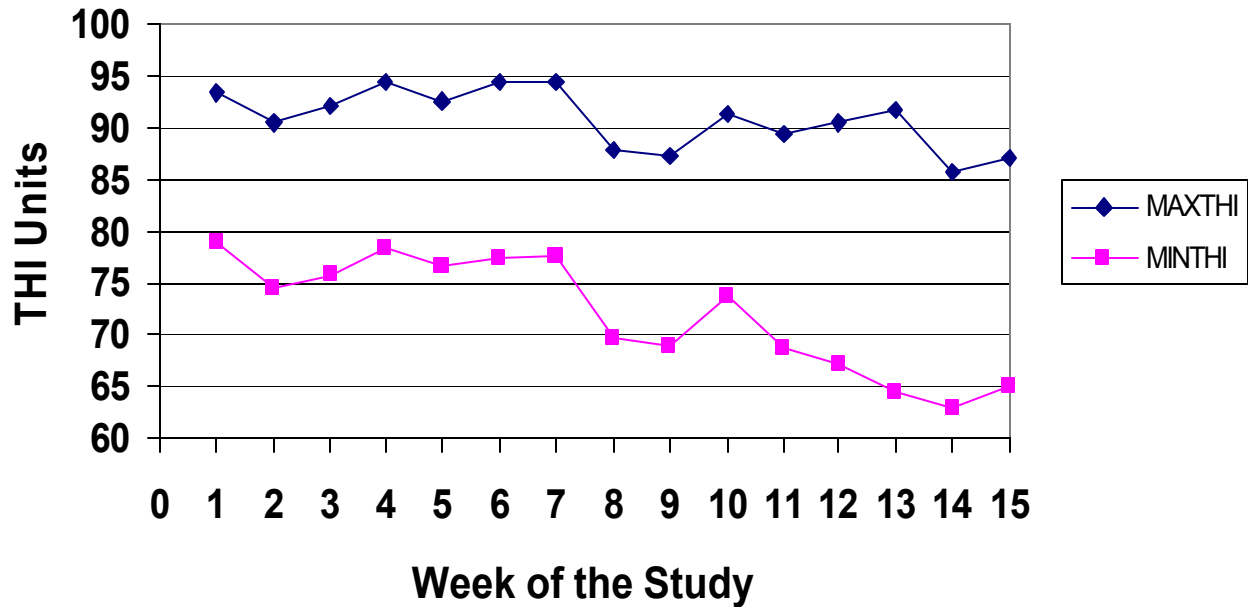


Figure 1. Average minimum (MINTHI) and maximum (MAXTHI) temperature-humidity index during the 15 weeks of study.

Table 1. Least square means for respiration rate (RR) and rectal temperature (RT) by time of the day for cooled and non-cooled cows during the dry period.

Time of the day	variable	cooled	non-cooled	se	probability
10:00	RR	53.6	61.5	1.3	0.0001
	RT	38.7	38.9	0.07	0.0003
14:00	RR	56.0	72.3	1.3	0.0001
	RT	38.9	39.2	0.08	0.0001
18:00	RR	60.8	70.3	1.7	0.0001
	RT	39.1	39.5	0.10	0.0003

Table 2 Least square means for calf birth weight and milk yield at 8 week period for cooled and non-cooled cows.

Variable	cooled Mean $\pm$ S.E.	non-cooled Mean $\pm$ S.E.	probability
Calf birth weight	37.9 + 2.6	31.8 + 3.4	0.180
Milk Yield	24.9 + 1.3	19.4 + 1.1	0.002

# GROWTH AND PUBERTAL RESPONSES OF FEMALE OFFSPRING PRODUCED BY EWES TREATED WITH PROPYLTHIOURACIL AND MELATONIN DURING PREGNANCY

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**ABSTRACT:** Thyroid hormones influence onset of anestrus in ewes. Effects of altering maternal thyroid status during pregnancy on reproductive patterns of female offspring are unknown. In this study, 24 Rambouillet ewe lambs were used to examine effects on ewe lamb performance after maternal treatment with propylthiouracil (PTU) and melatonin during gestation. Beginning January 2 (first day of treatment at  $76.8 \pm 4.7$  d of gestation), ewes received 0 or 40 mg PTU/kg BW daily (gavage) for 15 d. After 15 d, the 40 mg dosage was decreased to 20 mg/kg BW for 20 d, and melatonin was given (I.M., 5 mg/d) for 30 d. Five and 19 female lambs were produced by PTU-treated and control ewes, respectively. Lambs were weighed at birth, and blood samples were collected on d 0 (parturition), 14, and 28 and were evaluated for serum thyroxine. Lambs were weaned at 60 d of age and were allowed free access to shade, salt, water, and alfalfa hay. Ground corn was fed at 0.3 kg/animal daily until 7 mo of age. Blood samples were collected from ewe lambs three times weekly beginning in mid-August, and serum progesterone ( $P_4$ ) was measured to determine date of puberty (day when serum  $P_4$  rose above 1 ng/mL for 2 consecutive samples). At birth, 60% of female lambs from PTU-treated dams had enlarged thyroid glands compared with none of the lambs from control ewes. However, serum thyroxine did not differ ( $P > 0.10$ ) in the two groups. Control ewe lambs ( $5.3 \pm 0.3$  kg) weighed more ( $P = 0.05$ ) at birth than did those from PTU-treated dams ( $4.6 \pm 0.3$  kg). This weight difference tended to be present ( $P = 0.08$ ) at weaning and at monthly weigh periods after weaning. Control ewe lambs reached puberty on October 17 ( $\pm 12$  d) while those from PTU-treated ewes were pubertal on November 4 ( $\pm 12$  d,  $P = 0.17$ ). Female offspring from control and PTU-treated ewes were 206 and 227 ( $\pm 12$ ) d of age at puberty, respectively ( $P = 0.13$ ). Maternal PTU treatment during gestation resulted in lower BW of female offspring and tended to delay onset of puberty.

Key Words: Sheep, Puberty, Thyroid

## Introduction

The management strategy of breeding spring born ewe lambs is utilized by sheep producers to increase total lifetime productivity from the ewe flock. The ability of the ewe lamb to reach puberty is determined by several factors including age, body size, nutrition, season of birth, and photoperiod (Yellon and Foster, 1986). Exposure to long days of spring and summer, followed by short days in autumn induces photoperiodic cues to stimulate the ewe lamb's first estrus (Foster et al., 1985; Herbosa et al., 1994). Ewe lambs must experience these conditions because of the

seasonal nature of the ovine reproductive cycle which is made up of a fall breeding season followed by a season of anestrus. During anestrus, pulsatile secretion of LH decreases due to a change in estradiol feedback on LH from positive to negative (Karsch et al., 1984). In mature ewes, the thyroid hormone thyroxine ( $T_4$ ) has been indicated to play a role in seasonal reproduction. Karsch et al. (1995) prevented estradiol from providing negative feedback by removing the thyroid gland prior to the end of the breeding season. This allowed the period of cyclicity to continue throughout the anestrus season. Administering  $T_4$  to thyroidectomized ewes caused decreases in LH and the eventual ending of cyclicity (Dahl et al., 1995). These data confirm that the thyroid gland is involved in seasonal anestrus. Studies by Hernandez et al. (1999) and Bollinger et al. (2000) examined effects of the thyroid inhibitor propylthiouracil (PTU) on pregnant ewes. In both experiments, oral administration of PTU was effective in lowering circulating  $T_4$  concentrations. Work done by Wells et al. (2003) demonstrated that administration of PTU to ewe lambs did not hasten puberty or improve pregnancy rates. Currently no data are available that demonstrate effects of altering maternal thyroid status during pregnancy on reproductive patterns of female sheep. Therefore the objective of this research was to examine effects on ewe lamb performance after maternal treatment with PTU and melatonin during gestation.

## Materials and Methods

### Maternal Treatments

Beginning on d 0 (January 2,  $76.8 \pm 4.7$  d of gestation), eight mature Rambouillet ewes received (gavage) 40 mg PTU/kg BW/d for 15 d. After 15 d, the 40 mg dosage of PTU was decreased to 20 mg/kg BW for an additional 20 d, and melatonin was given (i. m. injections at 5 mg/d) for 30 d. Past studies using pregnant ewes (Hernandez et al., 1999; Bollinger et al., 2000) demonstrated that dosages of 12 mg PTU or less did not decrease serum  $T_4$  below 20 ng. A more recent study (Hernandez et al., 2003) showed that a larger dosage of PTU (40 mg/kg BW) lowered serum  $T_4$  below 20 ng. Melatonin was administered at 1600 each day. This melatonin regimen was employed because Perez-Equia and Hallford (1994) showed it would elevate night time serum melatonin concentrations for 3 to 6 h after treatment. A complete description of maternal PTU treatments can be found in Gifford et al. (2004).

### *Female Offspring*

Five ewe lambs were produced by PTU-treated ewes and 19 ewe lambs were produced by contemporary ewes in the breeding flock. All lambs were weighed at birth and at regular intervals throughout the trial. Ewe lambs were weaned at 60 d of age and were maintained in a single pen (4 x 12 m) under ambient conditions with free access to shade, salt, water, and alfalfa hay. Ground corn was fed at 0.3 kg/animal daily until 7 mo of age. Lambs were vaccinated against tetanus and enterotoxemia at 30 d of age and again at weaning.

### *Blood Collection*

Initial ewe lamb blood samples were collected on d 0 (parturition), 14, and 28. Beginning in mid-August, blood samples were collected from ewe lambs three times weekly. Blood samples were collected before feeding into sterile vacuum tubes (Corvac 7, Kendall Health Care, St. Louis, MO). Samples were allowed to clot at room temperature for approximately 30 min before centrifugation at 1,500 x g for 15 min at 4°C to separate serum. Following centrifugation, serum was transferred to plastic vials and stored frozen at -20°C until analyzed. All animal procedures were approved by the Institutional Animal Care and Use Committee.

### *Hormone Analyses*

Serum  $P_4$  was quantified by solid phase RIA using components of commercial kits supplied by Diagnostic Products Corp. (DPC, Los Angeles, CA). Modifications to the  $P_4$  assay were described by Schneider and Hallford (1996). Date of puberty was determined by measuring  $P_4$  in samples collected three times weekly. Puberty was defined as the day that  $P_4$  rose above 1 ng/mL and stayed for two consecutive blood samples. Ewe lamb  $T_4$  serum at birth was quantified by RIA (Richards et al., 1999) utilizing components of a DPC kit. The within and between assay coefficients of variation were less than 10%.

### *Statistical Analysis*

Variables measured were ewe lamb birth weight, actual weaning weight, adjusted weaning weight, and age at time of puberty. Effect of maternal PTU treatment on these variables were determined by analysis of variance for completely random designs. Post weaning BW responses were compared by split plot analysis of variance for repeated measures on animals (Gill and Hafs, 1972). Maternal PTU effects were included in the main plot while time and the treatment x time interaction were in the subplot. Maternal PTU effects were tested using animal within treatment as the error term. All analyses were computed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC).

## **Results and Discussion**

Propylthiouracil decreased ( $P \leq 0.05$ ) maternal serum  $T_4$  on d 23 through 41 of the treatment period (Gifford et al., 2004). However, the decrease was not as pronounced as in previous studies (Hernandez et al., 1999; Bollinger et al., 2000). Gifford et al. (2004) also

demonstrated that the dams of these ewe lambs had decreased serum triiodothyronine in response to PTU. At birth, 60% of female lambs from PTU-treated dams had enlarged thyroid glands compared with none of the lambs from control ewes. However, serum thyroxine did not differ ( $P > 0.10$ ) in the two groups. These data demonstrate that maternal PTU treatment induced hypothyroidism in the dams, and the enlarged thyroid glands in the majority of their offspring suggests that our ewe lambs were exposed to hypothyroid conditions in utero.

At parturition, control ewe lambs ( $5.3 \pm 0.3$  kg) weighed more ( $P = 0.05$ ) than did those from PTU-treated dams ( $4.6 \pm 0.3$  kg). Actual weaning weight was similar ( $P = 0.26$ ) between groups; but when 60-d weaning weights were adjusted to a single ewe lamb, mature ewe basis (Scott, 1977) control lambs ( $27.3 \pm 1.4$  kg) tended to be heavier ( $P = 0.08$ ) than lambs produced by PTU-treated dams ( $24.4 \pm 1.4$  kg). Split plot analysis of post-weaning weight responses detected no maternal PTU treatment by time interaction ( $P > 0.10$ ) allowing examination of maternal treatment effects across the entire post-weaning period. Using this procedure revealed that control ewe lambs weighed more ( $P = 0.04$ ) after weaning than did those from PTU-treated ewes. At 42 d after weaning, control females weighed  $36.2 \pm 2.0$  kg compared with  $30.6 \pm 2.0$  kg for offspring from treated ewes ( $P = 0.03$ ). This magnitude of difference was consistent throughout the post-weaning period such that at approximately 7 mo of age (142 d after weaning), control ewe lambs weighed  $54.9 \pm 1.8$  kg while those from treated ewes weighed  $50.2 \pm 1.8$  kg ( $P = 0.03$ ).

The average date of birth was March 24 and March 22 (SE = 3 d) for control ewe lambs and those from treated ewes, respectively, indicating a very uniform group. However, the average date of puberty for controls was October 17 compared with November 4 (SE = 12 d,  $P = 0.17$ ) for female offspring of treated ewes. Based on day of birth and date of puberty, control lambs were  $206 \pm 12$  d of age when they attained puberty compared with  $227 \pm 12$  d ( $P = 0.13$ ) for ewe lambs produced by PTU-treated dams. Although these puberty estimates did not differ statistically between groups, the size of the numerical difference indicates a tendency for offspring from PTU-treated ewes to reach puberty later than control ewe lambs. However, this trend is most likely a reflection of the BW difference between the two groups of ewe lambs rather than a direct effect of maternal PTU treatment. Using a similar set of ewe lambs to those in the present experiment, Shirley et al. (2001) showed that heavier ewe lambs reached puberty at 200 d of age compared with 214 d of age ( $P = 0.06$ ) for ewe lambs classified as light.

## **Implications**

Propylthiouracil treatment during mid gestation resulted in a hypothyroid state in ewes. This maternal hypothyroidism resulted in lower weights of female offspring which also tended to delay onset of puberty.

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## Literature Cited

- Bollinger, J. L., D. M. Hallford, J. A. Hernandez, and K. L. Shirley. 2000. Reproductive, hormonal, and milk characteristics of ewes treated with propylthiouracil and melatonin during late gestation. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 51:374-379.
- Dahl, G. E., N. P. Evans, L. A. Thrun, and F. J. Karsch. 1995. Thyroxine is permissive to seasonal transitions in reproductive neuroendocrine activity in the ewe. *Biol. Reprod.* 52:690-696.
- Foster, D. L., S. M. Yellon, and D. H. Olster. 1985. Internal and external determinants of the timing of puberty in the female. *J. Reprod. Fertil.* 75:327-344.
- Gifford, C. A., R. L. Knight, J. L. Duffey, and D. M. Hallford. 2004. Effects of short-term propylthiouracil and melatonin treatment during gestation on serum thyroxine, postpartum reproduction, and lamb performance in fine-wool ewes. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 55:(In press).
- Gill, J. L., and H. D. Hafs. 1971. Analysis of repeated measurements in animals. *J. Anim. Sci.* 33:331-336.
- Herbosa, C. G., R. I. Wood, H. I'Anson, and D. L. Foster. 1994. Prenatal photoperiod and the timing of puberty in the female lamb. *Biol. Reprod.* 50:1367-1376.
- Hernandez, J. A., D. M. Hallford, J. L. Bollinger, K. L. Shirley, and J. B. Richards. 1999. Serum thyroxine, progesterone, and luteinizing hormone and body weight and milk characteristics in ewes treated with PGF<sub>2α</sub> and propylthiouracil during gestation. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 50:226-231.
- Hernandez, J. A., D. M. Hallford, and N. H. Wells. 2003. Ovarian cyclicity in thyroid-suppressed ewes treated with propylthiouracil immediately before onset of seasonal anestrus. *J. Anim. Sci.* 81:29-34.
- Karsch, F. J., E. L. Bittman, D. L. Foster, R. L. Goodman, S. J. Legan, and J. E. Robinson. 1984. Neuroendocrine basis of seasonal reproduction. *Recent Progress in Hormone Research* 40:185-225.
- Karsch, F. J., G. E. Dahl, T. M. Hachigian, and L. A. Thrun. 1995. Involvement of thyroid hormones in seasonal reproduction. *J. Reprod. Fertil. Suppl.* 49:409-427.
- Perez-Equia, E., and D. M. Hallford. 1994. Growth, reproductive and endocrine characteristics of ewe lambs after administration of exogenous melatonin. *Sheep & Goat Res. J.* 3:194-200.
- Richards, J. B., D. M. Hallford, and G. C. Duff. 1999. Serum luteinizing hormone, testosterone and thyroxine and growth responses of ram lambs fed locoweed (*Oxytropis sericea*) and treated with vitamin E/selenium. *Theriogenology* 52:1055-1066.
- Schneider, F. A., and D. M. Hallford. 1996. Use of rapid progesterone radioimmunoassay to predict pregnancy and fetal numbers in ewes. *Sheep & Goat Res. J.* 12(1):33-38.
- Scott, G. E. 1977. *The Sheep Man's Production Handbook* (2<sup>nd</sup> Ed.). Sheep Industry Development Program, Denver, CO.
- Shirley, K. L., J. A. Hernandez, D. M. Hallford, and M. G. Thomas. 2001. Serum luteinizing hormone, growth hormone and insulin-like growth factor-I after releasing hormone challenge in prepubertal ewe lambs selected for twinning. *Theriogenology* 56:867-877.
- Wells, N. H., D. M. Hallford, and J. A. Hernandez. 2003. Serum thyroid hormones and reproductive characteristics of Rambouillet ewe lambs treated with propylthiouracil before puberty. *Theriogenology* 59:1403-1413.
- Yellon, S. M., and D. L. Foster. 1986. Melatonin rhythms time photoperiod-induced puberty in the female lamb. *Endocrinology* 119:44-49.

# INCIDENCE OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* IN FECAL, WOOL, AND CARCASS SAMPLES IN FEEDLOT LAMBS

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**ABSTRACT.** The present study examined the incidence of *Escherichia coli* O157:H7 and *Salmonella* in lambs in the feedlot and at slaughter. We hypothesized *E. coli* O157:H7 and *Salmonella* organisms are prevalent in feces and on the pelt of feedlot lambs and pose a potential source of carcass contamination. Fecal, wool, and carcass samples were examined in 56 lambs for *E. coli* O157:H7 and *Salmonella* to evaluate potential carcass contamination sources. Fecal samples were collected at monthly intervals upon entering feedlot until slaughter. Prior to slaughter, belly wool samples were collected. Overall, *E. coli* O157:H7 prevalence in fecal, wool, and carcass samples was 9% (12 of 129). *Salmonella* prevalence in fecal, wool, and carcass samples was 19% (25 of 129). *E. coli* O157:H7 was isolated from 2 of 43 (5%) fecal, 9 of 43 (21%) wool, and 1 of 43 (2%) carcass samples. The single *E. coli* O157:H7 positive carcass had negative fecal and wool samples, suggesting cross contamination at slaughter. *Salmonella* was isolated from 4 of 43 (9%) fecal, 21 of 43 (49%) wool, and 0 of 43 carcass samples. A higher prevalence of *Salmonella* was seen in wool and that the wool is an important source of potential contamination. However, it appears that modern slaughter techniques and sanitation methods are effective in reducing carcass contamination.

**Keywords:** *E. coli* O157:H7, *Salmonella*, Feedlot Lambs

## Introduction

In the United States, 14% of the population (approximately 30 million people) will experience some form of food borne illness annually (Lundberg, 1997). Medical and productivity costs are high in both dollars and loss of life. Two pathogenic bacteria, *E. coli* O157:H7 and *Salmonella* account for a large proportion of the above losses. The presence of enterohemorrhagic *E. coli* O157:H7 and *Salmonella* in the intestinal tracts of various animal species, including ruminants, account for major reservoirs of human food-borne illnesses (Bielaszewska et al., 2000; Cornick et al., 2000; Fedorka-Cray et al., 1998). Common symptoms of *E. coli* O157:H7 infection in humans is painful, bloody diarrhea, which may lead to the hemolytic uremic syndrome or thrombotic thrombocytopenic purpura (Griffen and Tauxe, 1991). Numerous studies attribute cattle as a natural reservoir of virulent *E. coli* O157:H7 (Wang et al., 1996), however, recent investigations have detected naturally occurring *E. coli* O157:H7 in sheep (Kudva et

al., 1996). The selective use of sensitive culture techniques and appropriate season of sampling are necessary in detecting this human pathogen in sheep. Studies have shown increased *E. coli* O157:H7 fecal shedding during the summer months (Cornick et al., 2000). Environmental influences such as high temperatures and diet help to colonize virulent *E. coli* strains in various ruminant populations such as cattle and sheep (Kudva et al., 1996; Wang et al., 1996). Elder et al. (2000) investigated the correlation of enterohemorrhagic *E. coli* O157 prevalence in fecal, hide, and carcass samples of beef cattle. Their study determined fecal and hide prevalence were significantly correlated with carcass contamination, which further indicated a role for control of enterohemorrhagic *E. coli* O157 in cattle. Results from our earlier study on the incidence of *E. coli* O157:H7 in feedlot lambs on the New Mexico State University Farm, found a higher prevalence of the *E. coli* O157:H7 in wool samples (56%) than in fecal samples (40%), however there were no positive carcass samples. The overall prevalence of *E. coli* O157:H7 in lambs found in the study was 37% (22 of 60 samples). *Salmonella* species are commonly reported and costly cause of food borne disease in humans with food borne transmission accounting for approximately 95% of all Salmonellosis cases in the United States (Mead et al., 1999). *Salmonella* causes illness in many animals, including ruminants. Clinical signs of illness include diarrhea and fever in adults and neonates occasionally leading to death. Cattle and sheep have been shown to be asymptomatic carriers of *Salmonella*, shedding this pathogen into the environment (Fedorka-Cray et al., 1998). At present, studies investigating the incidence of *E. coli* O157:H7 and *Salmonella* in fecal, wool, and carcass samples in ovine species are limited. The aim of this study was to determine if fecal and wool samples testing positive for *E. coli* O157:H7 and *Salmonella* are a potential source for carcass contamination.

## Materials and Methods

**Animals.** For this study, ewe and wether lambs were sampled from a feedlot in San Angelo, Texas during the summer and fall seasons between June and October 2003. Under a typical feedlot management program, all lambs were maintained in a common pen in drylot conditions. The institutional animal care and use committee approved all procedures.

**Sample Collection.** Initially, 56 feedlot lambs from various Texas ranches were selected for sampling from lambs entering the feedlot. Samples were collected during the months of June, August, September, and October 2003. Initial fecal samples were collected from lambs in June. Between August and October, fecal, wool, and carcass samples were collected. Fecal grabs were obtained via rectal palpation and placed in a sealed bag. Wool samples were shorn from the ventral midline just before moving to packing plant and placed in a sealed bag. Carcasses were sampled after 24 h in the cooler. Carcasses were swabbed using a sponge moistened with sterile phosphate buffer in a bag (Nasco ® Whirl Pak, Modesto, CA). Sponges were used to swab the leg/flank, shoulder, and rectal regions of the carcass. Gloves and other sanitary precautions were used to protect samples from other bacterial contamination during the collection process.

### Culture Methods.

***E. coli* O157:H7.** All fecal, wool, and carcass samples were shipped to the USDA/ARS Texas Experiment Station in College Station, TX for further analysis. Fecal samples (10 g) were enriched in 90 mL of gram-negative broth containing vancomycin, cefixime, and cefsulodin for 6 h at 37° C. *E. coli* O157:H7 was isolated using an immunomagnetic separation technique using anti-*E. coli* O157 antibody-labeled paramagnetic beads (Dynabead anti-*E. coli* O157, Dynal Inc., Lake Success, N.Y.). The resulting suspension (50 µL) was spread onto a sorbitol MacConkey agar plate, containing cefixime and potassium tellurite and incubated for 18 h at 37° C. Colonies exhibiting typical *E. coli* O157:H7 colony phenotype were selected from each plate and confirmed as O157:H7 using Reveal microbial screening tests according to the manufacturer's instructions (Neogen Corporations, Lansing, MI). Wool and carcass swab specimens were enriched in 20 mL of sterile 22% brilliant green bile broth and incubated for 6 h at 37° C. The remainder of the isolation techniques was the same as described above for fecal samples. Identified *E. coli* O157:H7 isolates were stored in glycerol-TSB at -80° C.

***Salmonella.*** Fecal material (5–10 g) was enriched in 90 mL of tetrathionate broth for 24 h at 37° C. The above suspension (200 µL) was added to 5 mL Rapport-Vassilisis R10 broth, incubated at 42° C for 24 hours, and plated on brilliant green agar containing novobiocin. *Salmonella* samples were characterized biochemically using lysine agar and triple sugar iron agar if typical *Salmonella* morphology was displayed. Slide agglutination using SM-O antiserum poly A-I and V-I was used to confirm *Salmonella* positive samples.

**Statistical Analysis.** Summary statistics were conducted using Frequency procedure of SAS (SAS Institute Inc., Cary, NC). Statistical summaries were reported for presence or absence of *E. coli* O157:H7 and *Salmonella* in fecal, wool, and carcass samples.

## Results and Discussion

Overall, the prevalence of *Salmonella* organisms (19%) was a higher than *E. coli* O157:H7 organisms (9%) in fecal, wool, and carcass samples. Of 43 wool samples, 21 (49%) samples tested positive for *Salmonella* and 9 (21%) tested positive for *E. coli* O157:H7. *Salmonella* prevalence in fecal samples was 9% (4 of 43), while *E. coli* O157:H7 prevalence in fecal samples was 5% (2 of 43). *Salmonella* was not isolated from any carcass samples, however, *E. coli* O157:H7 was isolated from 1 of 43 (2%) carcass samples. Of eight Texas ranches involved in the study, one ranch contributed more ( $P < .0001$ ) lambs with fecal samples testing positive for *E. coli* O157:H7. However, these lambs tested negative for *E. coli* O157:H7 at the second sampling period approximately 1 mo later. *Salmonella* present in the feces did not differ ( $P > 0.20$ ) among the ranches. When final fecal, wool, and carcass samples were analyzed, a positive fecal sample did not always correlate with a positive wool sample during sample collection. Data suggest that cross contamination occurred between fecal and wool material at a higher incidence than carcass cross contamination at slaughter. The single positive *E. coli* O157:H7 carcass had negative fecal and wool samples. In a similar study on *E. coli* O157:H7 prevalence in beef cattle, Elder et al. (2000) reported an approximate prevalence of 28% in beef cattle sampled in the United States. Zschock et al. (2000) reported approximately 20% of feedlot cattle in Europe are carriers of *E. coli* O157:H7. Elder et al. (2000) demonstrated a direct correlation between fecal populations of *E. coli* O157:H7 and the level of carcass contamination. Elder et al. (2000) later demonstrated that *E. coli* O157:H7 can be isolated from the oral cavity, hide surface, and feces. Elder et al. (2000) suggested that bacterial culture of feces alone generally underestimates the percentage of fed beef cattle positive for *E. coli* O157:H7. Even though the number of human outbreaks of *E. coli* O157:H7 that are attributed to ovine rather than bovine sources are far less, the research indicates sheep are a reservoir for *E. coli* O157:H7. Results from our study investigating the incidence of *E. coli* O157:H7 in feedlot lambs on the New Mexico State University farm, confirmed a higher prevalence of *E. coli* O157:H7 in wool samples than in fecal samples (Long et al., 2003). Of 18 wool samples, 10 samples (56%) tested positive for *E. coli* O157:H7. Of the 30 fecal samples, 12 (40%) were positive for *E. coli* O157:H7. All carcass swab samples tested negative for *E. coli* O157:H7 contamination, therefore, we did not determine any cross contamination from fecal and wool to carcass. Several reasons may attribute to this finding. First, carcass contamination may not have occurred in lambs on this study, suggesting sanitary conditions were maintained. Second, negative carcass samples may be due to sampling technique. Sampling technique and procedures may not have been effective in removing *E. coli* O157:H7 organisms from the carcasses.

## Implications

As reported earlier, data from the current study indicate that sheep are a natural reservoir for food borne pathogenic *E. coli* O157:H7 and *Salmonella* bacterial organisms. Our hypothesis that wool was an important source of potential contamination was confirmed in this study. Carcass contamination was very low, suggesting modern slaughter techniques and sanitation methods are effective during slaughter.

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## Literature Cited

- Bielaszewska, M., H. Schmidt, A. Liesegang, R. Prager, W. Rabsch, H. Tschape, A. Cizek, J. Janda, K. Blahova, and H. Karch. 2000. Cattle can be a reservoir of sorbitol fermenting shiga toxin producing *Escherichia coli* O157:H- strains and a source of human diseases. *J. Clin. Microbiol.* 38:3470-3473.
- Cornick, N.A., S.L. Booher, T.A. Casey, and H.W. Moon. 2000. Persistent colonization of sheep by *Escherichia coli* O157:H7 and other *E. coli* pathotypes. *Appl. Environ. Microbiol.* 66:4926-4934.
- Elder, R.O., J.E. Keen, G.R. Siragusa, G.A. Barkocy Gallagher, M. Koohmaraie, and W.W. Laegreid. 2000. Correlations of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Nat. Acad. Sci.* 97:2999-3003.
- Fedorka-Cray, P.J., D.A. Dargatz, L.A. Thomas, and J.T. Gray. 1998. Survey of *Salmonella* serotypes in feedlot cattle. *J. Food Prot.* 61:525-530.
- Griffen, P.M. and R.V. Tauxe. 1991. The epidemiology of infections caused by *E. coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* 13:60-98.
- Kudva, I.T., P.G. Hatfield, and C.J. Hovde. 1997. Characterization of *Escherichia coli* O157:H7 in microbial flora of sheep. *J. Clin. Microbiol.* 34: 431-433.
- Long, M., T.T. Ross, T.S. Edrington, J.D. Thomas, and K.L. Christensen. 2003. Incidence of *Escherichia coli* O157:H7 contamination in fecal, wool and carcass samples from feedlot lambs. *Proc. Wes. Sec., Amer. Soc. Anim. Sci.* 54: 46-48.
- Lundberg, G.D. 1997. Putting money where the U.S. mouth is: Initiative on food safety gets underway (editorial). *J. Am. Med. Assoc.* 277:1340-1342.
- Mead, P.S., L. Slutsker, V. Dietz, L.F. McCraig, J.S. Bresee, C. Shapiro, P.M. Griffin, and R.V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Inf. Dis.* 5(5):1-39.
- Wang, G., T. Zaho, and M.P. Doyle. 1996. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. *Appl. Environ. Microbiol.* 62: 2567-2570.
- Zschock, M., H.P. Hamann, B. Kloppert, and W. Wolter. 2000. Shiga-toxin producing *Escherichia coli* in feces of healthy dairy cattle, sheep, and goats: Prevalence and virulence properties. *Lett. Appl. Microbiol.* 31:203-208.

## USE OF ULTRASOUND TO DETERMINE BODY COMPOSITION OF BEEF COWS NUTRIENT RESTRICTED DURING EARLY TO MID-GESTATION

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**ABSTRACT:** One hundred-sixteen multiparous Angus  $\times$  Gelbvieh cows (initial BW =  $571 \pm 63$  kg, BCS =  $5.4 \pm 0.7$ ) were blocked by BW, assigned to one of 18 pens, and received one of two dietary treatments from d-31 to 125 of gestation (Exp. 1). Control (C) cows were fed native grass hay fortified with vitamins and minerals at recommendations for a mature cow to gain 0.72 kg/d for the first 120 d of gestation. Nutrient restricted (NR) cows were fed one half C minerals and vitamins, and millet straw at 68.1% of NE<sub>m</sub> requirements. Along with BW and BCS, ultrasound measurements of ribeye area (REA), 12th rib fat thickness at the 12<sup>th</sup> rib (BF), and percent i.m. fat of the LM (IMF) were collected every 14 d. In Exp. 2, 96 cows from Exp. 1 were re-blocked according to BW and BCS and assigned to one of 16 pens. Control cows continued to be fed as in Exp. 1, while NR cows were realimented with the target of achieving BCS similar to C cows by 60-d parturition. Body weight and BCS were measured every 14 d, whereas REA, BF, and IMF were measured every 28 d. A subset of cows from Exp. 1 ( $n = 20$ ) and Exp. 2 ( $n = 10$ ) were harvested to determine correlations between ultrasound and carcass measurements of REA ( $r = 0.49$ ,  $P = 0.006$ ), BF ( $r = 0.85$ ,  $P < 0.001$ ), and IMF ( $r = 0.69$ ,  $P < 0.001$ ). In Exp. 1, treatment  $\times$  day of sampling interactions were noted ( $P \leq 0.001$ ) for all variables. Body weight, BCS, BF, IMF, and REA were reduced ( $P < 0.05$ ) by d-59, 45, 59, 73, and 73 of gestation, respectively. In Exp. 2, BW and BF remained less ( $P < 0.002$ ) for NR than C cows throughout the realimentation period. Cow BCS and REA were lower ( $P \leq 0.03$ ) for NR versus C cows until d-164 of gestation, but were similar ( $P = 0.11$  and  $P = 0.58$ , respectively) by d-192 of gestation. Ultrasound may be a useful technology to predict changes in body composition associated with a beef cow's nutritional plane.

**Key words:** Nutrient Restriction, Ultrasound, Beef Cow

### INTRODUCTION

Beef cows grazing rangelands in the western United States may consume low-quality forage during early to mid-gestation (DelCurto et al., 2000), and thus may experience periods of undernutrition (NRC, 1996). Maternal nutrient deficiencies during critical times of pregnancy can permanently affect development of fetal tissues (Barker, 1995). Furthermore, calf birth weight has been reduced when their dams were fed on a low plane of nutrition from mid-gestation through early lactation (Freetly et al., 2000). Beef cow producers may curtail the possibility of cows experiencing nutrient deficits by implementing feed supplementation practices. The BCS system (Wagner et al.,

1988) has been adopted by the beef industry as a method to assess a cow's plane of nutrition by subjectively evaluating her body energy reserves. On a one-to-nine scale, differences from BCS 1 to 4 are primarily the result of variation in energy stored as muscle protein whereas changes from BCS 5 to 9 are mostly related to external body fat (Mathis et al., 2002). Due to the subjectivity of the BCS systems, however, ultrasound measurements may also be used in commercial cow operations to aid in the assessment of cow energy reserves. Ultrasound measurements can provide estimates of some body energy reserves in a fast, inexpensive, and repeatable fashion. Ultrasound technology has been successfully employed to as a management tool in the feedlot (Houghton and Turlington, 1992; Smith et al., 1992; Greiner et al., 2003) and replacement female sectors (Tait, Jr. et al., 2004) of the beef industry. Although Bullock et al. (1991) demonstrated that ultrasound measurements can be used to predict cow body energy reserves, this technology has yet to be fully exploited to evaluate changes in body energy reserves in mature beef cows maintained on various planes of nutrition. The first objective of this study was to determine the correlation between ultrasound and carcass measurements of beef cows. The second objective was to evaluate BW, BCS, and ultrasound measurements of ribeye area, 12<sup>th</sup> rib fat thickness, and percent i.m. fat in pregnant beef cows fed various planes of nutrition.

### MATERIALS AND METHODS

All procedures were conducted in accordance with an approved University of Wyoming Animal Care and Use Committee. In Exp. 1, on d 31 of gestation, 116 multiparous, Angus  $\times$  Gelbvieh cows (initial BW,  $571 \pm 63$  kg; initial BCS,  $5.4 \pm 0.7$ ) were stratified by weight, assigned to one of nine blocks, and allotted to one of two pens within each block (5 to 7 cows/pen). Control cows were fed native grass hay (12.1% CP, 70.7% TDN on a DM basis) fortified with vitamins and minerals at NRC (1996) recommendations for a mature cow to gain 0.72 kg/d during the first 125 d of gestation. Nutrient Restricted cows were fed one half the Control minerals and vitamins, and millet straw (9.9% CP, 54.5% IVDMD) to provide 68.1% NE<sub>m</sub> and 86.7% of metabolizable protein requirements during the first 120 d of gestation (NRC, 1996). Feed intake was adjusted every 14 d based on average pen weight. Body weight was measured and BCS measurements were calculated as the average of three trained individual's estimates on d 31, 45, 59, 73, 87, 101, and 115 of gestation. Ribeye area, 12<sup>th</sup> rib fat, and percentage i.m. fat were measured on d 31, 45, 59, 73, 87, 101, and 115 of gestation

via ultrasound using the "New" Aloka SSD-500 with 17.2-cm transducer (Aloka Co., Ltd, Wallingford, CT). Images were collected on Beef Image Analysis (BIA) software (Designer Genes Technologies, L.L.C.).

In Exp. 2, 96 cows from Exp. 1 were re-blocked according to BW and BCS. Cows were assigned to one of 16 pens (5 to 7 cows/pen). Control cows were fed the same diet as in Exp. 1. Nutrient restricted cows were fed the Nutrient Restricted hay, Control minerals and vitamins, and a corn-based supplement (Table 1) to achieve a BCS equal to the Control cows by d 220 of gestation. Body weight and BCS were measured on d 136, 150, 164, 178, and 192 of gestation. Ribeye area, 12<sup>th</sup> rib fat thickness, and percentage i.m. fat were measured on d 136, 164, and 192 of gestation via ultrasound as in Exp. 1.

A subset of cows from Exp. 1 ( $n = 20$ ) and Exp. 2 ( $n = 10$ ) were slaughtered at the end of each experiment. Cows were withheld from feed over night, slaughtered using normal industry procedures, and chilled at 2 to 4°C for 48 h. Forty-eight hours postmortem, the left side of each carcass was ribbed between the 12<sup>th</sup> and 13<sup>th</sup> ribs and 12<sup>th</sup> rib fat and ribeye area measurements (Boggs et al., 1998), and marbling scores (USDA, 1989) were recorded. These values were used to determine correlations between live animal ultrasound estimates and actual measurements of ribeye area, 12<sup>th</sup> rib fat, and percentage i.m. fat. All data were analyzed using the GLM procedures of SAS (SAS Inst. Cary, NC) using a model for a split-block design. Dietary treatment was the main plot tested against the treatment  $\times$  block interaction (error a), with the period and treatment  $\times$  period interaction as the subplot tested against residual error (error b).

## RESULTS AND DISCUSSION

Ultrasound and carcass measurements were moderately to highly correlated and significant. Carcass measurements versus ultrasound-predicted ribeye area, 12<sup>th</sup> rib fat thickness, and percent i.m. fat were all positively correlated with  $r$ -values of 0.49, 0.85, and 0.69, respectively. In their review of the literature, Houghton and Turlington (1992) found that correlation coefficients for actual vs. ultrasound-predicted ranged from 0.20 to 0.94 for ribeye area, 0.55 to 0.96 for 12<sup>th</sup> rib fat thickness, and 0.21 to 0.91 for percentage i.m. fat. Smith et al. (1992) attributed their moderately low correlation coefficient for ribeye area ( $r = 0.43$ ) to improper placement of the transducer, poor image resolution, or inaccurate interpretation of the image. The potential for error caused by improper placement of the transducer cannot be eliminated, but we suspect that poor images resulting from insufficient surface contact in the cows with lower BCS may explain our moderate correlation coefficient for ribeye area. Bullock et al. (1991) reported a high correlation coefficient ( $r = 0.79$ ) for 12<sup>th</sup> rib fat thickness for cows ranging in mean BCS from 2.9 to 7.1. The slightly higher correlation coefficient ( $r = 0.85$ ) noted herein could be attributed to more uniform BCS for the cows on this study (BCS ranged from 4.1 to 6.7). Harada et al. (1985; as cited by Houghton and Turlington, 1992) reported correlation coefficients of 0.78 and 0.24 for percentage i.m. fat in serial scans of Japanese black bulls. These researchers attributed the

inability to consistently measure percentage i.m. fat on the premise that i.m. fat is a very mobile energy reserve that is highly affected by environment. Our findings, combined with previous research would imply that the use of ultrasound is an acceptable method of tracking changes in cow 12<sup>th</sup> rib fat, percentage i.m. fat, and ribeye area. However, there are limitations to the use of correlation coefficients in the reporting of ultrasound accuracy (Houghton and Turlington, 1992). Two limitations that potentially explain the observed results would be that populations with larger than normal variations will produce high correlations, and correlation coefficients do not necessarily reflect the bias associated with consistently over or underestimating measurements (Houghton and Turlington, 1992; Greiner et al., 2003). Houghton and Turlington (1992) also suggest that there is potential that position of the hanging carcass influences measurements, therefore influencing the perceived accuracy of ultrasound.

### Exp. 1

Treatment  $\times$  period interactions were noted ( $P \leq 0.001$ ) for all variables. By design, BW of Nutrient Restricted cows was less than that of Control cows by d 59 of gestation, and remained lower ( $P < 0.001$ ) for the duration of the restriction period (Table 2). Body condition score was also reduced ( $P < 0.001$ ) by d 45 of gestation and remained lower ( $P < 0.001$ ) for the duration of the restriction period (Table 2). Freetly et al. (2000) noted similar reductions in cow BW and BCS with nutrient restriction during mid to late gestation. Lalman et al. (1997) and Buskirk et al. (1992) noted that each unit change in BCS corresponded with a 33 kg and 40 kg change in BW, respectively. Additionally, the NRC (1996) suggests that more weight is associated with a change in BCS for cows with a BCS  $> 5$ . In the current study, Control cows would have changed 122 kg in BW for each unit change in BCS, whereas Nutrient Restricted cows would have changed 108 kg for each corresponding unit change in BCS. Some of the discrepancy between BW and associated BCS change in our study could be attributed to the increased weight gain associated with pregnancy. In a companion abstract, Vonnahme et al. (2004) reported that Control cows had heavier fetuses than Nutrient Restricted cows. Estimated weights increases associated with the gravid uterus (Ferrell et al., 1976; Prior and Laster, 1979) ranged from 3.66 to 8.22 kg for this same period. A large proportion of the weight change for the Nutrient Restricted cows can be attributed to the loss in internal organ mass. In a companion paper, Molle et al. (2004) reported that Nutrient Restricted cows had reduced weights of the rumen, omasum, heart, pancreas, liver, and kidney. Therefore, apparent associations between changes in BW and BCS in our study may not have been similar to previous reports because of the confounding influence of the gravid uterus and (or) the mass of visceral tissue.

Twelfth-rib fat, percentage i.m. fat, and ribeye area were reduced ( $P \leq 0.05$ ) for Nutrient Restricted cows by d 59, 73, and 73 of gestation, respectively (Table 2), and tended to be different ( $P \leq 0.10$ ) on d 45 and 59 for 12<sup>th</sup> rib fat and percentage i.m. fat, respectively. Reduced fat cover over the 12<sup>th</sup> rib occurring sooner than reduced i.m. fat or ribeye area

indicates that s.c. fat reserves were more readily mobilized than intramuscular adipose tissue or muscle energy reserves. Consistent with our observations, Bullock et al. (1991) reported decreased back fat thickness and reduced ribeye area as cow BCS decreased from 7.1 to 2.9. Our findings also support the suggestion that i.m. fat is a mobile energy reserve (Harada et al., 1985 as cited by Houghton and Turlington, 1992).

#### Exp. 2

In our second experiment, previously restricted cows were fed to achieve a BW similar to Controls by d 220 of gestation. Cow BCS was lower ( $P \leq 0.001$ ) for Nutrient Restricted versus Control cows through d 178, but only tended to be lower ( $P = 0.09$ ) on d 192, although BW remained less ( $P < 0.001$ ) for Nutrient Restricted than Control cows throughout the realimentation period (Table 3). Therefore, Nutrient Restricted cows were on track to be similar in weight to Control cows by d 220 of gestation. Control cows gained 36.2 kg but only increased BCS by 0.06 units, whereas Nutrient Restricted cow BW increased 70.9 kg with 0.47 units in BCS. This would equate to one unit in BCS gain for every 603 kg gain in BW for Control cows and one BCS unit for every 151 kg increase in BW for Nutrient Restricted cows. Expected gain associated with the growth of the gravid uterus from d 115 to 192 of gestation was between 12.2 (Prior and Laster, 1979) and 17.0 kg (Ferrell et al., 1976). Thus, increased BW of cows was partially attributed to growth of the gravid uterus. Data from our companion paper (Molle et al., 2004) indicated that realimenting the Nutrient Restricted cows increased total digestive tract weight by 22.0%, which compared to a 10.9% increase for Control cows. These same cows exhibited an increase of 43.3% and 14.3% (Nutrient Restricted and Control, respectively) in the combined weights of the lung, heart, pancreas, liver, and kidney weight from d 125 to 250 of gestation. Increased BW without a concomitant increase in BCS was likely due to a combination of increased weight of the gravid uterus and increased internal organ mass, especially for the Control cows.

Freetly et al. (2000) noted that cows nutrient restricted during gestation will regain BCS when realimented either during the last third of gestation or during the first 28 d of lactation. Over the course of the realimentation period, Nutrient Restricted cows in our study increased ( $P \leq 0.001$ ) in BCS. Although i.m. fat percentage did not change ( $P = 0.18$ ) over the course of Exp. 2, Nutrient Restricted cows tended to have a lower ( $P = 0.11$ ) percentage i.m. fat than Control cows. Likewise, 12<sup>th</sup> rib fat thickness for Nutrient Restricted cows remained less ( $P < 0.001$ ) than Control cows throughout the realimentation period. Cow ribeye area was lower ( $P \leq 0.001$ ) for Nutrient Restricted versus Control cows until d 164 of gestation, but were similar ( $P = 0.58$ ) by d 192 of gestation. Our findings support the contention of Harada et al. (1985; as cited by Houghton and Turlington, 1992), who suggested that i.m. fat percentage is a mobile energy reserve that is highly affected by environment. The ability of the realimented cows to regain ribeye area indicates increased gain of bodily protein reserves (Mathis et al., 2002; NRC, 1996). The inability of

the realimented cows to regain s.c. fat by d 192 of gestation was consistent with our observation that BCS tended to be lower in these cows. Buskirk et al. (1992) demonstrated that body lipid content increased to a greater degree than body protein as cow BCS increased. Thus, ultrasound measurement of back fat thickness at the 12th rib may be a sensitive estimate of a cow's body energy reserve.

#### IMPLICATIONS

Ultrasound is a useful technology to predict changes in body composition associated with a beef cow's nutritional plane. Utilizing ultrasound measurements to predict body energy reserves will aid producers in making informed decisions about cow nutritional management programs.

#### LITERATURE CITED

- Barker, D. J. P. 1995. Fetal origins of coronary heart disease. *Br. Med. J.* 311:171-174.
- Boggs, D. L., R. A. Merkel, and M. E. Doumit. 1998. *Livestock and Carcasses An Integrated Approach to Evaluation, Grading, and Selection*. 5th ed. Kendall/Hunt Publishing Co. Dubuque, IA.
- Buskirk, D. D., R. P. Lemanager, and L. A. Horstman. 1992. Estimation of net energy requirements ( $NE_m$  and  $NE_\Delta$ ) of lactating beef cows. *J. Anim. Sci.* 70:3867-3876.
- Bullock, K. D., J. K. Bertrand, L. L. Benyshek, S. E. Williams, and D. G. Lust. 1991. Comparison of real-time ultrasound and other live measures to carcass measures as predictors of beef cow energy stores. *J. Anim. Sci.* 69:3908-3916.
- DelCurto, T., B. W. Hess, J. E. Huston, and K. C. Olson. 2000. Optimum supplementation strategies for beef cattle consuming low-quality roughages in the western United States. *Proc. Am. Soc. Anim. Sci.* 1999. available at: <http://www.asas.org/jas/symposia/proceedings>.
- Ferrell, C. L., W. N. Garrett, and N. Hinman. 1976. Growth, development and composition of the udder and gravid uterus of beef heifers during pregnancy. *J. Anim. Sci.* 42:1477-1489.
- Freetly, H. C., C. L. Ferrell, and T. G. Jenkins. 2000. Timing of realimentation of mature cows that were feed-restricted during pregnancy influences calf birth weights and growth rates. *J. Anim. Sci.* 78:2790-2796.
- Greiner, S. P., G. H. Rouse, D. E. Wilson, L. V. Cundiff, and T. L. Wheeler. 2003. The relationship between ultrasound measurements and carcass fat thickness and longissimus muscle area in beef cattle. *J. Anim. Sci.* 81:676-682.
- Harada, H., K. Moriya, and R. Fukuhara. 1985. Early prediction of carcass traits of beef bulls. *Jpn. J. Zootech. Sci.* 56(3):250.
- Houghton, P. L., and L. M. Turlington. 1992. Application of ultrasound for feeding and finishing animals: A review. *J. Anim. Sci.* 70:930-941.

- Lalman, D. L., D. H. Keiser, J. E. Williams, E. J. Scholljegerdes, and D. M. Mallett. 1997. Influence of postpartum weight and body condition change on duration of anestrus by undernourished suckled beef heifers. *J. Anim. Sci.* 75:2003-2008.
- Mathis, C. P., J. E. Sawyer, and R. Parker. 2002. Managing and feeding beef cows using body condition scores. New Mexico State University. Cooperative Extension Service. Circular 575.
- Molle, J. D. C., E. J. Scholljegerdes, S. L. Lake, V. Nayigihugu, R. L. Atkinson, L. R. Miller, S. P. Ford, W. J. Means, J. S. Caton, B. W. Hess. 2004. Effects of maternal nutrient restriction during early- to mid-gestation on cow and fetal visceral organ measurements. *Proc. West. Sect. Am. Soc. Anim. Sci.* 55:(Submitted).
- NRC. 1996. Nutrient requirements of beef cattle, 7<sup>th</sup> rev. ed. Natl. Acad. Press, Washington, DC.
- Prior, R. L., and D. B. Laster. 1979. Development of the bovine fetus. *J. Anim. Sci.* 48:1546-1553.
- Smith, M. T., J. W. Oltjen, H. G. Dolezal, D. R. Gill, and B. D. Behrens. 1992. Evaluation of ultrasound for prediction of carcass fat thickness and longissimus muscle area in feedlot steers. *J. Anim. Sci.* 70:29-37.
- Tait, Jr., R. G., G. H. House, D. R. Maxwell, M. L. Spangler, and P. B. Wall. 2004. A comparison of serially scanned replacement and feedlot Angus sired heifers for body composition traits: Ribeye area, fat cover, and percent intramuscular fat. *Iowa State Univ. Anim. Industry Rep.* A.S. Leaflet R1871.
- USDA. 1989. Official United States standards for grades of carcass beef. Agri. Marketing Service, United States Dept. of Agriculture, Washington, D.C.
- Vonnahme, K. A., S. P. Ford, M. J. Nijland, L. P. Reynolds. 2004. Alteration in cotyledonary (COT) vascular responsiveness to angiotensin II (ANG II) in beef cows undernourished during early gestation. *Proc. Soc. Study Reprod.*, Vancouver, B.C., Canada, (In Press)
- Wagner, J. J., K. S. Lusby, J. W. Oltjen, J. Rakestraw, R. P. Wettemann, and L. E. Walters. 1988. Carcass composition in mature Hereford cows: Estimation and effect on daily metabolizable energy required during winter. *J. Anim. Sci.* 66:603-612.

Table 1. Realimentation supplement fed to cows d 125 through d 192 of gestation

Ingredient	Ration composition, %
Corn	79.6
Soybean meal	6.1
Sunflower meal	5.3
Molasses	4.2
Safflower meal	2.6
Dried skim milk	1.6
Chemical composition	-----% DM-----
CP	13.2
IVDMD	77.6

Table 2. Effects of nutrient restriction on body composition of multiparous beef cows during early to mid gestation (Exp. 1)

Measurement	Day of gestation							SE	P<*
	31	45	59	73	87	101	115		
Body weight, kg									
C	573.8 <sup>aef</sup>	570.9 <sup>ce</sup>	575.9 <sup>af</sup>	581.2 <sup>ag</sup>	587.6 <sup>ah</sup>	593.1 <sup>ai</sup>	598.3 <sup>aj</sup>	1.4	0.001
NR	580.0 <sup>be</sup>	567.2 <sup>df</sup>	563.9 <sup>bf</sup>	559.0 <sup>bg</sup>	556.1 <sup>bg</sup>	546.3 <sup>bh</sup>	541.2 <sup>bi</sup>		
BCS (1-9) **									
C	5.4 <sup>e</sup>	5.5 <sup>afi</sup>	5.5 <sup>af</sup>	5.5 <sup>af</sup>	5.6 <sup>ag</sup>	5.8 <sup>ah</sup>	5.6 <sup>agi</sup>	0.03	0.001
NR	5.4 <sup>e</sup>	5.4 <sup>be</sup>	5.2 <sup>bf</sup>	5.1 <sup>bg</sup>	5.0 <sup>bh</sup>	4.9 <sup>bh</sup>	5.1 <sup>bg</sup>		
Ribeye area, cm <sup>2</sup> ***									
C	69.0 <sup>ef</sup>	68.3 <sup>e</sup>	72.2 <sup>fh</sup>	76.4 <sup>ag</sup>	75.7 <sup>ag</sup>	76.3 <sup>ag</sup>	74.8 <sup>agh</sup>	1.5	0.001
NR	69.3 <sup>ch</sup>	69.0 <sup>eh</sup>	74.8 <sup>f</sup>	71.9 <sup>befg</sup>	68.7 <sup>befg</sup>	67.8 <sup>bh</sup>	67.9 <sup>bh</sup>		
12th rib fat, cm ***									
C	0.49 <sup>e</sup>	0.51 <sup>ce</sup>	0.55 <sup>af</sup>	0.56 <sup>afi</sup>	0.58 <sup>afgh</sup>	0.60 <sup>agh</sup>	0.58 <sup>ahi</sup>	0.01	0.001
NR	0.46 <sup>ef</sup>	0.47 <sup>def</sup>	0.44 <sup>beg</sup>	0.48 <sup>bf</sup>	0.43 <sup>bg</sup>	0.41 <sup>bg</sup>	0.43 <sup>bg</sup>		
Intramuscular fat, %***									
C	3.98 <sup>e</sup>	3.93 <sup>e</sup>	4.04 <sup>cef</sup>	4.14 <sup>af</sup>	4.29 <sup>agh</sup>	4.30 <sup>ag</sup>	4.17 <sup>afh</sup>	0.1	0.001
NR	4.07 <sup>e</sup>	3.87 <sup>f</sup>	3.89 <sup>df</sup>	3.76 <sup>bfg</sup>	3.83 <sup>bf</sup>	3.79 <sup>bfg</sup>	3.70 <sup>bg</sup>		

<sup>a,b</sup> Means in a column with different superscripts differ ( $P < 0.05$ ).

<sup>c,d</sup> Means in a column with different superscripts differ ( $P < 0.10$ ).

<sup>e,f,g,h,i,j</sup> Means in a row with different superscripts differ ( $P < 0.10$ ).

\*  $P$ -values for treatment  $\times$  day interaction

\*\* Body Condition Score 1 = emaciated, 9 = very obese (Wagner et al., 1988).

\*\*\* Ultrasound measurements.

Table 3. Effects of realimentation on body composition of multiparous beef cows during early to mid gestation (Exp. 2)

Measurement	Day of gestation						SE	$P <^*$
	115	136	150	164	178	192		
Body weight, kg								
C	600.6 <sup>ae</sup>	613.0 <sup>af</sup>	628.4 <sup>cg</sup>	630.0 <sup>ag</sup>	636.5 <sup>ah</sup>	636.8 <sup>ah</sup>	2.7	0.001
NR	546.7 <sup>be</sup>	566.9 <sup>bf</sup>	579.1 <sup>dg</sup>	586.4 <sup>bh</sup>	601.4 <sup>bi</sup>	617.6 <sup>bj</sup>		
BCS (1-9) **								
C	5.6 <sup>ae</sup>	5.9 <sup>af</sup>	5.7 <sup>ae</sup>	5.7 <sup>ae</sup>	5.7 <sup>ae</sup>	5.7 <sup>ce</sup>	0.05	0.001
NR	5.1 <sup>be</sup>	4.8 <sup>bf</sup>	4.8 <sup>bf</sup>	5.0 <sup>be</sup>	5.3 <sup>bg</sup>	5.6 <sup>dh</sup>		
Ribeye area, cm <sup>2</sup> ***								
C	75.4a <sup>e</sup>	77.8 <sup>ae</sup>	-	80.8 <sup>afg</sup>	-	77.6 <sup>cg</sup>	1.4	0.03
NR	68.4 <sup>be</sup>	68.7 <sup>be</sup>	-	73.1 <sup>bf</sup>	-	78.7 <sup>g</sup>		
12th rib fat, cm ***								
C	0.58 <sup>ae</sup>	0.65 <sup>af</sup>	-	0.70 <sup>ag</sup>	-	0.69 <sup>afg</sup>	0.02	0.002
NR	0.42 <sup>be</sup>	0.42 <sup>be</sup>	-	0.45 <sup>be</sup>	-	0.57 <sup>bf</sup>		
Intramuscular fat, %***								
C	4.21	4.11	-	4.27	-	4.35	0.08	0.18
NR	3.70	3.87	-	3.74	-	3.94		

<sup>a,b</sup> Means in a column with different superscripts differ ( $P < 0.05$ ).<sup>c,d</sup> Means in a column with different superscripts differ ( $P < 0.10$ ).<sup>e,f,g,h,i,j</sup> Means in a row with different superscripts differ ( $P < 0.10$ ).\*  $P$ -value for treatment  $\times$  day interaction

\*\* Body Condition Score 1 = emaciated, 9 = very obese (Wagner et al., 1988).

\*\*\* Ultrasound measurements.

**EVALUATION OF FOUR FEEDING SYSTEMS FOR HOLSTEIN CALVES IN THE MEXICALI VALLEY, MEXICO**

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**ABSTRACT:** In order to evaluate the effect of four different systems of liquid feeding on daily weight gain and to compare their costs, 200 dairy calves (95 females and 105 males) of two days of age were assigned to one of four groups: group 1 (n=67) fed with 4 L of whole milk; group 2 (n=58) fed with 0.4 kg of replacer master milk mixed in 3.6 L of warm water; group 3 (n=35) fed with 0.4 kg of replacer hi-bloom mixed 3.6 L of warm water, and group 4 (n=40) fed with 0.46 kg of replacer ultra milk mixed 3.54 L of warm water. Calves consumed 2 L of colostrum at 6 and 12 h after birth. Calves starter was offered to all calves from the first week of age. Calf birth weight was recorded as well as weights of calves each 10 d until weaning (60 d of age). Statistical analysis was performed using SAS. Average weight of calves at 60 d (W60) was higher ( $P<.05$ ) in group 1 than in groups 2, 3, and 4 ( $67.0 \pm 0.89$  vs  $62.5 \pm 1.00$ ,  $59.2 \pm 1.24$ , and  $58.72 \pm 1.17$  kg, respectively); W60 in group 2 was higher ( $P<.05$ ) than groups 3 and 4. Daily weight gain (DWG) was higher ( $P<.05$ ) in group 1 than in groups 2, 3, and 4 ( $0.58 \pm 0.01$  vs  $0.51 \pm 0.01$ ,  $0.46 \pm 0.02$ , and  $0.43 \pm 0.02$  kg, respectively), and DWG for group 2 was higher ( $P<.05$ ) than groups 3 and 4. However, average daily feeding cost per animal was higher in group 1 (1.09 and 1.23 dollars) than the rest of the groups considering two different costs: costs in Mexicali valley and costs of the milk plant in Mexicali city. Regarding cost of milk replacers, group 3 was the most economical alternative (0.71 dollars). Even though calves fed with whole milk showed better performance, it was the most expensive feeding system. These results show that the use of milk replacers could be an alternative to reduce feeding costs for dairy calves.

**Key Words:** Milk replacer, Daily weight gain, Economical analysis

**Introduction**

In Mexico, there has been a great interest to become milk production systems into more profit business, due to the lack of milk supply and its great demand from a growing human population. One way to solve this problem is growing dairy calves with small amounts of whole milk or using commercial milk replacer (MR) for a short period of time (Dalzell and Allen, 1970; De Peters et al., 1986; Plaza and Fernandez, 1991). Milk replacers are an excellent feed for calves before weaning. When formulated properly MR are cheaper than whole milk. However, MR are designed to supply adequate nutritive components and to promote an aggressive concentrate intake in order to provide an acceptable growth of calves (Quigley, 1997). Feeding calves with MR is possible to obtain good adaptation to balanced diets, because MR stimulate calf starter and this fact has an important effect on consumption, rumen development, and calves performance before and after weaning (Luchini et al., 1993; Plaza and Fernández 1994a,b). Whole milk is an essential feed for a calf; however, the demand of this product for human consumption has promoted the use of MR, reducing liquid feed costs (Plaza and Fernandez, 1994a). There are some studies about MR evaluation in the Mexicali Valley, Baja California, showing good results in weight gain and economical profit (Guerrero et al., 1998; Saucedo et al., 1999). Nevertheless, it is necessary to continue with these kind of studies in order to assess new commercial MR that are available for milk producers in Baja California. The objective of this study is to evaluate four different systems of liquid feeding and to compare their costs.

**Materials and Methods**

The study was carried out at the experimental dairy herd unit of the Instituto de Ciencias Agrícolas of the UABC. This herd is located in the Ejido Nuevo Leon, Baja California, situated 42 km SW of Mexicali city. Two hundred Holstein calves were used (95 females and 105

males) from 2 to 60 d of age born from august, 1997 to march, 2002. All calves were fed 2 L of colostrum using nursing bottle during the first 6 to 12 h after birth. Calves were distributed randomly into four groups: group 1 (n=67) fed with 4 L of whole milk; group 2 (n=58) fed with 0.4 kg of replacer master milk mixed in 3.6 L of warm water; group 3 (n=35) fed with 0.4 kg of replacer hi-bloom mixed in 3.6 L of warm water, and group 4 (n=40) fed with 0.46 kg of replacer ultra milk mixed in 3.54 L of warm water. All groups were fed with nursing bottle twice a day (2 L am and 2 L pm). Milk replacers used for this study are sold at regular livestock stores and contain 20% of PC and 20% fat. After birth, calves were separated from their dam and housed individually. Subsequently, they were assigned to the groups. Calves were weight at birth and every 10 d until 60 d of age. Calf starter (18% PC) and alfalfa hay was offered ad libitum from the first week of age. The economic analysis was performed as follows: for whole milk, the cost per liter was calculated from two sale prices: a) 0.27 dollars, sale price at the UABC, and b) 0.30 dollars, sale price at the milk plants in Mexicali city. The costs for MR was obtained according the technical recommendation of the factory: a) Master Milk Replacer, sack of 25 kg provides 62.5 d of liquid feeding; cost per sack is 49.54 dollars and 250 L of the final mixed gives 0.20 dollars per liter; b) Hi-Bloom replacer, sack of 20 kg provides 50 d of liquid feeding; cost per sack is 35.9 dollars and 200 L of the final mixed gives 0.18 dollars per liter, and c) Ultra Milc Replacer, sack of 20 kg provides 43.47 d of liquid feed; cost per sack is 38.18 dollars and 173.91 L of the final mixed gives 0.22 dollars per liter. Data were subject to analysis of variance using a completely randomized design by using GLM procedure (General Linear Models) from the Statistical Program SAS (1991).

### Results and Discussion

Live weights (LW) and daily weight gains (DWG) of calves at 40, 50 and 60 d per group are shown in Table 1. LW at 40 d of group 1 (whole milk,  $51.60 \pm 0.59$  kg) was greater ( $P<.05$ ) than LW of groups 2 (master milk,  $47.46 \pm 0.59$  kg), 3 (hi-bloom,  $46.40 \pm 0.82$  kg) and 4 (ultra milc,  $45.97 \pm 0.77$  kg). Meanwhile, LW at 60 d was greater ( $P<.05$ ) in group 1 ( $67.04 \pm 0.89$  kg) than in groups 2, 3 and 4 ( $62.53 \pm 1.00$ ;  $59.20 \pm 1.24$ ;  $58.72 \pm 1.17$  kg respectively). Among MR, LW at 60 d was higher ( $P<.05$ ) in group 2 than in groups 3 and 4; however, there were no significant difference ( $P>.05$ ) in groups 3 and 4.

The results obtained in the present study are higher than those found in the literature, where milk replacers have protein source from vegetal origin (Perez et al., 1986; Plaza and Fernandez, 1994, 1997). When these results were compared with MR of protein source from animal origin,

LW's were similar (Pérez et al., 1986; Plaza y Fernández, 1991; Guerrero et al., 1998; Saucedo et al., 1999). DWG are also shown in Table 1. Calves with higher LW obtained the higher DWG at 40, 50 and 60 d of age. DWG at 60 d was higher ( $P<.05$ ) in group 1 ( $0.58 \pm 0.01$  kg) than in groups 2, 3 and 4 ( $0.508 \pm 0.02$ ;  $0.455 \pm 0.02$  and  $0.443 \pm 0.02$  kg respectively). Among MR, group 2, had higher ( $P<.05$ ) DWG than groups 3 and 4. The results obtained in this study agree with the fact that whole milk is the ideal feed for calves growth because its nutrients are more efficiently used than nutrients found in MR. However, feeding costs using whole milk are almost 50% higher when it is included in calves nursing programs (Plaza and Fernandez, 1994b). Table 2 shows the economical analysis based on the two sale prices mentioned. Costs of liquid feeding in group 2 were 27.33 and 35.88% lower than whole milk; and costs of group 3 were 34.20 and 41.91% lower than costs of whole milk. On the other hand, group 4 was 19.66 and 29.11% lower than whole milk. This proves that feeding and handling calves during preweaning period using MR is more economical than whole milk (Plaza y Fernández, 1997).

### Implications

Calves live weight and daily weight gain was higher in the group of animals feed with whole milk, but the most economical cost of liquid feeding was obtained by hi-bloom milk replacer.

### Literature Cited

- De Peters, E. J., L. J. Fisher y J. LL. Stone. 1986. Effect of adding drained whey to starter diet of early and late weaned calved. *J. Dairy Sci.* 69:181.
- Guerrero R. J., Saucedo Q. J. S., Pérez M. A. y González G. H. 1998. Comparación de tres sistemas de alimentación líquida en la crianza artificial de becerros Holstein. In: Mem. 8va. Reunión Anual Internacional Sobre Producción de Carne y Leche en Climas Cálidos. 24 y 25 de Septiembre. Mexicali, B. C. pp. 80-84.
- Luchini, N.D., S.F. Lane, and D.K. Combs. 1993. Preweaning intake and postweaning dietary energy effects on in take and metabolism of calves weaned at 26 days of age. *J. Dairy Sci.* 76:255.
- Pérez, L. O., D. A. Avila, S. J. M. Pérez y P. H. Román. 1986. Comportamiento de becerros de razas lecheras criadas en condiciones de clima tropical subhúmedo. *Memorias del XXX Congreso Nacional de Buiatría. Tampico. Tamps.* Pág. 741.

Plaza J. y E. Fernández. 1991. Efecto de la cantidad de yogur y la edad destete en el comportamiento de terneros Holstein. Rev. Cubana Ciencias Agrícolas. 25:171.

Plaza J. y E. Fernández. 1994a. Efecto de la sustitución de leche integra por reemplazador lechera (RL) en el comportamiento de los terneros. Rev. Cubana Ciencias Agrícolas. 28:51.

Plaza J. y E. Fernández. 1994b. Efecto del sistema de alimentación en el comportamiento de los terneros. Rev. Cubana Ciencias Agrícolas. 28:175.

Plaza J. y E. Fernández. 1997. Crianza artificial de terneros en fincas lecheras. Rev. Cubana Ciencias Agrícolas. 31:32.

S.A.S. 1989. SAS/STAY User's guide: Statistic (ver. 7de). SAS Inst. Inc., Cary, N.C.

Saucedo Q. J. S., Pérez M. A., Cervantes R. M. y González G. H. 1999. Crianza de becerros Holstein con adición parcial y total y sustituto de leche. In: Memorias 9va. Reunión Internacional Sobre Producción de Carne y Leche en Climas Cálidos. 22 al 24 de septiembre de 1999. Mexicali, B. C. México. Pp 259-262.

Table 1. Live Weight (LW) and Daily Weigh Gain (DWG) of calves in three age periods according to their liquid feeding.

VARIABLES	GROUPS*			
	G 1	G 2	G 3	G 4
No. Animals	67	58	35	40
LW (kg):	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
40 d	51.60 $\pm$ 0.59 <sup>a</sup>	47.46 $\pm$ 0.59 <sup>b</sup>	46.40 $\pm$ 0.82 <sup>b</sup>	45.97 $\pm$ 0.77 <sup>b</sup>
50 d	58.77 $\pm$ 0.75 <sup>a</sup>	54.34 $\pm$ 0.83 <sup>b</sup>	52.15 $\pm$ 1.04 <sup>bc</sup>	51.91 $\pm$ 0.98 <sup>c</sup>
60 d	67.04 $\pm$ 0.89 <sup>a</sup>	62.53 $\pm$ 1.00 <sup>b</sup>	59.20 $\pm$ 1.24 <sup>c</sup>	58.72 $\pm$ 1.17 <sup>c</sup>
DWG (kg):				
40 d	0.487 $\pm$ 0.01 <sup>a</sup>	0.386 $\pm$ 0.01 <sup>b</sup>	0.361 $\pm$ 0.02 <sup>b</sup>	0.347 $\pm$ 0.01 <sup>b</sup>
50 d	0.532 $\pm$ 0.01 <sup>a</sup>	0.446 $\pm$ 0.01 <sup>b</sup>	0.405 $\pm$ 0.02 <sup>bc</sup>	0.396 $\pm$ 0.02 <sup>c</sup>
60 d	0.580 $\pm$ 0.01 <sup>a</sup>	0.508 $\pm$ 0.01 <sup>b</sup>	0.455 $\pm$ 0.02 <sup>c</sup>	0.443 $\pm$ 0.02 <sup>c</sup>

\* Groups: G 1= Whole Milk; G 2= Master Milk Replacer; G 3= Hi-Bloom Replacer; G 4= Ultra Mile Replacer.

<sup>a, b, c</sup> Rows with different letters indicate a significant difference (P<.05).

Table 2. Economic aspects of four liquid feeding systems of calves during breeding period

VARIABLES	GROUPS <sup>a</sup>			
	G 1 <sup>b</sup>	G 2	G 3	G 4
No. de Animals	67	58	35	40
Intake/Animal/day (liters)	4	4	4	4
Cost/liter (dll)	0.27 0.30	0.20	0.18	0.22
Cost/animal/day (dll)	1.09 1.23	0.80	0.72	0.88
Intake/animal in 59 days (liters)	236	236	236	236
Cost/Animal/Treatment in 59 days (dll)	64.36 72.94	46.77	42.37	51.70
Saving/Animal/Treatment in 59 days (%)		27.33 35.88	34.20 41.91	19.66 29.11

<sup>a</sup>Groups: G 1= Whole Milk; G 2= Master Milk Replacer; G 3= Hi-bloom Replacer; G 4= Ultra mile Replacer..

<sup>b</sup>Sale cost in US currency at UABC (0.27 dollars) and sale price of milk plants in Mexicali city (0.30 dollars).

## BRINGING BREAK-EVEN ANALYSIS ONE STEP FURTHER

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**ABSTRACT:** Financial stability of Western cow/calf producers hinge on determining break even costs of production and analyzing marketing opportunities. In the West, producers rely on three common marketing/production opportunities; selling or back grounding calves, retaining or purchasing stockers, and retaining ownership through the feedlot phase. As beef producers moved into a world of technologies and changes, they found that break even tools have progressed from paper and pencil, to DOS based computer programs, and to Windows based decision making tools. During the 1980's Universities, consultants, and computer users developed software tools for use in the decision making process. However, as operating systems were developed, many of these tools were not updated to function and take advantage of technology changes. University of Nevada Cooperative Extension established a procedure to modify and bring three of these types of DOS based decision making tools into the Windows environment. CALFWNTR (renamed CALFBACK), GRASSFAT, and FEEDLOT, which are partial budgeting DOS based computer programs, have been updated into Windows based operating systems and are available for producer use. The new programs are interactive and dynamic; with instant updates to results as new datum is input. The programs have the input section and output section on one screen, results are immediately visible. The programs will also save previously developed scenarios. The programs were modified and Beta tested by producers and Extension personnel and published following a blind review process. The programs are available for user download at <http://www.ag.unr.edu/cabnr/Resources.htm> and <http://agecon.uwyo.edu/RiskMgt/>. Utilizing web based tracking information downloads of the programs are tracked. To date 707 Downloads of the 3 programs, in 37 states, plus 4 foreign Countries has been documented.

Key Words: Decision Aids, Computer Programs, Management Software

### Introduction

Management and marketing are key components of success and profitability of any beef cow/calf operation. A major concern of most beef cow/calf producers is to determine the value of a cow because making a profit is their number one goal (Carson et al., 1992) and the value of a cow is largely determined by management schemes in selling calves. There are many factors involved in management of a cow/calf operation and in marketing its products. The sale price is a function of supply and demand, and is largely established by the market value of

comparable classes and quality of cattle (Price, 1985). These factors interact in a complex manner making any attempt to separate their effects, when predicting profitability as a function of management and marketing decisions, difficult and impractical. Therefore, our approach was to develop and update a series of simple computer programs (CalfBack, Feedlot, and GrassFat, running under Windows98™ or later versions) to enable producers to evaluate various management practices and their potential impacts on profitability. These programs link the management and marketing variables for calves commonly found in a cow/calf operation in an interactive way. This results in an immediate response to any changes in the input data and, therefore, provides the users with the ability to test many "what if scenarios" and their subsequent effects on profitability. This lets producers check rapidly many different scenarios and possible prices, costs, etc., and how they will affect profitability. These programs evaluate management ideas and profit potential of chosen scenarios. These programs consider money borrowed, production parameters, and other variables to evaluate profit potential. Varying any one of these give insights to management practices that could be emphasized to increase profit. The program allows investigation of the effects of costs relative to other inputs in decisions about managing and selling livestock particularly calves. One of the problems with current input/output models with regard to cow/calf management and/or marketing is the failure to account for individual operator goals such as reducing the weaning weights under desert conditions (Melton and Colette, 1993). These programs are flexible enough in their input sections to allow for varying management strategies.

### The Programs

These Programs are Windows98 based (and later versions) and are written and compiled in Delphi. The program supports all of Windows98 conventions, and Windows98 or later versions of Windows required for their use. On line help is compiled into the programs to help users with questions concerning its operation.

These programs are unique in that they are highly interactive. By the use of slider bars, any variable may be immediately changed to reflect a new scenario. The output is immediately and dynamically updated which greatly increases potential as an interactive teaching tool and a management evaluator.

Only one screen appears with these programs. The top half of the screen is the input area using slider bars and

the bottom half is the output area in a dynamically updated table. Various scenarios may be saved or retrieved from previous sessions, deleted or printed by the use of large buttons in the lower right hand corner. Input is by slider bar only. This prevents the use of an inappropriate number.

The series consists of three programs, each dealing with a different aspect of managing calves for a profit. Budgeting production alternatives on the computer before investing in these alternatives may save you money. These programs were developed to help producers compare the economics of alternative production and marketing strategies. The program allows you to develop and customize a partial budget for backgrounding calves. This could include keeping weaned cattle for a period of time in order to increase weight and take advantage of different prices at a later marketing date, or evaluating costs of feeding cattle. The decision to retain ownership of weaned cattle through backgrounding periods requires careful consideration of the added costs and added returns of this marketing strategy. These programs can be used to budget this marketing alternatives on paper before committing funds to retain ownership of cattle that may or may not make money. Numerous analyses can quickly be performed to evaluate under what market conditions retained ownership through the backgrounding phase would be the most profitable marketing strategy.

*GrassFat.* The first program is called GrassFat and deals with pasturing calves. Pasturing cattle, primarily yearlings, is a means of keeping your cattle through the third stage of the production process. Profit potential exists each time cattle change hands. Some advantages of pasturing are that it allows you to vertically integrate and diversify your operation, spread risk by marketing cattle at different times of the year, and more closely evaluate the performance capabilities and genetic potential of your cattle.

*CalfBack.* CalfBack is a backgrounding decision aid program. Backgrounding is keeping your cattle through more stages of the production process. Profit potential exists each time cattle change hands. Some advantages of backgrounding are that it allows you to vertically integrate and diversify your operation, spread risk by marketing cattle at different times of the year, and more closely evaluate the performance capabilities and genetic potential of your cattle. Numerous factors affect the economics of backgrounding. Some of the major considerations include initial weight of cattle, sex, breed, body type, background (nutritional status, disease exposure), shrink, price spread, and environmental factors. Because so many factors influence the performance and economic potential of putting cattle into a backgrounding program, a careful evaluation of this management option should be made before cattle enter the feedlot.

*Feedlot.* The third program is called feedlot and is a computer program for estimating the economics of retained ownership through the feedlot. The decision to retain ownership of yearlings through the feedlot requires careful consideration of the added costs and added returns of this marketing strategy. Use the Feedlot program to budget this marketing alternative on paper before committing funds to retain ownership of cattle that may or may not

make you money. Numerous analyses can quickly be performed to evaluate under what market conditions retained ownership would be the most profitable marketing strategy.

## Software Applications

*Extension.* These programs were originally developed for extension educators to use in helping beef producers to make management decisions. Individual producers also have adopted them, with the help of extension personnel. Extension personnel also have used them in conjunction with loan officers at banks to make loan decisions.

*Research.* These programs are useful to researchers interested in the applied aspects of beef production by helping them discover areas of research that may be of economic importance in impact. It is an aid to pin point areas that will be the most productive in impacting beef herd management.

*Teaching.* These programs are useful in beef production classes and classes that form the basis for continuing education for high school agricultural instructors. The ability to pose many "what if" scenarios is very helpful in stimulating discussion and increasing the understanding of the links between management and profit.

## Summary

The programs were modified and Beta tested by producers and Extension personnel and published following a blind review process. The programs are available for download at <http://www.ag.unr.edu/cabnr/Resources.htm>. Utilizing web based tracking information downloads of the programs are tracked. To date 707 Downloads of the 3 programs, in 37 states, plus 4 foreign Countries has been documented. A number of these downloads have been by instructors for class distribution.

Using these programs can give valuable insights into different selling programs for calves. These programs can help producers avoid costly mistakes by trying marketing alternative programs out on the computer first. They are available free for download at [http://www.ag.unr.edu/vetmed/Extension/Ext\\_Pubs.htm](http://www.ag.unr.edu/vetmed/Extension/Ext_Pubs.htm) and <http://agecon.uwyo.edu/RiskMgt/>.

## Literature Cited

- Carson, W. W., J. C. Paschal, and C. W. Hanselka, 1992. Comprehensive ranch management for profit (CRMP) program South Texas ranchers interviews: results and implications. *J. Anim Sci.* 70 (Suppl. 1):276 (Abstr.).
- Melton, B. E., and W. A. Colette. 1993. Potential shortcomings of output:input ratios as indicators of economic efficiency in commercial breed evaluations. *J. Anim. Sci.* 71:579-586.
- Price, D. P. 1985. Beef production. Southwest Scientific, University Park, NM, 88003.

## THE MONTANA BEEF NETWORK

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**ABSTRACT:** The Montana Beef Network was initiated in 1999 as a joint partnership between the Montana Stockgrowers Association and Montana State University. The objectives of this partnership are to 1) provide Beef Quality Assurance education, 2) help ranchers voluntarily certify that their weaned calves have followed defined health management protocols and 3) return carcass information on their calves. Since inception, approximately 750 producers have become BQA-certified after undergoing a one hour program followed by a graded exam. The BQA educational materials are available by either a written text book, an interactive CD or via the internet ([mbn.montana.edu](http://mbn.montana.edu)). Twelve interactive state-wide television programs and four intensive two-day programs have been presented on topics of interest to producers; BSE, marketing, nutrition, and carcass evaluation. During the past five years, approximately 60,000 cattle have been certified through this program. During 2001, certified calves were worth \$1.63/cwt more ( $P<.05$ ) than calves which were not certified. Data collected at the ranch includes source (ranch location) and process information (weight, gender, vaccines, implants). Calves are identified with an electronic ear tag and the information is transferred via e-mail to a central data management service (eMerge, Inc.). Data are confidential and are returned to the producer through an internet site. The success in carcass data return from the packing plant has ranged from approximately 30% to 41%. During 2003, 71% of the producers received information on 41% of their calves. Reasons for low data return include buyers that are not interested in sharing data, information was lost because calves were mixed with non-tagged calves at time of sale or mixed in feedlot pens with cattle that were not part of the program. After summarizing carcass data on 4,700 calves from 1999 through 2001, average carcass values for calves enrolled in the program were: 62.5% Ch- or better and 36.1% were Se. Ninety one percent of all carcasses had a YG less than 3.9. Data returned to the producer are intended to determine the percentages of nonconforming cattle and potential solutions for improvement.

**Key Words:** Beef quality assurance, Montana beef network

### Introduction

Americans are demanding information about the food they eat. Consumers are making (beef, poultry and pork) purchasing decisions based upon nutrition, safety, price, and convenience. One industry response to these

challenges is the voluntary beef quality assurance (BQA) education programs for producers.

The National Cattlemen's Beef Association (NCBA) established the Quality Assurance program to maximize consumer confidence in and acceptance of beef by focusing the industry's attention on beef quality assurance through the use of science, research and educational initiatives. BQA programs in all 50 states are an industry effort to encourage cattlemen to follow certain production practices and quality-control measures that exceed U.S. Department of Agriculture and Food and Drug Administration requirements. Through research and education efforts conducted by state university and extension staff, the program educates cattlemen on the proper uses of pharmaceuticals, cattle handling, feed purchasing, testing and other procedures aimed at improving the overall safety and quality of cattle and beef.

### The Montana Beef Network

The Montana Beef Network (MBN) is a producer-driven partnership between the Montana Stockgrowers Association and Montana State University, and uses a systems approach to help producer's market consistent, source and process-verified feeder cattle and establish an information network to provide production feedback. The Network also utilizes its resources to undertake research and educational issues that are of concern to Montana beef producers.

The Montana Beef Network has three primary objectives; 1) educational programs aimed at meeting beef quality assurance standards, production and marketing goals and providing additional educational programs through interactive-video conferencing, 2) certification of feeder calves that have met defined management protocols and 3) information feedback from the feedlot and packing plant to the cow-calf producer showing if the feeder calves met industry requirements for quality, consistency, safety and red meat yield.

*Beef Quality Assurance.* A Beef Quality Assurance training manual, interactive CD and an interactive web site ([MBN.montana.edu](http://MBN.montana.edu)) were developed to train beef cattle producers about the concepts of quality assurance. County agents were trained to provide this educational program to producers at the local level. The training has been presented to over 1600 producers in the state and more than 750 are currently certified.

One of the continuing projects has been to survey producers every twenty four months to determine attitudes toward issues of importance in the industry. The most recent survey was conducted during August of 2003 (two months after the BSE case in Alberta, CA).

The data in Table 1 show the type of records maintained by producers and methods of animal identification. Differences were found between BQA certified producers and non-BQA certified producers regarding the types of records maintained on the ranch.

Table 1. Types of records and animal identification methods by BQA Certified and Non-BQA certified Montana producers (Duffey et al. 2004)

	BQA, %	Non-BQA, %	t value*
<b>Type of records maintained</b>			
No records kept	3.5	7.8	
Animal number and description	86.1	69.0	3.87*
Where animal was born	33.8	23.3	2.01*
Cowherd records	73.6	49.1	4.64*
Calf birth records	77.9	64.7	2.66*
Vaccination records	84.0	59.5	5.20*
Feed records	38.5	25.0	2.53*
BQA records	45.0	4.3	8.44*
Animal purchases and sales	77.1	66.4	2.13*
Names of suppliers and buyers	43.3	29.3	2.54*
<b>Identification Methods</b>			
No Identification	0	0	
Hot Iron	80.1	64.7	3.17*
Freeze Brand	9.5	9.5	
Ear notch	13.4	15.5	
Ear Tattoo	20.8	12.1	2.00*
Plastic Tag	91.8	77.6	3.78*
Electronic Tag	10.8	1.7	3.02*
Metal Tag	8.2	6.9	

\*P < .05.

More BQA producers kept track of animal numbers and descriptions than non-BQA producers. BQA producers (74%) kept more (P<.05) cowherd records than non-BQA producers. Eighty-four percent of the BQA producers maintained vaccination records compared to 59.5% of the non-BQA respondents. Only 43.3% of BQA respondents and 29.3% of non-BQA respondents reported maintaining records of suppliers and buyers of their cattle. The majority of cattle in MT were identified either with a plastic ear tag and(or) a hot iron brand.

Table 2. Vaccination preferences and location of injections for BQA certified and non-BQA certified MT producers (Duffey et al., 2004)

	BQA %	Non-BQA %	t value
<b>Preferred Vaccine Type</b>			
Killed	36.8	33.6	
Modified Live	62.8	50	2.29*
Chemical	0.9	0	
No Preference	5.2	6.9	
Killed Booster Given	94 <sup>†</sup>	87 <sup>†</sup>	
<b>Vaccine Location</b>			
Neck	96.5	87.1	3.39*
Armpit	6.9	12.1	
Shoulder	1.3	4.3	
Upper rear leg	0	0.9	
Side or ribs	0.9	0	
Lower rear leg	0	0	

\*P < .05

The majority of producers indicated they preferred to give a modified live vaccine product (Table 2). Although both populations administered vaccinations in the neck, more BQA producers (97%) vaccinated in this area compared to non-BQA producers (87%). Relatively few producers gave vaccinations in any rear area of the body. The usual administrator of the vaccinations was the producer, with 84% of BQA producers giving their own vaccinations compared to 76% for non-BQA producers.

With the discovery of BSE in Alberta followed by another case in Mabton, WA, MT producers were very concerned about food safety issues (Table 3). Producers who were BQA certified were more (P<.05) interested in a national animal identification program than were non-BQA producers.

Table 3. Perception of issues facing the beef industry by BQA-certified and Non-BQA certified producers (Duffey et al., 2004)

Issues	BQA	Non-BQA
<b>Food Safety</b>		
Very Concerned	64.9	59.9
Somewhat concerned	32.5	30.2
Not Concerned	0.9	4.3
<b>National Animal ID Program?</b>		
Yes	59.3	46.6*
No	15.2	20.7
Don't Know	21.6	24.1

\*P < .05.

Additional educational programs which have been initiated by the MBN include:

- One to two day short courses are held each year, in which issues pertinent to the beef industry are presented.
- Interactive television short courses use the broadcast studios at MSU and allow producers in 14 MT cities to attend programs at their local hospitals. These programs were aimed at carcass evaluation, genetic management, opportunities for backgrounding calves, BSE update, animal handling and welfare, BQA certification and marketing options.
- A project with 20 cooperating ranches was initiated to determine if a standardized protocol including vaccines and nutrition could reduce morbidity and mortality of weaned calves.
- A project with 2400 weaned calves was started to determine if an experimental E. coli vaccines would reduce the incidence of shedding of E. coli O157:H7 between weaning and harvest. This is part of an approach to improve beef safety through a pre-harvest intervention strategy.
- A study to determine if vaccinating pregnant cows with an experimental vaccine would provide antibody titers to the newborn calf to reduce E. coli O157:H7 shedding.

As part of the cooperative effort between Montana State University, Montana Stockgrowers Association, and Montana Grain Growers Association, a livestock component to the Montana MarketManager™ program was developed. This program is offered to Montana producers for the purpose of improving their marketing skills via three components including:

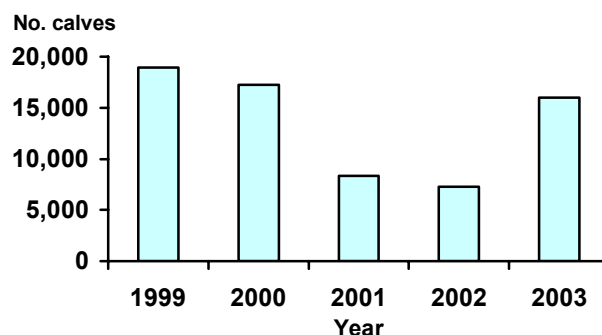
- a) Marketing Clubs –Thirty five marketing clubs are currently operating around Montana where agricultural producers organize to provide a forum for education and discussion about their marketing strategies.
- b) Marketing Workshops/Seminars – marketing fundamentals and strategies are presented by industry experts to producers throughout Montana via interactive television.
- c) Website – a website serves as the information hub of the Montana MarketManager™ program and includes specific news, pricing, weather, education and references, and links to other marketing resources.

*Calf Certification and Tracking.* The purpose is to establish an electronic network of communication exchange between the livestock producer, feedlot manager and the packer. This objective is accomplished through two approaches. First, for producers who only want to certify that their weaned calves have received required vaccinations can elect to use a plastic ear tag so that at time of sale, the buyer will know that the calves have been preconditioned and are certified. The second approach is designed to return information from the packing plant to a centralized database. The data base is maintained by eMerge, Inc. A producer attaches a uniquely numbered electronic ear tag (EID) to each individual animal, preferably while still on the premises. The protocol requires systematically following that animal through the production chain while collecting and recording statistically significant production related data (ADG, F/G, carcass weight, yield grade, quality grade) and ultimately downloading that data into the eMerge, Inc. database which is designed to analyze various production aspects of a specific group of cattle.

Approximately 18,000 calves were certified during the first year, 17,000 the second year, 8,000 the third year, 7,000 the fourth year and 16,000 during 2003 (Figure 1). To be able to sell calves under the MBN program, the producer must first be BQA certified and then follow a defined protocol for vaccinations. The main emphasis of this certification is documentation that vaccines were given. Information gathered at the ranch includes the producer's BQA certification number, identification numbers of certified calves, ranch of origin, breed combination, range of calf ages, and health protocol. Weaned calves (Option A) must be dehorned and castrated and must be vaccinated 21-60 days prior to shipping with IBR, BVD, PI-3, BRSV, clostridial, pasturella and H. Somnus. Calves under Option B must

receive a similar vaccination protocol plus a booster and are backgrounded for 45 d prior to sale.

Figure. 1. Calves enrolled in Montana Beef Network (1999-2003)



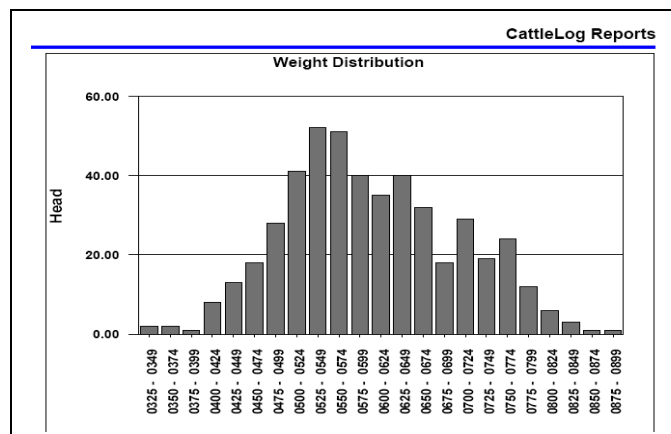
Data summarized by Brester (2001, unpublished) suggested that BQA-certified calves were worth significantly more ( $P < .05$ ) money than non-certified calves (\$1.62/cwt for steers and \$1.46/cwt for heifers Table 3).

Table 3. Effect of MBN membership on sale value (\$/cwt) of weaned calves (Brester, 2002, unpublished)

Gender	BQA	Non-BQA	P<.05
Steers	\$98.04	\$96.42	Yes
Heifers	\$93.28	\$91.82	Yes

If a producer decided to market his calves through the MBN, it would cost \$2.00/head to purchase electronic ear tags (RFID). Personnel from MSU or county extension agents travel to the ranch to attach RFID tags and will weigh calves and help with vaccinations. Data collected at the chute includes: electronic ID number, visual ear tag number, gender, and weight. Collected data are sent via email to the data management company eMerge® in FL where the information is stored. The following figure (Figure 2) shows an example of a summary of calf sale weights from a ranch in central MT.

Figure 2. Example for summary of sale weight range for 169 steers from a MBN ranch in central MT (eMerge Interactive®)



Reports such as this are useful in identifying non-conforming calves (13 head weighed less than 425 lbs). The owner maybe able to determine the causes of poor performance (born late in the season, calves born to heifers, calves out of older cows, sickness etc).

During 2002, carcass data were summarized from 4700 calves collected during 1999, 2000 and 2001. The following table (Table 4) shows that approximately 62.5% of the calves were fed to a choice quality grade and 91% had a yield grade of 3 or less.

Table 4. Average carcass quality and yield grade measurements for 4700 calves enrolled in the MBN (Brester, 2002, unpublished)

Yield Grade	Quality Grade, %				Totals
	Prime	Choice	Select	Standard	
1	0.0	1.8	3.4	0.4	5.6
2	0.2	22.6	19.5	0.5	42.8
3	0.8	30.0	11.6	0.3	42.8
4	0.2	5.8	1.4	0.1	7.5
5	0.0	1.0	0.2	0.0	1.2
Totals	1.2	61.3	36.1	1.3	

Recently, MBN personnel have begun to summarize data for ranchers who have been enrolled in the program for three or more years. Figures 3 (yield grade) and 4(quality grade) begin to show trends in the collected carcass data.

Figure 3. Changes in yield grade for ranch number 1151 over the past four years (Skinner et al., 2004,unpublished)

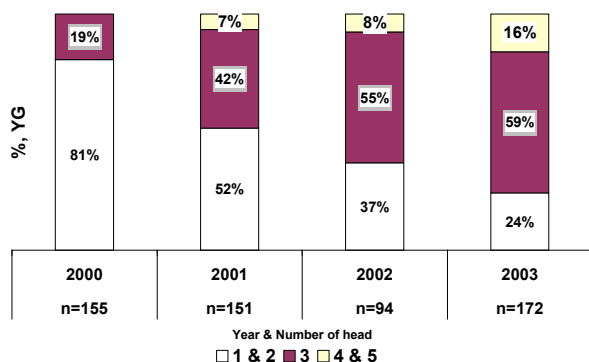
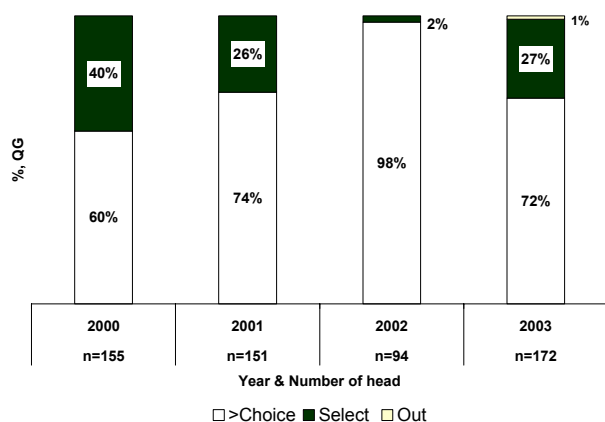


Figure 4. Changes in quality grade for ranch number 1151 over the past four years (Skinner et al., 2004,unpublished)



It appears that this producer's calves have declined in yield grades from a high percentage of 1's and 2's to an average YG of 3 in 2003. As the YG have decreased, the percentage of choice quality grade has tended to increase. It could be speculated that either the cattle feeder has changed the way the animals are finished in the feedlot (due to changing market conditions) or there has been a change in breeding objectives at the ranch.

Duffey et al. (2004) reported that ranchers used carcass data primarily for a) information only, b) to cull cows or c) to change bull genetics (Table 5).

Table 5. How MT ranchers indicated that they were using carcass data on their operations (Duffey et al., 2004)

	BQA-certified, %	Non BQA certified, %	Significance P<.05
Information only	34	23	Yes
To cull cows	21	12	Yes
Change bull genetics	35	24	Yes

### Summary

The beef industry is becoming more consumer-focused and specific quality and consistency targets are being established in all segments of the industry. To satisfy customer concerns over food safety/quality and return additional revenue to cattle producers, a systems network must be in place to ensure that a consistent product is being produced. Central to this networking approach is the exchange of information from the producer to the customer (feedlot, packing plant). This systems approach for information transfer is the foundation of the Montana Beef Network. The Network also utilizes its resources to undertake research and educational programs that are requested by MT beef producers.

The responsibility for quality beef lies with each segment of the beef production chain, from the genetics produced by the seedstock producer all the way to beef handling and preparation by the consumer. Gary Smith from Colorado State University said (MT Stockgrowers Magazine) "If every part of the production chain would work together and listen to the demands of each other, we have a much better chance of producing something the end consumer will keep buying".

## INTERACTIVE TRAINING IN SUSTAINABLE RANGELAND LIVESTOCK PRODUCTION SYSTEMS

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**ABSTRACT:** Community-based agricultural advisors (CBAA), typically employed by state Cooperative Extension Services (CES) and the Natural Resources Conservation Service (NRCS), fill an essential role in disseminating reliable and useful information to clientele. Successful execution of the land-grant mission is enhanced by CBAA who are technically-competent, confident and skillful communicators. Range livestock production systems are a primary agricultural enterprise for western North (ND) and South Dakota (SD). However, many CBAA are not trained with an appreciation for complete and holistic incorporation of production systems information. We provided a two year training course for CBAA in sustainable livestock production, emphasizing the key result areas of 1) natural resource and rangeland management, 2) livestock husbandry, nutrition and behavior, 3) economics, 4) system dynamics, and 5) adult education techniques. Thirty-two CBAA from ND-CES, SD-CES, ND-NRCS and SD-NRCS participated in the training. Training occurred in hands-on field and classroom sessions. During year one, basic information was presented on the key result areas using a teach-coach-mentor (TCM) style for adult learners. Experiential learning situations were developed to teach CBAA effective methods of conveying information to clientele. Year two training transitioned to application of concepts. Teams developed plans for a real world ranch management problem to enhance understanding of the complexity in sustainable range livestock production systems. Considerable attention was given to complex interrelationships among production components. This exercise facilitated the maturation of TCM relationships. Project participants are better trained, have increased confidence and have built an inter-state, inter-agency network through which they can more readily assist producers to improve profitability and sustainability.

Key words: systems, training, sustainability

### Introduction

In 1998, recognizing the changing fabric of rural South Dakota, the leadership of the College of Agriculture and Biological Sciences at South Dakota State University undertook an organizational review of the Cooperative Extension Service (CES). A key component of the management plan adopted as a result of this review, entitled "Extension Vision for the 21<sup>st</sup> Century" (SD CES, 1998), was the empowerment and

training of community based Extension Educators by Extension Specialists and other SDSU faculty. The Department of Animal and Range Sciences, in cooperation with other departments, has adopted this approach in developing training programs.

It is common for community based agriculture advisors (CBAA) working for both the United States Department of Agriculture-Natural Resources Conservation Service (NRCS) and the CES of Land Grant Universities to find themselves unprepared to deal with the complexities of situations facing them in rural America. Rural communities continue to shrink as farms and ranches increase in size and the supporting institutions of government, small business, education, health care, and churches consolidate in larger communities or simply close. Society in general is re-defining its social contract with farmers and ranchers from one of food and fiber production to one that also includes natural resource protection and enhancement, the production of renewable sources of energy, and recreation (Kephart, 2001). Trained in narrow disciplines with reductionist methodologies, CBAA find themselves dealing with complex integrated situations (Janssen and Goldsworthy, 1995). For CBAA to be effective in dealing with these, they require skills and techniques that they simply have not been exposed to or trained in. Consequently, CBAA are often viewed by a frustrated community as unable or unwilling to help with situations where complexity often escapes even the community members themselves. This has manifested itself in reduced public support, both politically and financially. CBAA respond in frustration with a negative self-image and short tenure. Critical educational opportunities provided by CBAA to their communities are often poorly attended, and the important material covered on topics like farm management, marketing, range and pasture management, and livestock production goes unheard and is not adopted (example: NAHMS, 1998).

*Project Overview.* The objectives of this project were to 1) provide information and hands-on training in the diverse areas, and more importantly the interrelationships among those areas, impacting sustainable livestock production systems on rangelands of the western Dakotas, 2) provide CES and NRCS staff with experiential learning situations, teaching them effective methods for transferring information to their clientele, and 3) evaluate the success of this training and modify these approaches for future training opportunities. This project involved 32 participants and 10 trainers representing CES and NRCS in both SD and ND.

Training was provided in four sessions, all of which were attended by most of the participants

## Materials and Methods

### *Assumptions and Guiding Principles.*

1. Sustainable range and pasture livestock production is a desired and positive outcome for farmers and ranchers, rural communities, and society in general.
2. Principles governing complex systems can be successfully adapted to range and pasture livestock production and the people and communities whose lives and livelihoods are based on it.
3. Principles of the Teach/Coach/Mentor diffusion methodology are effective and positive.
4. With exposure to new knowledge and techniques, CBAA are an integral part of the solution to the problems and challenges of ranchers, farmers, and rural communities.
5. The knowledge, concepts and skills gained by CBAA can be successfully transferred and replicated in their communities and within their institutions.

*Activities.* The first training session was held at the SDSU Antelope Range Livestock Research Station in northwestern South Dakota in June 2001. In this session participants learned basic concepts in range inventory, grazing management, livestock production, adult learning styles, and the Teach/Coach/Mentor (TCM) philosophy of education (Department of the Army, 1996). In the TCM approach to education, principles are taught and then students are coached in application of these principles. When students have the confidence and competence to effectively apply principles and concepts to their situations, teachers step back and establish themselves as mentors to their former student. Our goal was to establish trainers in the mentorship role with participants, and to establish experienced CBAA in coaching/mentor relationships with their peers. This allows training to have impact far beyond project activities.

The second session was held in December 2001 at the SDSU West River Ag Center in Rapid City, SD. In this session, topics introduced in the first session were expanded, however the focus of the training was on two topics: ranching systems and the impact of personality types on professional relationships. Systems thinking and its application to ranching systems was introduced using the "Ranch Wheel," a diagrammatic representation of all the components affecting ranching systems (B.H. Dunn, unpublished). The "Wheel" includes many of the components easily associated with these systems (e.g. natural resources, production, finances). It also includes components that are often left unconsidered, such as cultural and spiritual issues, social/political impacts and family. Personality types were examined using a modification of the Strength Deployment Inventory (Porter and Rollins, 1996). Participants learned the characteristics of four major types of personalities and the way each type interacts with others and acquires information. Participants found important insights in this exercise, recognizing how conflicts or miscommunication

with clientele and colleagues might have been avoided through understanding personality types.

The third session was held at Dickinson State College in Dickinson, ND in August 2002. In this session, instructors continued to expand on the training that had occurred in previous sessions, especially regarding systems thinking. The major focus of this session, however, was on the development of a plan to solve a ranching system problem. Participants were divided into six teams. All teams were challenged with the same ranching system problem. Teams were expected to work cooperatively to develop plans which addressed short-term, mid-drought condition as well as a long term plan. The TCM concept was encouraged and implemented within each team. A presentation of the plan was developed for presentation at the subsequent December session.

The fourth and final session was held in Mandan, ND in December 2002. Teams had developed their plans over the 4-month interval since August, and each provided an oral presentation of their plan to the entire group at this session. Presentations provided both an audience for teaching and learning opportunities, and an opportunity to practice teaching. The teachers/coaches/mentors provided feedback on presentation techniques and skills. Each plan was different and some were more fully developed than others.

## Results and Discussion

*Outcomes.* Most teams demonstrated a substantial understanding of the interrelationships within ranching systems, and most used the expertise of the team members to great advantage. This exercise not only required them to operate in a team environment and utilize the breadth of their collective knowledge, but it also demonstrated for the participants the fact that there may be more than one solution to systems problems.

In addition:

- 1) Multi-state, interagency networks to support future clientele problem solving were developed.
- 2) Participants' team skills and desire to work in teams to solve problems improved.
- 3) Participants' understanding of adult learning styles increased.
- 4) Participants' understanding of the role personality types play in working with colleagues and clientele improved.
- 5) Confidence of participants in their knowledge and ability to provide sound information to clientele increased. Several participants, as a result of this training, have gone from relying on Specialists to speak at programs to confidently assuming that role themselves. They continue to use instructors as coaches and mentors, but have moved into the role of teaching and coaching others.
- 6) Participants' understanding and appreciation for the disciplines outside their own training that are integral to ranching systems was enhanced.
- 7) Participants' understanding of the complexities and interrelationships in ranching systems improved.

## **Conclusion**

Participants were very enthusiastic about the training they received, and are dedicated to providing their clientele with quality assistance in sustainable livestock production. Participants have also requested additional training. We are confident that new participants will be added to the project. Interest has been expressed by CBAA not involved in this training to have access to similar training opportunities.

## **Literature Cited**

- Department of the Army. 1996. A leader's guide to lane training. Training Circular #25-10.
- Janssen, W., and P. Goldsworthy. 1995. Multidisciplinary research for natural resource management: conceptual and practical implications. Agricultural Systems. Elsevier Science Limited, Great Britain. 259-279.
- Kephart, K.D. 2001. Director's Comments. Farm and Home Research. SDSU. 52 (1):1.
- NAHMS, 1998. Part.III. Reference of 1997 Beef Cow-calf Production Management and Disease Control. pp 28 In: National Animal Health Monitoring System, USDA-APHIS, Ft. Collins, CO.
- Porter, E.H. and M.L. Rollins. 1996. Relationship Awareness Theory. Personal Strengths Publishing, Inc. Carlsbad, CA.
- SD CES. 1998. Extension Vision for the 21<sup>st</sup> Century. South Dakota State University. South Dakota State University Cooperative Extension Service. <http://sdces.sdstate.edu>

## NEW MEXICO FORAGE MINERAL SURVEY

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**ABSTRACT:** Forage minerals were surveyed across New Mexico to enhance Extension ruminant nutrition education programs by measuring mineral content and mineral variation of forages on New Mexico rangelands. New Mexico has nine major land resource areas. Seven of the areas are divided into two to four subresource areas. Forage samples were collected at two locations within each subresource area in fall and late winter of 2001 and 2002. Approximately 100 g of forage was hand-plucked at each location. Following collection, air-dry forage samples were sent to a commercial laboratory and analyzed for calcium, phosphorous, magnesium, potassium, sodium, sulfur, aluminum, cobalt, copper, iron, manganese, molybdenum, selenium, and zinc. The average concentration and range, respectively, for each mineral were: calcium (0.46%; 0.13-1.59%), phosphorous (0.07%; 0.01-0.18%), magnesium (0.09%; 0.03-0.36%), potassium (0.37%; 0.09-1.38%), sodium (0.05%; 0.01-0.57%), sulfur (0.10%; 0.03-0.29%), aluminum (1059 ppm; 147-5820 ppm), cobalt (0.46 ppm; 0.01-3.57 ppm), copper (12.6 ppm; 2.0-50.2 ppm), iron (876 ppm; 113-7450 ppm), manganese (75.5 ppm; 14.2-222 ppm), molybdenum (1.12 ppm; 0.09-2.90 ppm), selenium (0.10 ppm; 0.03-1.05 ppm), and zinc (23.7 ppm; 5.1-75.0 ppm). Forage mineral concentration varied greatly, both within and across major land resource areas of New Mexico. In most cases, average mineral concentrations were higher in the fall than in late winter. Primary consideration should be given to phosphorous, potassium, magnesium, copper, sodium, sulfur, and zinc in New Mexico mineral nutrition educational efforts. The observed magnitude of variation in forage mineral concentration highlights the importance of site-specific forage analysis to develop cost-effective mineral supplementation programs. However, in the absence of site-specific forage mineral analysis, these results provide a valuable foundation for addressing mineral supplementation in New Mexico Extension education by providing general guidelines from which to make recommendations.

Key Words: Forage, Mineral, Extension

### Introduction

Livestock have requirements for water, energy, protein, vitamins, and minerals. Grazing cattle are expected to acquire the majority of required nutrients (other than water) from forage. New Mexico range forages may be seasonally deficient in required nutrients; therefore, supplemental nutrients must be provided to meet production goals. Nutrient supplementation can be costly and, in the interest of maximizing profitability, should be approached

strategically. Informed decisions about mineral supplementation to grazing livestock require knowledge of the dynamic nature of forage mineral concentration and animal requirements. Animal requirement guidelines such as *Nutrient Requirements of Beef Cattle* (1996) have been published. However, dietary mineral content for animals grazing on specific sites is challenging to pinpoint. It is important for producers to identify the minerals that are most likely to be deficient and hamper performance.

This survey was conducted to develop a general measure of mineral content and variation in forages on New Mexico rangelands in order to aid producers in nutritional management of range livestock.

### Materials and Methods

New Mexico has nine major land resource areas. Seven of the nine major resource areas are further divided into two to four subresource areas. In an effort to best represent the rangelands of New Mexico, two locations within each subresource area were identified for sampling (i.e., 42 locations statewide) and marked using global positioning technology. Forage samples were collected in the fall (mid-October through mid-December) and late winter (February through early March) of 2001 and 2002. Not all locations were sampled four times because of snow cover or lack of forage production. Fall samples were collected to represent mineral content at the end of the growing season, while late winter samples were collected to represent mineral content after plants had become completely dormant. Forage samples were collected while plants were becoming dormant or during dormancy because it is during this period that forages are least likely to supply adequate minerals to meet the requirement of grazing livestock.

Approximately 100 g of forage was hand-plucked at each location to best represent selectivity of grazing beef cattle. Following collection, air-dry forage samples were sent to a commercial laboratory (SDK Laboratories, Hutchinson, KS) and analyzed for calcium, phosphorous, magnesium, potassium, sodium, sulfur, aluminum, cobalt, copper, iron, manganese, molybdenum, selenium, and zinc using inductively coupled plasma procedures.

### Results and Discussion

The only mineral measured to exceed the NRC (1996) requirements for beef cattle in all forage samples was iron (Table 1 and 2). In most cases, minerals were measured in higher concentration in the fall (Table 3) than in late winter (Table 4) when the forage was completely dormant. This is expected due to weathering and leaching losses that

commonly occur during winter. The range in concentration for each mineral measured exceeded ninefold. Iron was the only mineral that was measured to be above the concentration that might be considered problematic.

### *Macrominerals*

*Calcium.* Calcium concentration was below the NRC (1996) requirement for a gestating beef cow in 23% of samples and below the requirement for a lactating beef cow in 34%. The majority of fall and late winter samples contained sufficient calcium to meet the requirements of a gestating beef cow.

*Phosphorous.* Phosphorous content of all samples was below the requirement (NRC, 1996) for all classes of beef cattle. However, average forage phosphorous content in the fall was substantially higher than in late winter.

*Potassium.* Forage potassium concentration was below the NRC (1996) requirement for a gestating beef cow in 83% of samples and for a lactating beef cow in 89% of samples. But, phosphorous concentration was sufficient in at least one sample from six of the regions sampled in the fall. In late winter, as a result of normal decline in forage potassium leading into dormancy, less than 2% of samples was deficient in potassium for a gestating beef cow.

*Magnesium.* Magnesium concentration was below the NRC (1996) requirement for a gestating beef cow in 77% of samples, and below the requirement of a lactating beef cow in 97%. Magnesium was observed to be higher in the fall than late winter in most cases; however, the magnitude of difference between fall and late winter was not as great for magnesium as for phosphorous and potassium. In the fall, average magnesium concentration from five of the regions sampled were not sufficient to meet the requirement of a gestating beef cow, but only two of those regions were insufficient, on average, to meet the requirements of growing cattle. In late winter, average magnesium content for all regions was not sufficient for a gestating beef cow, and ranged from 20 to 50 percent of the requirement for a lactating beef cow. Magnesium absorption is compromised when dietary potassium or nitrogen concentrations are high (Greene et al., 1983). Usually, neither of these antagonists is high enough to significantly hinder magnesium absorption in cattle on dormant New Mexico range.

*Sodium.* Sodium concentration of samples collected from different locations was observed to vary greatly. Sodium concentration was substantially less than required (NRC, 1996) for a gestating or lactating beef cow in 91% and 93% of samples, respectively. However, sodium was recorded to exceed those requirements at four locations. At three of the four locations with sufficient sodium, the concentration was more than threefold the requirement. At almost all other locations sodium content was substantially less than the requirement. Although sodium concentration of forage is usually low, other than a few exceptions, it is important not to overlook the contribution drinking water makes to dietary sodium intake. For example, if an 1100-pound cow drinks 8 gallons of water that contains 150 mg/L of sodium, approximately 40 percent of the sodium requirement is fulfilled by the drinking water.

*Sulfur.* Sulfur content was below the NRC requirement for beef cattle in 92% of samples, and average sulfur concentration was observed to be lower in the late winter than fall in all regions. Sulfur concentration exceeded the requirement in at least one sample from four of the regions sampled in the fall, and from two of the regions sampled in the late winter. In late winter, sulfur concentration most commonly fell between 40 and 70 percent of the requirement for a beef cow. As with sodium, sulfur in drinking water often significantly contributes to the dietary supply of the mineral. For example, if an 1100-pound cow drinks 8 gallons of water containing 300 ppm of sulfates, 24 percent of the sulfur requirement will be supplied by the drinking water alone. However, excessive dietary sulfur, in drinking water or from supplemental feed sources can also reduce the absorption of copper or potentially cause polioencephalomalacia (Underwood and Suttle, 1999). Sulfur is considered toxic when it exceeds 0.4 percent of the diet (NRC, 1996), and performance may be suppressed at levels as low as 0.25 percent of the diet (Zinn et al., 1997).

### *Microminerals*

*Aluminum.* Aluminum concentration in forages did not follow the pattern of being higher in the fall than late winter like most other minerals measured. Extremely high aluminum values (greater than 3000 ppm) are likely the result of soil contamination of the forage sample. Aluminum toxicity has been reported (Underwood and Suttle, 1999), but is usually not a concern with grazing ruminants.

*Cobalt.* Cobalt concentration was below the NRC requirement (1996) for beef cattle in 8% of samples, but was not uniformly higher in the fall than late winter. In the fall, only two of the regions had at least one sample that was insufficient to meet the cobalt needs of a beef cow. However, in late winter seven of the regions sampled had at least on sample that was deficient in cobalt.

*Copper.* Copper concentration was deficient in 40% of samples overall, yet 89% of fall samples contained sufficient copper to meet the requirements of beef cattle (NRC, 1996). Samples collected in late winter were generally 20 to 50 percent less than fall samples, with deficient samples recorded for every region. The magnitude of deficiency in the late winter was marginal, with regional average values ranging from 74 to 104 percent of the requirement. The determination of copper content in the diet or in grazed forage has no diagnostic value in ruminants unless other elements that are antagonistic to copper availability are analyzed concurrently (Underwood and Suttle, 1999). More specifically, sulfur intake and the ratios of copper:molybdenum and iron:copper should be evaluated.

*Iron.* The iron content of all samples was sufficient to meet the NRC (1996) requirement of beef cattle. In fact, in five of the regions sampled in the fall and seven of the regions sampled in the late winter, at least one sample was collected that exceeded 1000 ppm, which is considered to be toxic to beef cattle. Iron:copper ratios of less than 50:1, 50:1 to 100:1, and greater than 100:1 are considered low, marginal and high risk for antagonism problems

(Underwood and Suttle, 1999). Using these risk categories 49% of samples were low risk, while 19 and 32% were classified as marginal and high risk, respectively. These findings indicate that iron may impact copper availability in many areas of the state.

**Manganese.** Forage manganese content was below the NRC (1996) requirement for a beef cow in only 16% of samples; however, it was sufficient to meet the substantially lower requirement of growing cattle in more than 98% of samples. Average manganese concentration for all regions sampled in the fall and late winter exceeded the requirement of beef cattle. Although average values were considered sufficient, some individual samples had insufficient manganese concentrations. Manganese concentration was measured to be less than the requirement for a beef cow in only three of the regions sampled in the fall, but seven of the regions sampled in the late winter had at least one sample considered deficient.

**Molybdenum.** Molybdenum is not an essential mineral required by mammals. Molybdenum can reduce copper absorption (Underwood and Suttle, 1999), and is considered toxic when dietary concentration exceeds 5 ppm (NRC, 1996). The highest molybdenum value recorded was less than 3 ppm, and 90% of samples contained less than 2 ppm. Molybdenum concentration was higher in the fall than late winter in most cases. Copper concentration exhibited the same general seasonal pattern, so that the ratio of copper to molybdenum was not drastically different between fall and late winter. It is recommended that the potential for copper depletion resulting from molybdenum interaction be evaluated using the copper:molybdenum ratio. Ratios greater than 3:1, 1:1 to 3:1, and less than 1:1 are classified as low, marginal, and high risk, respectively (Underwood and Suttle, 1999). Using the copper:molybdenum ratio, 95% of samples were classified as low risk, and the remaining 5% as marginal risk. These findings indicate that molybdenum is not a widespread copper antagonist issue within New Mexico.

**Selenium.** Selenium concentration was highly variable, and was below the NRC (1996) requirement for beef cattle in 92% of samples. Selenium concentration was deficient for beef cattle in at least one sample from four of the regions sampled in the fall, and from seven of the regions sampled in late winter. Average regional selenium content was not consistently different between fall and late winter. The Arizona and New Mexico Mountains, Southeastern Arizona Basin Range, and High Intermountain Valleys regions were noticeably lower in average forage selenium content than other regions.

**Zinc.** Zinc concentration was below the NRC (1996) requirement for beef cattle in the 77% of samples. More specifically, average regional zinc concentration was above the requirement for only three of the regions sampled in the spring, and was never sufficient in the late winter.

### Implications

Forage mineral concentration varied greatly. Most minerals were in lower concentration in late winter than fall. In New Mexico, primary consideration should be given to phosphorous, potassium, magnesium, copper, and zinc

when developing a mineral supplementation program for cattle because forage samples were almost always deficient. Selenium concentration was highly variable and should also be considered. Site-specific water analysis should be incorporated when interpreting the potential importance of low forage sodium and sulfur concentration at specific locations. Calcium, cobalt, and manganese were less frequently observed to be deficient, but should not be completely disregarded.

The observed magnitude of variation in forage mineral concentration highlights the importance of site-specific forage analysis to develop cost-effective mineral supplementation programs. In the absence of site-specific forage mineral analysis, the results of this survey may serve as a general guide.

### Literature Cited

- Greene, L. W., K. E. Webb, Jr., and J. P. Fontenot. 1983. Effect of potassium level on site of absorption of magnesium and other macroelements in sheep. *J. Anim. Sci.* 56:1214-1221.
- NRC. 1996. *Nutrient Requirements of Beef Cattle* (7<sup>th</sup> Ed.). National Academy Press, Washington, DC.
- Underwood, E. J., and N. F. Suttle. 1999. *The Mineral Nutrition of Livestock* (3<sup>rd</sup> Ed.). CABI Publishing, New York.
- Zinn, R. A., E. Aloarez, M. Mendez, M. Montano, E. Ramirez, and Y. Shen. 1997. Influence of dietary sulfur level in growth, performance and digestive function in feedlot cattle. *J. Anim. Sci.* 75:1723-1728.

**Table 1.** Percentage of samples for each macromineral not meeting NRC (1996) requirement for gestating and lactating beef cows.

	Gestating	Lactating
Macromineral	-----%-----	
Calcium	23	34
Phosphorus	100	100
Potassium	83	89
Magnesium	77	97
Sodium	91	93
Sulfur	92	92

**Table 2.** Percentage of samples for each essential micromineral not meeting NRC (1996) requirement for beef cattle.

Micromineral	-----%-----
Cobalt	8
Copper	40
Iron	0
Manganese	16
Selenium	47
Zinc	77

**Table 3.** Average fall forage mineral concentration from New Mexico major land resource areas.

Item	Mineral Concentration										
	WP <sup>a</sup>	ND <sup>b</sup>	AN <sup>c</sup>	SA <sup>d</sup>	SD <sup>e</sup>	RM <sup>f</sup>	HV <sup>g</sup>	CP <sup>h</sup>	HP <sup>i</sup>	Low	High
Number of samples	6	2	8	4	10	4	0	15	12		
Calcium, %	0.60	0.39	0.49	0.29	0.48	0.47		0.56	0.56	0.16	1.59
Phosphorus, %	0.08	0.14	0.11	0.06	0.08	0.10		0.07	0.08	0.03	0.18
Potassium, %	0.76	1.21	0.53	0.30	0.61	0.39		0.52	0.46	0.13	1.38
Magnesium, %	0.17	0.18	0.10	0.05	0.12	0.10		0.10	0.09	0.04	0.32
Sodium, %	0.15	0.27	0.03	0.02	0.03	0.08		0.03	0.03	0.01	0.57
Sulfur, %	0.15	0.26	0.11	0.08	0.13	0.10		0.13	0.11	0.06	0.29
Aluminum, ppm	772	316	571	636	1204	1445		951	1127	202	3330
Cobalt, ppm	0.17	0.34	0.25	0.12	0.48	0.97		0.40	0.44	0.01	1.91
Copper, ppm	13.2	10.4	13.8	16.9	15.7	29.3		17.5	20.9	4.3	50.2
Iron, ppm	580	325	507	417	932	1390		816	909	113	3490
Manganese, ppm	81	52	71	68	124	83		92	65	31	202
Molybdenum, ppm	1.49	0.96	1.66	1.69	1.28	2.18		1.30	1.46	0.55	2.90
Selenium, ppm	0.13	0.22	0.05	0.04	0.13	0.13		0.13	0.11	0.04	0.65
Zinc, ppm	19.3	23.9	51.7	20.9	26.2	35.6		29.9	30.4	9.3	75.0

<sup>a</sup>WP=New Mexico and Arizona Plateaus and Mesas. <sup>b</sup>ND=San Juan River Valley, Mesas, and Plateaus. <sup>c</sup>AN=Arizona and New Mexico Mountains. <sup>d</sup>SA=Southeastern Arizona Basins and Range. <sup>e</sup>SD=Southern Desertic Basins, Plains, and Mountains. <sup>f</sup>RM=Southern Rocky Mountains. <sup>g</sup>HV=High Intermountain Valleys. <sup>h</sup>CP=Pecos-Canadian Plains and Valleys. <sup>i</sup>HP=Southern High Plains.

**Table 4.** Average late winter forage mineral concentration from New Mexico major land resource areas.

Item	Mineral Concentration										
	WP <sup>a</sup>	ND <sup>b</sup>	AN <sup>c</sup>	SA <sup>d</sup>	SD <sup>e</sup>	RM <sup>f</sup>	HV <sup>g</sup>	CP <sup>h</sup>	HP <sup>i</sup>	Low	High
Number of samples	11	3	7	4	12	8	2	16	10		
Calcium, %	0.43	0.35	0.28	0.25	0.37	0.37	0.47	0.53	0.54	0.13	1.14
Phosphorus, %	0.04	0.04	0.05	0.05	0.06	0.08	0.07	0.05	0.06	0.01	0.13
Potassium, %	0.28	0.19	0.20	0.23	0.28	0.26	0.21	0.22	0.21	0.09	1.01
Magnesium, %	0.10	0.06	0.06	0.04	0.07	0.09	0.10	0.10	0.07	0.03	0.36
Sodium, %	0.10	0.04	0.03	0.03	0.03	0.09	0.03	0.03	0.03	0.01	0.57
Sulfur, %	0.11	0.13	0.06	0.07	0.10	0.09	0.09	0.09	0.09	0.03	0.26
Aluminum, ppm	843	532	486	452	1041	1706	1172	1766	1033	147	5820
Cobalt, ppm	0.39	0.04	0.42	0.11	0.38	1.10	0.66	0.60	0.41	0.01	3.57
Copper, ppm	9.6	7.5	8.0	10.4	7.4	8.0	9.5	9.6	8.8	2.0	28.6
Iron, ppm	729	504	565	319	718	1833	1074	1282	846	136	7450
Manganese, ppm	49	42	49	56	97	87	69	76	51	14	222
Molybdenum, ppm	0.87	1.30	0.38	1.42	0.67	1.55	0.98	0.59	0.76	0.09	2.89
Selenium, ppm	0.12	0.15	0.04	0.06	0.10	0.13	0.07	0.22	0.18	0.03	1.05
Zinc, ppm	12.5	25.4	14.0	17.6	16.9	20.0	25.1	19.1	17.4	5.1	52.7

<sup>a</sup>WP=New Mexico and Arizona Plateaus and Mesas. <sup>b</sup>ND=San Juan River Valley, Mesas, and Plateaus. <sup>c</sup>AN=Arizona and New Mexico Mountains. <sup>d</sup>SA=Southeastern Arizona Basins and Range. <sup>e</sup>SD=Southern Desertic Basins, Plains, and Mountains. <sup>f</sup>RM=Southern Rocky Mountains. <sup>g</sup>HV=High Intermountain Valleys. <sup>h</sup>CP=Pecos-Canadian Plains and Valleys. <sup>i</sup>HP=Southern High Plains.

## 2003 LIVESTOCK FEED ASSISTANCE PROGRAM AND FEEDING NON-FAT DRIED MILK

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**ABSTRACT:** On April 8, 2003, the USDA offered surplus stocks of non-fat dried milk (NDM) to beef producers in drought stricken areas as a form of drought assistance. By June 13, 2003, based on drought index information, 134 counties across 10 western states were eligible for over 104 million kg of NDM. Increased drought severity following the initial announcement increased the eligible area to include 12 western states. The NDM allocations for each county and state were determined by estimating the total number of livestock (beef cattle, bison, sheep and goats), offering a 30-d supply of NDM based on providing .91 kg/d for cattle and bison and 0.23 kg/d for sheep and goats. Producers enrolled in the program were given the option of having their allocation delivered to the feed company of their choice and receiving approximately \$.09/kg NDM credit (\$80/ton) applied towards the purchase of a NDM-containing feed, or taking actual delivery of the NDM to their ranch. Based on laboratory analysis and limited studies, NDM is an excellent source of supplemental protein, well-suited for grazing or confined feeding situations, provided the proper feed handling equipment. Crude protein levels range from 35 – 37%, with average energy values of 1.76 Mcal/kg NEm and 1.15 Mcal/kg NEg. Non-fat dried milk can be used as a protein and energy source when included in rations and range supplements. However, the physical characteristics of the product make it challenging to feed. Some of these characteristics include the hygroscopic nature of powdered milk, settling and separation issues, hardening qualities of NDM when used in commercial feeds and supplements, as well as storage and handling concerns. Successful feeding methods include limiting daily intake of NDM and improving the handling qualities by diluting it with feed grains and byproduct feeds, decreasing the dustiness and improving handling by adding vegetable oils, and minimizing the product handling challenges by adjusting the sequence of ingredients added to the feed mixer or wagon.

Key Words: Non-fat Dried Milk, Supplement, Protein

### Introduction

The USDA Livestock Feed Assistance Non-Fat Dry Milk (NDM) program was approved in 2003 for parts of several western states, including 17 counties in Wyoming. This program provided Non-Fat Dry Milk (NDM) for producers in designated counties at the rate of 0.91 kg/d NDM per head for cattle, and 0.23 kg/d for sheep and goats for a 30-d period. The non-fat dried milk product was valued at \$80/ton. Participating operations were given the option of either having their allotment of NDM delivered to

the feed company of their choice, and the estimated value of the NDM (\$80/ton) applied towards the purchase of manufactured feed containing the NDM, or taking actual delivery of the NDM for their ranch. Additional items included in the livestock feed assistance program included: A) The NDM was made available to each state at a rate of \$1.00 per truck lot with transportation costs paid by the Commodity Credit Corporation (CCC) to distribution points selected by the state. Actual amounts delivered were determined by using the U.S. National Drought Monitor. Counties that were listed as part of the drought area over a six month period were eligible for the program. Each month the CCC, using the U.S. Drought Monitor, re-evaluated whether a county meets the drought criteria. B) The quantity of NDM to be made available by CCC was based on ag census data estimating the total number of livestock (cattle, bison, sheep and goats) in each county, and providing the 30 d supply of NDM based on the previously mentioned feeding levels. Actual title to the NDM and risk of loss was transferred to the State upon delivery by CCC. C) The NDM sold by CCC could only be used for feeding foundation livestock herds, and the producer may sell or exchange the NDM to acquire feed containing NDM for their foundation livestock herd. All uses are permitted subject to the following limits: (1) the NDM may not be used as a replacement for whey or whey products; (2) the NDM may not be processed for or used for human consumption; (3) ultimate consumption of the NDM must be in the state(s) allocated the NDM.

While many producers chose to receive feed credits for their NDM allotment, additional operations chose direct delivery of their non-fat dried milk. The idea of receiving the free NDM product seemed quite attractive, but NDM also poses several handling challenges to producers, which are addressed below. Following the completion of the livestock feed assistance program, several private feed companies have continued to market NDM as an alternative protein source, pricing it competitively with other protein feeds.

### Nutritional characteristics

As several popular press articles and publications have emphasized, NDM is an excellent feed resource (Table 1) with high levels of degradable protein. Protein associated with NDM has been reported to be 80% casein and 20% whey (Stock et al., 1986a; Taniguchi et al., 1995; Köster et al., 1996), and would work very well in a grazing situation, supplying high quality protein to grazing livestock (Hendrix et al., 1973; Procop et al., 1976; Stock et al., 1986b). One of the major concerns are that NDM also contains readily available sugars and proteins that can cause

digestive upset if large amounts are consumed. In addition, readily available sugars have a negative effect on low quality forage digestion, counter-acting any positive effects of the protein. Most recommendations are to limit daily intake of NDM to around 0.91 kg/d when feeding NDM as a protein supplement to grazing cattle, and to reduce feeding and handling concerns by diluting it with other feeds, such as cereal grains, byproduct feeds including soybean hulls, wheat middlings, and corn gluten feed.

**Table 1. Nutrient Analysis of Non-Fat Powdered Milk, as-fed basis.**

Item	USDA specs	Erickson et al. (2003)
Dry Matter, %	96.8	
Energy		
TDN, %	74	90
NEm, Mcals/lb	.80	
NEg, Mcals/lb	.52	
Protein, %	36.16	34 – 37
Fat, %	0.77	.6 – 1.25
Minerals		
Calcium, %	1.257	1.35
Phosphorous, %	0.968	1.1
Iron, g/lb	0.00145	
Magnesium, g/lb	0.50	
Potassium, g/lb	8.14	1.7%
Zinc, g/lb	0.0185	
Copper, g/lb	0.000186	

### Handling Concerns with Non-Fat Dried Milk

*Hygroscopic nature of powdered milk.* Non-fat dried milk is extremely dry and powdery, with bagged feed averaging 98% DM. One of NDM's unique characteristics is its ability to attract moisture. This can lead to several concerns in handling feed. Once moisture comes in contact with the NDM, it has a tendency to "set up". If it is not mixed or blended with other feeds, it has the potential to clog feed augers, cake feeders, etc. It may also be a problem if producers choose to hand-mix powdered milk into mineral supplements. If large amounts are hand-mixed into mineral supplements, it may lead to over-consumption of the mineral. If moisture comes in contact with the mineral, it will tend to harden, and large amounts of moisture may create conditions where the NDM-based feed will sour.

Because the NDM is hygroscopic, and attracts moisture, it is sometimes difficult to mix the dry form into mixed rations and supplements. If there are any wet ingredients, the NDM "clings" to the wet, resulting in uneven distribution and clumps. Producer comments and field observations both indicate mixing difficulties, especially when trying to combine NDM in prepared feeds and sacked TMR diets containing molasses.

*Settling and separation concerns.* Because the powdered milk is extremely fine, it has a tendency to settle out when it is mixed with grain and other coarse feeds. This could create problems if the NDM is included in grain mixtures for self-feeders or calf creep feeders. The NDM

will tend to settle to the bottom, causing feeding problems, especially if it comes in contact with moisture.

*Hardening qualities of NDM.* Many producers and feed mill managers in the Western U.S. received a crash course in incorporating NDM in supplements this fall and winter. One of the lessons learned was that high levels of NDM in range cubes and cakes increased the hardness of the product. Most feed mills producing range cubes restrict the level of NDM to a maximum of 10 to 15% to avoid extremely hard range cubes.

*Palatability concerns when NDM is fed at higher levels.* In nearly all cases, dried non-fat milk works well in total rations that contain a smaller percentage of NDM. However, field observations suggest that rations and supplements containing high levels of NDM may lead to reductions in feed intake. Situations where this may occur include limit-fed rations, or top-dress supplements containing high levels of NDM. This may be an important factor to consider when intake is important, such as high-protein receiving supplements for weaned calves.

*Uses for supplemental NDM.* Determining how to effectively utilize NDM in summer grazing situations is challenging. While high protein supplements are very effective at improving digestion of low quality forages, improving intake and overall energy status, it may be difficult to provide additional NDM to cattle on remote summer grazing allotments. Because of this challenge, many producers chose delayed delivery of their drought assistance program NDM for late summer, fall and winter supplementation programs.

*Storage Concerns.* If producers choose to store NDM through the fall and winter, actual storage of the NDM may be difficult. Its hygroscopic nature, as well as insect and rodent concerns, create a challenge when trying to store the product for long periods of time. Keeping the product dry and well protected is essential for longer-term storage.

### Recommended Winter Rations with NDM

Several states have published winter feeding recommendations for feeding NDM, depending on available feed resources. University of Nebraska recommendations (Erickson et al., 2003; based on 1100 lb cow) are as follows:

Gestation (amounts on an as-fed basis):

1. 47 lb corn silage, 2 lb NDM
2. 25 lb grass hay (53% TDN, 6% CP), 2 lb NDM
3. 22 lb crop residue (45% TDN, 4% CP)  
2 lb NDM, 0.8 lb DDG, 1.7 lb grain.
4. 9 lb crop residue, 2 lb NDM, 9.15 lb grain.

Early Lactation (as-fed basis):

1. 60 lb corn silage, 2 lb NDM, 0.55 lb DDG
2. 25.5 lb grass hay (53% TDN, 6% CP),  
2 lb NDM, 2.8 lb DDG
3. 21 lb crop residue (45% TDN, 4% CP)  
2 lb NDM, 5.5 lb DDG, 2 lb grain.
4. 9 lb crop residue, 2 lb NDM, 6.7 lb DDG,  
6.7 lb grain.

Late Lactation (as-fed basis):

1. 55 lb corn silage, 2 lb NDM
2. 28 lb grass hay (53% TDN, 6% CP),  
2 lb NDM, 1.6 lb DDG
3. 23 lb crop residue (45% TDN, 4% CP) 2 lb NDM,  
4.5 lb DDG
4. 9 lb crop residue, 2 lb NDM, 3.3 lb DDG,  
8.3 lb grain.

### Feeding Recommendations

Common suggestions with handling and feeding NDM include: 1) Limiting non-fat dried milk levels to less than .91 kg/d when feeding as a supplement. However, several backgrounding and finishing operations have had success feeding higher levels to confined animals receiving total mixed rations. 2) Use vegetable oils and fats to condition NDM rations, helping to reduce dust and eliminate problems with “setting up”. Experience at the University of Wyoming would suggest that 2.5 to 5% oil works extremely well. Water and/or molasses would also work to condition the supplement or feed, but require much higher levels. 3) When including NDM in total mixed rations, the order of feed ingredients during mixing is critical. Wet ingredients must be thoroughly mixed before adding NDM to prevent clumping and poor distribution. Several large feeding operations have avoided the mixing challenges of NDM by adding the non-fat dried milk directly to their silage during the packing process. 4. Feeding NDM in the dry form, by top-dressing on long stemmed hay, has proved to have little success. Non-fat dried milk is extremely fine, and is prone to wind loss or settling. Animals that inhale the fine powder may increase their risk to additional respiratory problems.

### Literature Cited

- Erickson, G., T. Klopfenstein, I. Rush, R. Rasby, and D. Adams. 2003. Non-Fat Dry Milk in Drought Diets. Nebr. Coop. Ext. Pub. NF03-572. [www.ianr.unl.edu/pubs/beef/nf572.htm](http://www.ianr.unl.edu/pubs/beef/nf572.htm).
- Hendrix, K., and T. Klopfenstein. 1973. Whey additions to corn silage. Nebr. Beef Cattle Rept. EC 73-218:17-18.
- Köster, H. H., R.C. Cochran, E.C. Titgemeyer, E.S. Vanzant, I. Abdelgadir, and G. St-Jean. 1996. Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass-prairie forage by beef cows. J. Anim. Sci. 74:2473-2481.
- Prokop, M., T. Klopfenstein and L. Peterson. 1976. Adding whey to roughage rations. Nebr. Beef Cattle Rept. EC 76-218:37-38.
- Stock, R., T. Klopfenstein, D. Brink, R. Britton and D. Harmon. 1986. Whey as a source of rumen-degradable protein I. Effects on Microbial protein production. J. Anim. Sci. 63: 1561-1573.
- Stock, R., T. Klopfenstein and D. Brink. 1986. Whey as a source of rumen-degradable protein II. For growing ruminants. J. Anim. Sci. 63: 1574-1580.
- Taniguchi, K., G.B. Huntington, and B.P. Glenn. 1995. Net nutrient flux by visceral tissues of beef steers given abomasal and ruminal infusions of casein and starch. J. Anim. Sci. 73:236-249.

## **EDUCATING COW-CALF PRODUCERS THROUGH A RETAINED OWNERSHIP PROGRAM**

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### **ABSTRACT:**

The Nebraska IRM retained ownership program has been on-going for the past 6 years. The primary objectives of the program is to educate cow-calf producers about the entire beef industry. They gain knowledge of all segments of the beef industry, plus knowledge of their calves' value. The program consists of each producer consigning at least 5 head of weaned (at least 30 days) steer and heifer calves and given preweaning immunizations. Arrival weights are collected and then after approximately 2-3 weeks of feed and health adjustment, an initial weight is taken plus midterm on feed weight and final shipping weights. The cattle are ultrasounded at midterm weight where outcome groups are identified and then all cattle are sold on a carcass grid in 3 groups separated by approximately 2 week intervals. Data on cattle given back to producers includes individual feedlot gain and carcass characteristics, plus profit for each individual animal is estimated. The program has had between 200-400 head each year with 20-35 producers from Nebraska, South Dakota, Wyoming and Colorado. Marketing telephone conference calls are held at least monthly for all participants. At least 3 educational meetings are held during the feeding period where experts from all segments of the industry make presentations. These have included sessions on cattle handling (Temple Grandin), IRM concepts, new case-ready beef product development, individual cattle identification, the packing industry operation and challenges, beef retail products and value, genetics for systems of beef production from conception to harvest, plus many others. One key to the success of this program is the cooperative efforts of a

multidiscipline coordinating team and a feedlot manager.

**Keywords:** retained ownership, beef cattle, producer education

### **Introduction**

Cow-calf producers have done an excellent job of producing high quality weaned calves in a large area of ranch country. Farmers and ranchers who are predominately cow-calf producers have a great incentive to produce heavy, high quality calves because oftentimes the sale of these calves is their once in the year pay day. In the past the majority of ranchers sold their calves at weaning and often either didn't know or had little interest in subsequent feedlot performance and carcass attributes. It was sometimes assumed "it's not my problem." Price differences between the highest and poorest performing cattle were not greatly different and if a premium was paid it was often not based on past performance.

In more recent years considerably more ranchers are maintaining ownership of their calves through harvest. Some have become involved in alliances that positions or aligns all of the segments, including the producer in the beef industry. Many more ranchers have become more interested in calf performance after weaning even if they were sold off the ranch. Marketing grids have been in place for several years which sent signals to producers that carcass traits were going to be significant economic traits in the future. Ultrasounding live animals give indications to producers of the differences in carcass merits.

Even though there was an increasing interest in the entire beef industry by leading cow-calf producers, many did not want to take the risk of maintaining ownership through the finishing phase. One of the reasons for not maintaining ownership was lack of knowledge or confidence of what would occur in the feedyards and at the packing plant that would affect their profitability. Because of lack of understanding in some cases, it opened the door to lack of trusting the feeding and packing industry. In some cases, even though producers felt they were producing an excellent calf at weaning they were unsure how their cattle would actually perform in the feedlot or when evaluated on carcass characteristics. To help producers gain information, many states established retained ownership programs. The objective of the Nebraska IRM retained ownership was primarily to educate cow-calf producers of all aspects of the beef industry that affected profitability of producing beef. A second objective was to allow cow-calf producers to get a snapshot on how their calves would perform in the feedlot and in the packing plant and to compare them to other ranchers' cattle in the region.

### **Materials and Methods**

Basically, the feeding program is very similar to many other state programs. At least 5 head of weaned steers or heifers are placed on feed in the fall and fed to harvest where carcass data is collected. Team members include a county educator from predominately cow-calf country, a beef specialist, an agricultural economist, an extension veterinarian, and a feedlot owner and manager. Major emphasis of the program team is to offer leading-edge educational programming throughout the year. No recognition is given for "high performance cattle".

An initial meeting is held in July or August to solicit interest in the IRM retained ownership program. Presentations are made on the importance of thinking in terms of a beef industry instead of just producing calves. Another presentation will explain the basics of the program and to solicit ideas for changes or improvements from past years

or other programs. The highlight of the first meeting is a presentation of projected closeouts by the feedlot operator. At this meeting producers are given information on weaning management on the ranch as the program requests that all calves be weaned at least 45 days with a proper vaccination program before delivering to the feedlot. For four years producers were offered the opportunity to take blood samples when the calves were given the preweaning vaccinations to evaluate the effect of the vaccines given. Cattle were again bled upon arrival at the feedlot so titer change could be evaluated on each ranch. Producers were encouraged to consult with their practitioner for a recommended vaccination program, thus resulting in a variety of different health and vaccination programs.

Cattle are delivered to the feedlot on one day in the middle of October where they are processed with needed vaccinations and parasite control plus are weighed individually. Producers and some resource people gather for informal discussions at this time.

During the summer of 2002 severe drought conditions existed in the region being served by this program so an offer was made to allow the early weaned calves to enter the program in early September. One hundred and twenty-four head of early weaned calves entered the program in 2002. The same option was offered in 2003 with fewer numbers weaned early.

The cattle are again individually weighed after approximately two weeks after arrival at the feedlot and implanted at that time. Midway through the feeding program the cattle are again individually weighed, ultrasounded, and reimplanted with a terminal implant. The ultrasound data is used to separate the cattle into 3 outcome sale groups. The cattle are all maintained in the same pen with different ear tag markings for sale groups. Cattle are sorted and individually weighed 1-2 days before shipping to harvest. All cattle are sold on carcass grid basis.

Marketing conference calls are conducted approximately monthly. The purpose of these calls

are to aid producers in understanding various marketing options available to aid in offsetting risks, gain a better understanding of methods of marketing, including forward contracting, live sale, "in the beef" and various grid offerings. In addition, the group discusses and votes on purchasing marketing risks, e.g. options and futures. If enough producers request, an educational meeting is presented by the agricultural economist to educate on the details of the various marketing and risk tools available.

An educational meeting is held in a large heated shop at the feedlot soon after the midterm weights and ultrasound data is collected. Producers have an opportunity to view the cattle plus a chute side demonstration is conducted before lunch. Chute side demonstrations have included quality assurance techniques, ultrasound demonstrations, individual identification techniques and proper cattle handling. The midterm programs include presentations concerning marketing decisions and current market positions, individual cattle health and performance, plus presentations from industry leaders to address various cutting edge topics and issues. During the time of cattle harvest opportunities are given to producers to visit the slaughter plant where they can observe the retained ownership cattle graded, plus a tour of the slaughter facilities.

At the end of the test after all performance and carcass data is collected and summarized, a final educational meeting is held in the ranching community. Again, discussions on all data collected and marketing decisions are discussed. Profitability of each individual animal is estimated by utilizing the NRC '96 equation number 7a and 7-1 to estimate intake and then all feedlot costs plus the weighted value of the weaned calf is subtracted from the actual sale price of the carcass. Industry leaders are invited to discuss pertinent information concerning the beef industry.

The cooperating feedyard sends feed and health bills to each producer every two weeks which not only gives a progress report but gives the experience of receiving frequent feed bills when cattle are retained in a feedlot. Feed costs are based

on the percent of weight that the producer has in the lot. The percentages are adjusted each time the cattle are weighed.

## **Results**

The program has been conducted for 6 years with 200-400 cattle consigned with 20-35 producers each year. Over a 4 year period overall results have been relatively similar (Table 1). Entry weights were lower in the fall of 2002 primarily due to drought conditions. Also daily gain was slightly lower probably due to a longer feeding period as the final weights were essentially the same each year. As with all retained ownership programs when comparisons are made from owner to owner there is a tremendous range in all traits measured and even much greater range when individual cattle are evaluated (Table 2). The range in estimated profit from the high and low producer was \$33 to \$205, a \$172 difference. This is largely due to the value of the carcasses and this is largely due to the severe discounts for "out" or undesirable carcasses such as, dark cutters, overfat carcasses, standard quality grade, or too light or heavy carcasses. Efforts to find indicators of profitability of feeder cattle have been disappointing. A small positive relationship is found with incoming weight, with the heavier groups of cattle showing slightly more profitability. This is most likely due to heavier carcass weight when sold. Initial weights vary considerably even though all calves are spring born and producers are encouraged to bring calves in the 550-600 lb range.

Early weaned calves gained slightly less than later weaned, however they were fed on an average of 23 days longer (Table 3). The early weaned calves appeared to have a slightly higher percent choice and prime, however the increase in quality grade did not increase the value enough to offset the added value of carcass weight and thus the late weaned calves were about \$15 more profitable in the feedlot.

The educational efforts have been very rewarding. As a result of this program several producers are now retaining ownership on a portion or all of their calf crop. In some cases they bring the entire steer calf crop to the cooperating feedlot or ship to a similar commercial feeder. In a few

cases producers have chosen to finish their cattle at home. Those that choose to finish cattle at home have learned that marketing cattle is relatively easy and simple if they market on a carcass grid.

Outside speakers have had a tremendous impact on the participants. Many producers will relate how much more they understand about the importance of quality lean beef and how the value is determined in the hanging carcass. This has resulted because of presentations, demonstrations and discussions on quality and yield grades, presentations by various resource people in the packing industry, and perhaps the most educational is the visits at the packing plants where they observe the grading process.

Quality assurance practices have changed on the ranch. Many presentations have dealt with the need to utilize quality assurance recommendations and why it is important when they consider beef sold to the consumer. Coupled with quality assurance is the knowledge gained in cattle handling. Experts have made presentations on not only the importance of proper handling of cattle and how it relates to cattle performance, dark cutters, etc. Demonstrations were also held on how to effectively handle cattle as well as proper equipment design. One year temperament scores were assigned while cattle were held in the squeeze chute. This was done at the time the cattle were started on test. These scores were correlated with performance and presented.

Producers have a much greater understanding of new convenience beef products that are developed for the busy consumer. Presentations were made in the meetings by companies that were developing new microwaveable products plus the products were served for dinner. Presentations on marketing alternatives have made producers much more aware of the many options available plus factors that influence the cattle market. Even though many efforts have been devoted to educating producers about the feeding, packing and beef processing industry, how this all relates to the profitability of the cow herd has continued to be emphasized. Resource people have addressed topics of how all

the feedlot and carcass performance relates to the production and profitability of the basic cow herd. This has involved both university animal geneticists and also leading cow-calf producers who have retained ownership for several years.

Issues such as individual animal identification have been presented by both demonstrations of optical reading, electronic ear tags, plus much discussion on individual animal identification. Issues such as catastrophic disease outbreaks have also been discussed by experts.

### **Implications**

Cow-calf producers can benefit greatly by participating in a retained ownership program in two ways. First, they will gain greater knowledge of the challenges and opportunities that other segments of the beef industry experience, and they will think in terms of being in the beef industry and not just producing calves. Second, with a representative sample of their calves fed with other producers' calves they will gain some understanding of how their calves perform in the feedlot and of their carcass merit. They can then learn of traits that they excel in and perhaps traits that need improvement.

Table 1. Summary of last 4 years of steer performance.<sup>1</sup>

Year	Head	Arrival weight, kg		Start wt, kg	Final wt, kg	ADG kg/day
		Early wean	Late wean			
2000-01	169	--	256	274	570	1.67
2001-02	180	--	265	281	570	1.67
2002-03	230	217	245	261	567	1.48
2003-04	120	245	265	286	--	--

<sup>1</sup>Performance trends of heifers were similar.

Table 2. Variation encountered in 2002-03 steer data.

	Average	Range
Producer groups		
In weight, kg	245	193 - 364
Final weight, kg	567	524 - 611
ADG, kg	1.48	1.34 - 1.59
Profit, \$/hd	\$144	\$33 - \$205
Individual steers		
In weight, kg		137 - 409
Final weight, kg		429 - 696
ADG, kg		.86 - 1.95
Profit, \$/hd		(\$170) - \$307

Table 3. Comparison of early and late weaned steer calves in 2002-03.<sup>1</sup>

	Date of Weaning	
	Early	Late
In weight, kg	217	245
Days fed	245	222
Final weight, kg	560	572
ADG, kg	1.40	1.47
% Choice or >	66.1	53.4
Fat, cm	.22	.22
Total steer value, \$/hd	\$967	\$981
Estimated feedlot profit, \$/hd	\$115	\$130

<sup>1</sup>Heifer mates showed same trends.

## COMPARISON OF BEEF CATTLE MANAGEMENT PRACTICES FOR PLANNING BEEF QUALITY ASSURANCE EDUCATION

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**ABSTRACT:** A mail-in survey was conducted to determine Beef Quality Assurance educational needs for record keeping, health management, marketing strategies, and perceptions of industry issues by MT beef producers. A 38-question survey was mailed to 1,000 beef producers; 500 were Beef Quality Assurance (BQA) certified (231 surveys returned or 46%) and 500 were not BQA (non-BQA) certified (116 surveys returned or 23%). Results of *t*-test comparisons showed BQA producers kept more ( $P<.05$ ) records (cowherd inventory, description, calf birth weights, vaccination, feed) than non-BQA producers. Ninety-seven percent of BQA producers vaccinated in the neck compared to 87% of non-BQA producers ( $P>.<.05$ ). BQA producers used modified live vaccines more ( $P<.05$ ) than non-BQA producers. Fifty-nine percent of all producers indicated they implanted calves with growth promotants. There were no differences ( $P>.10$ ) between treatment groups in the method of marketing feeder calves with the most common method reported being the order buyer (46%) followed by the auction market (26%). Eighty-six percent of all producers marketed their calves to the same buyer two or more years. Thirty-three percent of all producers indicated that their buyer required information on the calves with vaccination history requested most frequently (36%) followed by BQA certification. The most common method for identifying calves was the plastic ear tag followed by a hot iron brand. A majority of all producers (94%) indicated they were either somewhat to very concerned about food safety issues (BSE, *E. coli* O157:H7) in the industry. More ( $P<.05$ ) BQA producers (59%) than non-BQA producers (47%) indicated that a national animal identification program should be implemented. However, respondents were equally divided as to whether the program should be voluntary or mandatory. Results of this survey will be used to better determine the BQA educational programming needs of MT cattle producers.

Key Words: Beef quality assurance, Beef cattle, Extension programming

### Introduction

Reflective of our information-based society, Americans are demanding more information about the food they eat and how it was produced. Consumers are making beef, poultry and pork purchasing decisions based upon nutrition, safety, price, and convenience (Montana Beef Quality Assurance, 2003). The industry continues to change and evolve to meet the challenges of international

trade requirements, food safety issues (*E. coli* O157:H7 and BSE), dietary trends (high protein diets), and consumer demand for a safe, consistent, and quality beef product. One industry response to these challenges has been the voluntary beef quality assurance (BQA) educational programs for producers.

The National Cattlemen's Beef Association (NCBA) established the Quality Assurance program in 1987 to maximize consumer confidence in and acceptance of beef by focusing the industry's attention on beef quality assurance through the use of science, research and educational initiatives. Programs in all 50 states are an industry effort to encourage cattlemen to follow certain production practices and quality-control measures that exceed U.S. Department of Agriculture and Food and Drug Administration requirements. Through research and education efforts conducted by state university and extension staff, the program educates cattlemen on the proper uses of pharmaceuticals, cattle handling, feed purchasing, testing and other procedures aimed at improving the overall safety and quality of cattle and beef (NCBA, 1996).

This survey was conducted to measure the management practices of beef producers in order to assess the impact of current BQA programming and determine what educational needs are required in the future.

### Materials and Methods

Thirty-eight questions for this three-page survey were grouped into six categories: records management, health management, winter nutritional programs, marketing, perceptions on industry issues, and demographic information. The survey was peer reviewed by individuals familiar with survey design and the beef industry in the state. The resulting survey instrument was mailed to 1000 beef producers.

The survey population was derived from two mailing lists kept by the Montana State University beef extension specialist. The first was a list of producers who had completed Beef Quality Assurance (BQA) training and were identified as the BQA certified group. The second were producers who received the *Beef: Questions and Answers* newsletter published by the beef extension specialist approximately five times a year and were identified as the non-BQA group. The lists were compared to eliminate any duplication.

Surveys were mailed to producers between August 1 and August 4, 2003. Surveys were color coded to maintain treatment identification. No follow-up procedures were followed. Respondents were given a

deadline of September 1, 2003 to return the surveys. Surveys were analyzed September 15, 2003 and all surveys received after this date were not included in the data set. Responses from BQA and non-BQA producers were compared using an independent measures *t* test and were considered significant at  $P<.05$  (Statistical Package for Social Sciences ® 12.0).

## Results

Of the 500 surveys mailed to BQA certified producers, 231 were returned for a 46% response rate. Non-BQA certified producers returned 116 of the 500 surveys for a 23% response rate.

### *Demographics*

The majority of producers (82% BQA, 70% non-BQA) indicated they were cow-calf producers. Twenty percent of the BQA respondents indicated they backgrounded while 22% of non-BQA producers background calves. Only 2.6% of BQA and 4.3% of non-BQA producers finished their calves. The size of the operations reported by respondents varied evenly across the options provided. The majority of BQA producers (55%) had operations with fewer than 200 head of cows. Seventeen percent of non-BQA producers had over 500 cows.

The majority of the respondents for both groups were between the ages of 30 and 60 (79% BQA, 72% non-BQA). When asked about level of education, responses between the two populations were again comparable. The only difference ( $P<.05$ ) was that more BQA producers had Master's degrees than non-BQA producers.

### *Records Management*

The data in Table 1 shows the type of records maintained by producers. More ( $P<.05$ ) BQA producers (86%) kept records of animal numbers and descriptions than non-BQA producers (69%). BQA producers (74%) kept more ( $P<.05$ ) cowherd records than non-BQA producers (49%). More ( $P<.05$ ) BQA producers (84%) maintained vaccination records than non-BQA producers (60%). BQA producers (45%) kept more ( $P<.05$ ) BQA records than non-BQA producers (4%). Records on purchase and sale of animals were maintained by 77.1% of BQA and 66.4% of non-BQA respondents. Only 43.3% of BQA respondents and 29.3% of non-BQA respondents reported maintaining records of suppliers and buyers of their cattle.

Although Montana is a brand-law state, the survey asked respondents to describe how they identified their animals. Respondents were allowed to choose all answers that applied to their management situation. The data in Table 1 shows the animal identification methods used by producers. More ( $P<.05$ ) BQA producers (92%) used plastic ear tags for animal identification than non-BQA producers (78%). BQA producers (80%) were also different ( $P<.05$ ) from non-BQA producers (65%)

regarding the use of the hot iron brand. There was a difference ( $P<.05$ ) in the use of electronic identification tags between BQA producers (11%) and non-BQA producers (2%), which indicates a need for further education in light of the potential implementation of a national animal identification program that will likely use electronic identification technology.

Most of the respondents used some form of paper-based record keeping system for the information they kept. There was a difference ( $P<.05$ ) between BQA producers and non-BQA producers who use an on-farm electronic record keeping system, such as Excel, Quattro Pro, or similar program. The majority of the respondents kept records for more than two years, although there was a difference ( $P<.05$ ) between BQA producers and non-BQA producers. The low percentage of producers using electronic records (BQA, 20.3%; non-BQA, 8.6%) suggests a need for educational programming, especially in light of government and industry responses to the recently determined animal health and food safety risks.

### *Health Management*

When asked about vaccination protocols, the majority of producers indicated they preferred to give a modified live vaccine (Table 2). More ( $P<.05$ ) BQA producers (63%) preferred the modified live vaccine than non-BQA producers (50%). Although a majority of both populations administered vaccinations in the neck, there was a difference ( $P<.05$ ) between BQA producers (97%) and non-BQA producers (87%). Relatively few producers gave vaccinations in any rear area of the body. These data indicates that BQA educational programming has positively impacted the management practices of many producers in the state. The usual administrator of the vaccinations was the producer, with 84% of BQA producers giving their own vaccinations while 76% of non-BQA producers gave the vaccinations. One difference discovered was more ( $P<.05$ ) BQA spouses (27%) gave the vaccination compared to non-BQA spouses (15%).

More ( $P<.05$ ) non-BQA producers did not vaccinate their calves before sale than BQA producers. Additional education needs to be undertaken to show producers that calves perform better in feedlots when they have received viral vaccinations. Most of the producers from both populations vaccinated calves at least once before sale. There were no differences found in the implant protocols between the two populations with 42% of both groups not implanting steers or heifers.

### *Marketing*

Marketing strategies used by respondents are presented in Table 3. There were no differences between the two populations with regard to their preferences for marketing weaned calves. The most common method for both groups was the order buyer (BQA, 45%; non-BQA 48%) followed by the auction market (BQA, 25%; non-BQA 28%). When marketing cull cows, BQA producers

(95%) were more likely ( $P<.05$ ) to use the auction market than non-BQA producers (85%).

Although there were no differences between the two populations regarding the number of years the producers have marketed to the same buyer, the results indicate that a majority of producers have developed long-term relationships and have marketed to the same buyer for three or more years.

The results suggest that buyers are requiring producers to provide information about the calves they are purchasing. Vaccination records was the most common information requested (BQA, 39%; non-BQA, 31%) followed by additional health records (BQA, 13%; non-BQA, 10%). Of the respondents who are required to provide data to their buyers, there was a difference ( $P<.05$ ) between BQA producers and non-BQA who were asked to provide BQA certification. As the various sectors of the beef industry react to BSE, national animal identification program proposals, and country of origin labeling legislation, it is obvious that more records will be requested of producers. Future educational programming will be necessary to meet these needs.

#### *Perceptions on Industry Issues*

When asked about food safety issues, most of the respondents indicated they were either somewhat (BQA, 33%; non-BQA, 30%) to very concerned (BQA, 65%; non-BQA, 60%; Table 4). There were no differences between the populations regarding this issue. This level of concern suggests that respondents should be receptive to educational programming on food safety issues.

There was a difference ( $P<.05$ ) between the BQA producers (59%) who responded that there should be a national animal identification program compared to the non-BQA producers (47%) who also responded positively to this question.

## **Conclusions**

Beef quality assurance education has had an impact on the management strategies of producers. For example, injection-site lesions have caused enormous economic loss to the U.S. beef industry and have been a serious quality assurance problem (Roeber, et al., 2001). Due to BQA educational efforts, Roeber et al. (2001) reported a decline in the national incidence of injection-site lesions in top sirloin butts from 11.4% in November 1995 to 2.1% in July 2000. Improvements in record keeping and location of injections are evident with this survey compared to previous surveys conducted in MT.

Producers are aware of challenges facing the beef industry and have considered the possible actions needed to meet them. The majority of respondents are somewhat to very concerned about food safety issues. Fifty-nine percent of BQA producers and 47% of non-BQA producers believe a national animal identification program is needed. BQA training of producers will need to be supplemented with animal identification and records management regulations and options in addition to any new health protocol information. Preparing producers for this step will be best served by a comprehensive and dynamic BQA education program.

## **References**

- Roeber, D.L., R.C. Cannell, K.E. Belk, J.A. Scanga, G.L. Cowman, G.C. Smith. 2001. Incidence of injection-site lesions in beef top sirloin butts. *J. Anim. Sci.* 79: 2615-2618.
- Montana Extension Service. 2003. Montana Beef Quality Assurance. Manual, Montana State University, Bozeman.
- National Cattlemen's Beef Association. 1996. Cattlemen Launch Campaign on Quality Assurance Program. Available [http://www.beef.org/dsp/dsp\\_content.cfm?locationId=217&contentId=844&contentType=2](http://www.beef.org/dsp/dsp_content.cfm?locationId=217&contentId=844&contentType=2). Accessed December 31, 2003.

**Table 1. Types and format of records maintained by BQA certified and non- BQA certified producers**

	BQA		Non-BQA		<i>t</i> value*
	<i>f</i>	%	<i>f</i>	%	
Type of records maintained					
Animal number and description	199	86.1	80	69.0	3.87
Vaccination records	194	84.0	69	59.5	5.20
Calf birth records	180	77.9	75	64.7	2.66
Animal purchases and sales	178	77.1	77	66.4	2.13
Cowherd records	170	73.6	57	49.1	4.64
BQA records	104	45.0	5	4.3	8.44
Names of suppliers and buyers	100	43.3	34	29.3	2.54
Feed records	89	38.5	29	25.0	2.53
Where animal was born	78	33.8	27	23.3	2.01
No records kept	8	3.5	9	7.8	
Animal Identification Methods					
Plastic Tag	212	91.8	90	77.6	3.78*
Hot Iron	185	80.1	75	64.7	3.17*
Ear Tattoo	48	20.8	14	12.1	2.00*
Ear notch	31	13.4	18	15.5	
Electronic Tag	25	10.8	2	1.7	3.02*
Freeze Brand	22	9.5	11	9.5	
Metal Tag	19	8.2	8	6.9	
No Identification	0	0	0	0	

\**P* < .05.**Table 2. Vaccination protocols of BQA certified and non- BQA certified producers**

	BQA		Non-BQA		<i>t</i> value
	<i>f</i>	%	<i>f</i>	%	
Preferred Vaccine Type					
Killed	85	36.8	39	33.6	2.29*
Modified Live	145	62.8	58	50	
Chemical	2	0.9	0	0	
No Preference	12	5.2	8	6.9	
Killed Booster Given	80	94 <sup>†</sup>	34	87 <sup>†</sup>	
Vaccine Location					
Neck	223	96.5	101	87.1	3.39*
Armpit	16	6.9	14	12.1	
Shoulder	3	1.3	5	4.3	
Upper rear leg	0	0	1	0.9	
Side or ribs	2	0.9	0	0	
Lower rear leg	0	0	0	0	

<sup>†</sup>N for this category is the number of respondents that indicated they preferred the killed vaccine.

\**P* < .05**Table 3. Marketing preferences of BQA certified and non- BQA certified producers**

Non-BQA Certified Producers					
Marketing Options	BQA		Non-BQA		<i>t</i> value
	<i>f</i>	%	<i>f</i>	%	
Calf Marketing Preference					
Auction Market	57	24.7	32	27.6	3.46*
Order Buyer	103	44.6	56	48.3	
Video Auction	39	16.9	13	11.2	
Forward Contract	29	12.6	8	6.9	
Private Treaty	45	19.5	29	25.0	
Retained	19	8.2	7	6.0	
Ownership					
Cull Cow Marketing Preference					
Auction Market	220	95.2	98	84.5	3.46*
Order Buyer	26	11.3	12	10.3	
Retained	6	2.6	3	2.6	
Ownership					

\**P* < .05.**Table 4. Perception of issues facing the beef industry by BQA certified and non-BQA certified producers**

Issues	BQA		Non-BQA		<i>t</i> value
	<i>f</i>	%	<i>f</i>	%	
Food Safety					
Very Concerned	150	64.9	66	59.9	2.26*
Somewhat Concerned	75	32.5	35	30.2	
Not Concerned	2	0.9	5	4.3	
National Animal ID Program					
Yes	137	59.3	54	46.6	2.26*
No	35	15.2	24	20.7	
Don't Know	50	21.6	28	24.1	

\**P* < .05.

**A TO Z RETAINED OWNERSHIP, INC.: FACTORS AFFECTING  
PROFITABILITY IN THE INLAND NORTHWEST**

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**ABSTRACT:** Retained ownership is often used as a marketing alternative for beef cattle producers. The A to Z Retained Ownership, Inc. program has fed 5,286 head of cattle from over 120 ranches from Idaho, Oregon, Washington, and Montana. The objective of this study was to use regression techniques to determine the factors that have influenced the profitability of retained ownership decisions (from weaning to beef processing) during the period of 1992-2003. This analysis showed that feeder cattle price, average daily gain, whether the carcass graded choice or better, marbling score, adjusted back fat, marketing grid base price, days on feed, feed conversion, and corn price were significant variables ( $P < 0.025$ ) in explaining 62% of the variation in profitability over the 11-year time frame. The study also showed that heifers were \$12.21/head more profitable to feed than steers in the A to Z program. This difference in profitability between steers and heifers was explained by feeder cattle price, average daily gain, adjusted back fat, days on feed, and feed conversion.

**Key Words:** Beef Cattle, Retained Ownership, Profitability

### **Introduction**

Within geographic locations, cow-calf producers operate on similar calving, weaning, and marketing scenarios. In many cases, these seasonal patterns are determined by the seasonal availability of forage, weather, public and private land management plans, tradition, and a number of other factors. Since cow-calf producers operate in similar seasonal patterns within the Inland Northwest, the supply of weaned calves usually peaks in the fall, and corresponding decline in calf price occurs at that time (Gray, 2000).

In Idaho, it is typical to see calves born in the spring, grazed on summer forage, weaned, and sold in the fall. As a result of cattle price risk, small ranch size, finances, lack of information, and a number of other factors, the majority of Idaho ranchers sell weaned calves. Due to the abundant supply of marketed calves in the fall, prices tend to be lower. Back grounding calves through part of the winter and retaining animals in a feedlot throughout the winter could be a feasible solution to the problem of low fall prices. Prices for yearling and fed cattle are typically higher in the spring when beef supply is limited. By selling calves in the fall, ranchers are not only receiving a seasonally low price, but they are also forgoing potential

earnings (and additional risk) from owning the animal until it becomes processed beef.

A large portion of Idaho ranchers operate with fewer than 50 head of cattle (58 percent, Idaho Beef Council, 2003). Because these ranchers operate without the number of animals required to fill a pen at a feedlot, they have an additional barrier in retaining ownership of their cattle. If they decide to retain ownership of their cattle, they have an additional task of finding enough cooperating producers to fill a pen. Pens of cattle in feedlots generally entail the commitment of 200-500 head of cattle.

In the early 1990's, the University of Idaho, in cooperation with producers, feedlots, animal health practitioners, and lenders began the A to Z Retained Ownership, Inc. program. A to Z Retained Ownership, Inc. began in the fall of 1992 and is currently in its twelfth year of operation. The program was developed to give cow-calf producers information on how their cattle performed in the finishing and slaughter segments of the beef industry. The overall objective of A to Z was to educate ranchers in the Inland Northwest on issues regarding retained ownership, providing them with information processes for selecting a feedlot and financial institution, feedlot performance information, marketing alternatives and strategies, individual animal carcass information, and an economic evaluation of the factors that influence the profitability of feeding cattle (Momont et al., 1993). It is the intent of this research to provide an in-depth evaluation of the factors that influence cattle feeding profitability in the Inland Northwest.

### **Materials and Methods**

Feedlot closeouts and carcass data for 4,209 head of cattle consigned to the A to Z Retained Ownership, Inc. program between November 1995 and November 2003 were used for this study. The first two years which A to Z Retained Ownership, Inc. was in operation, carcasses were not priced on a marketing grid. Therefore, only the latter nine years were used for this study. The 2,558 head of steers and 1,651 head of heifers originated from over 117 cattle operations in Idaho, Montana, Oregon, and Washington. Individual animal data included incoming weight, outgoing weight, average daily gain, processing costs, gross returns, in date, out date, and days on feed. Hot carcass weight, back fat, grade, yield, carcass price, internal fat, marbling score, and ribeye area were obtained by University of Idaho faculty during the United States

Department of Agriculture (USDA) grading process at the processing plant. Marbling scores and yield grades were transformed into equivalent numerical values for analysis. All grade and yield data for the cattle were provided by the USDA Carcass Grading System.

Additional data included feeder cattle prices, feed conversion, and corn price. Feeder cattle prices were obtained from an electronic marketing report when the animals entered the feedlot (Jensen et al., 2003). Monthly corn prices were obtained from the USDA's National Agricultural Statistics Service (USDA/NASS, 1992-2003). Since Idaho publishes only yearly corn prices, national average prices for the months the cattle were in the feedlot were used in this study.

Calving, breeding, and weaning information was provided from the individual ranchers for each animal entered into the program. Total feed fed to each pen of cattle was provided monthly by the feedlot. Feed intake for each animal was based upon the animal's weight and gains during the warm-up and finish phases of the program. Feed intake and feed costs were allocated using the net energy for maintenance and gain equations of Owens et al., (1984). The equations derived by Owens et al., (1984) use weight, average daily gain, and daily feed intake to calculate net energy for maintenance and gain for animals on a dry diet. These equations were used to calculate the net energy for maintenance and gain for the animals consigned to the A to Z Retained Ownership, Inc.

Final yield grades were calculated using the procedures outlined in the Guidelines for Uniform Beef Improvement Programs. (Beef Improvement Federation, 1990). Profit per head was calculated by subtracting feed, yardage, transportation, processing, medical, death loss, and opportunity costs from the final value of the carcass. Opportunity costs included the initial value of the animal at weaning and forgone interest on the initial value, calculated using a 6% interest rate over the feeding period. Death loss was shared among the group and was calculated by summing the initial value of the dead animals less any costs incurred prior to death (Jensen et al., 2003).

Regression analysis was used to determine the significance of variables that explain the variation in profit over time. The regression model estimating profit per head is defined as:

$$\text{Profit} = \beta_0 + \beta_1\text{FCP} + \beta_2\text{ADGTOT} + \beta_3\text{GRADE} + \beta_4\text{MARSCOR} + \beta_5\text{ADJBF} + \beta_7\text{DOF} + \beta_8\text{FDCONVTL} + \beta_9\text{CORN}$$

Feeder cattle price (**FCP**) represents the opportunity cost, exclusive of forgone interest, the rancher incurred by retaining ownership of the animal. It is the input cost of the feeding phase for the cattle. Average daily gain (**ADGTOT**) is the average gain per day of an animal over the course of the feeding phase (warm-up = about 60 days; finish = about 100 days). Quality variables in the model included: grade (**GRADE**), marbling score (**MARSCOR**), and adjusted back fat (**ADJBF**). Grade was included in the model through the use of a dummy variable (Gujarati, 2003). If the carcass graded choice or prime, the

dummy variable was assigned a value of one (zero otherwise). Days on feed (**DOF**) and the price of corn (**CORN**) were representative of actual feeding costs. Feed conversion (**FCONVTL**) was expressed as kg of feed per kg of gain.

Past studies have suggested that heifers are less profitable to feed than steers. A study in the Midwest concluded that heifers (placed at 273 kg or greater) are \$12.30/head less profitable than steers (Lawrence et al., 1999). Due to pregnancy risk, lower average daily gain and various other factors, heifer prices are typically discounted in relation to steers as feeder cattle. The Kansas study (Lawrence et al., 1999) concluded that heifers are less profitable even after accounting for the discounted feeder cattle price. Langemeier et al., (1992) analyzed 2,600 pens of steers and 700 pens of heifers and found that approximately 86% of the variation in profit between steers and heifers was explained by differences in sale price, feeder price, feed conversions and average daily gains. In the present study, the issue of heifer vs. steer profitability was addressed by adding a dummy variable (**HEIFER**) to the previous model.

$$\text{Profit} = \beta_0 + \beta_1\text{FCP} + \beta_2\text{ADGTOT} + \beta_3\text{GRADE} + \beta_4\text{MARSCOR} + \beta_5\text{ADJBF} + \beta_6\text{BP} + \beta_7\text{DOF} + \beta_8\text{FDCONVTL} + \beta_9\text{CORN} + \beta_{10}\text{HEIFER}$$

In our formulation of this model, the difference in profitability between steers and heifers can be estimated. The **HEIFER** variable will serve as an intercept shifter and take on a value of one if the animal is a heifer and zero if the animal is a steer.

## Results and Discussion

Table 1 contains the results of the regression analysis of profitability from feeding cattle. All parameters were significant ( $P < 0.025$ ), and all had the expected sign. Sixty two percent of the variation in profitability associated with feeding cattle was explained by the model.

Profit increased by \$3.22/head for each dollar of increase in the base price. A one unit increase in marbling score increased profits by \$6/head, and a dollar increase in feeder cattle prices will reduce profit by \$2.97/head. A one kg increase in average daily gain will add \$97.94/head to profit. As feed conversion increases by one unit, profits will decrease by \$33.95/head, and \$44.71/head will be lost as corn price increases by one dollar per bushel. An additional \$29.73/head will be generated if the carcass graded choice or better. Each additional day on feed will cost roughly \$1 in profit, and each cm added to back fat will cause profits to drop \$28.46/head. Tests indicated no presence of multicollinearity among our independent variables.

Regression results testing the profitability difference between steers and heifers are reported in Table 2. The dummy variable for heifer was positive and significant ( $P < 0.025$ ). Over the 9 years of our study, heifers were \$12.21 more profitable than steers and this model formulation explained 63% of the variation in profit.

The \$12.21 difference in profitability was explained by differences in feeder cattle price, average daily gain, adjusted back fat, days on feed, and feed conversion. These findings support the analysis of steer and heifer data in separate models. Results are reported in Tables 3 and 4. All parameters were significant ( $P < 0.025$ ) and had the expected sign and explained 58% and 70% of the variation in profit for steers and heifers, respectively.

### Implications

This study examined factors that contributed to the variability in profits from feeding cattle in the Inland Northwest. Nine years of data and over 4,000 head of cattle consigned to the A to Z Retained Ownership Program were used for the study. Feeder cattle price, average daily gain, grade, marbling score, adjusted back fat, grid base price, days on feed, feed conversion, and corn price contributed to explaining the variability in profits. Overall, 62% of the variation in profitability was explained by these factors.

Profitability also differed between sexes. Results of this study show a \$12.21 dollar difference in profitability between steers and heifers, with heifers being more profitable. The \$12.21 dollar difference was largely explained by differences in feeder cattle price, average daily gain, back fat, days on feed, and feed conversion. Since heifers are typically marketed earlier than steers, their feeding costs are generally less than the feeding costs of steers. Higher carcass prices are also typically associated with earlier marketing dates. The early marketing of heifers takes advantage of these prices.

As a result, cattlemen need to consider the impact of the genetic base of their cattle (as embodied in back fat, marbling, those animals that grade USDA Choice or better), inputs and costs (feeder cattle prices, corn prices) and output prices (grid prices) on profitability when considering retaining ownership. Because of the movement toward grid pricing, cattlemen should also pay close attention to feedlot performance information and closeout data when choosing animals to sell and retain. It will also enhance the rancher's ability to selectively breed their herd to take advantage of premiums given by the grid pricing system. More specifically, standardized regression results indicate that feedlot average daily gain, marbling score, grade, adjusted back fat, feed conversion, feeder cattle prices, corn prices, and grid base price are equally important in explaining the variation in profits from feeding cattle. These should be monitored and considered equally when deciding to retain ownership of cattle.

Historically, feeder cattle prices for heifers have been heavily discounted in relation to steers. The rationale behind the discounted prices varies from risk of pregnancy to lower feedlot performance. However, our results show heifers are more profitable to feed. Since these results suggest that heifers are more profitable to feed, discounted heifer feeder cattle prices do not appear to be justified. By all rational economic reasoning, feeder cattle prices should be determined by market forces. If the market was allowed to determine feeder cattle prices, the initial price for the more profitable animal will increase until the economic profit of feeding both animals is equal. Thus, feeder heifer

prices should increase until there is no statistical difference in the economic profitability of feeding steers and heifers.

### Literature Cited

- BIF. 1990. Guidelines for uniform beef improvement programs. 6<sup>th</sup> ed. North Carolina State University. Raleigh, NC.
- Gray, C.W. 2000. Seasonal patterns of cattle prices. CL823 in Cow-Calf Management Guide and Cattle Producer's Library. University of Idaho, Moscow
- Gujarati, D.N. 2003. Basic Econometrics. 4<sup>th</sup> ed. McGraw-Hill, Boston, MA.
- Idaho Beef Council. 2004. Idaho's Beef Industry. Available: [http://www.idbeef.org/idbc\\_beefindustry.htm](http://www.idbeef.org/idbc_beefindustry.htm). Accessed: Jan. 15, 2004.
- Jensen, S., B. Wilhelm, N. Rimbey, G. Keetch, W. Cook, B. Glaze, W. Gray, J. Hawkins, E. Morrison, S. Williams, and P. Momont, 2003. A to Z retained Ownership, Inc: Year-end Summary. Available: <http://www.uidaho.edu/ag/caldwell/atoz.htm>. Accessed Jan. 15, 2004.
- Langemeier, M., T. Schroeder, and J. Mintert. 1992. Determinants of cattle finishing profitability. S.J. Agric. Econ. 24:41-48.
- Lawrence, J., Z. Wang, and D. Loy. 1999. Elements of cattle feeding profitability in midwest feedlots. J. Agric. Appl. Econ. 31(2):349-357.
- Momont, P., N. Rimbey, G. Keetch. 1993. A to Z retained Ownership, Inc.: Year-end summary. University of Idaho Coop. Ext. Sys. Bull. 763. Moscow, ID.
- Owens, F.N., W.M. Sharp, and D.R., Grill. 1984. Net energy calculation from feedlot performance data. Okla. Agric. Exp. Sta. Report MP-116. Stillwater, OK.
- USDA/NASS. 1992-2003. Agricultural Prices. Available: <http://www.nass.usda.gov>. Accessed Jan. 25, 2004.

**Table 1.** Regression results explaining the variation in profitability from feeding cattle consigned to A to Z Retained Ownership, Inc. from 1995 to 2003.

Variable <sup>1</sup>	Parameter Estimate	Standard Error
Intercept	103.52	21.74604
ADG	97.94	3.40964
FCP	-2.97	0.09775
FDCONVTL	-33.95	1.56470
GRADE	29.73	2.13336
MARSCOR	6.03	0.35068
BP	3.22	0.09686
DOF	-0.99	0.06344
CORN	-44.71	2.65206
ADJBF	-28.46	1.86739
n = 4209 F = 772.90 Adj. R <sup>2</sup> = 0.62		
<sup>1</sup> All variables significant (P<0.025)		

**Table 2.** Regression results specifying the difference in profitability between feeding steer and heifers consigned to A to Z Retained Ownership, Inc. from 1995 to 2003.

Variable <sup>1</sup>	Parameter Estimate	Standard Error
Intercept	41.22	23.27820
Heifer	12.21	1.69299
ADG	105.77	3.55893
FCP	-2.79	0.10050
FDCONVTL	-33.24	1.55838
GRADE	29.28	2.12140
MARSCOR	5.88	0.34925
BP	3.23	0.09628
DOF	-0.86	0.06538
CORN	-39.83	2.72165
ADJBF	-29.61	1.86314
n = 4209 F = 709.26 Adj. R <sup>2</sup> = 0.63		
<sup>1</sup> All variables significant (P<0.025)		

**Table 3.** Regression results explaining the variation in profitability from feeding steers consigned to A to Z Retained Ownership, Inc. from 1995 to 2003.

Variable <sup>1</sup>	Parameter Estimate	Standard Error
Intercept	192.24	32.32241
ADG	96.45	4.79062
FCP	-2.65	0.12994
FDCONVTL	-29.10	1.79783
GRADE	28.51	2.90374
MARSCOR	6.34	0.48424
BP	2.86	0.13490
DOF	-1.36	0.09647
CORN	-52.02	3.46559
ADJBF	-35.46	2.64088
n = 2587 F = 402.69 Adj. R <sup>2</sup> = 0.58		
<sup>1</sup> All variables significant (P<0.025)		

**Table 4.** Regression results explaining the variation in profitability from feeding heifers consigned to A to Z Retained Ownership, Inc. from 1995 to 2003.

Variable <sup>1</sup>	Parameter Estimate	Standard Error
Intercept	-18.81	33.35838
ADG	119.95	4.92474
FCP	-3.01	0.15747
FDCONVTL	-60.14	3.60818
GRADE	29.47	2.80114
MARSCOR	5.35	0.45242
BP	3.41	0.12521
DOF	-0.44	0.08022
CORN	-17.80	4.31387
ADJBF	-22.79	2.33461
n = 1620 F = 428.42 Adj. R <sup>2</sup> = 0.70		
<sup>1</sup> All variables significant (P<0.025)		

# EFFECT OF LIGHT TEST WEIGHT BARLEY VS. WHEAT MIDLINGS ON ADG AND CARCASS CHARACTERISTICS OF EARLY-WEANED CALVES<sup>1,2</sup>

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**ABSTRACT:** The objectives of this experiment were to compare the effects of starch-based (light test weight barley) vs. fiber-based (wheat middlings) diets on ADG and carcass characteristics of early-weaned calves. The calves were weaned at approximately 74 d of age (avg. wt. 39 kg) and adjusted to a ration containing 60% light test weight barley (17.25 kg·bu<sup>-1</sup>; **B**, n = 17) or 60% wheat middlings (**WM**, n = 18). The remainder of the diet was chopped grass hay, chopped barley hay, canola meal, and a protein/mineral supplement. At time of weaning, calves were weighed and measured for s.c. and i.m. fat and longissimus muscle area using ultrasound. Ultrasound measurements were repeated four times at approximately 28 d intervals. After 90 d, steers were placed on a similar growing ration for an additional 61 d and then transported to a commercial feedlot and fed a common finishing diet until harvest (382 d of age) when carcass data were collected. There were no differences in initial BW, ADG (two pens/treatment), longissimus muscle area, or s.c. or i.m. fat ( $P>0.05$ ). Calves fed **B** gained faster (1.29 kg·d<sup>-1</sup> vs. 0.99 kg·d<sup>-1</sup>;  $P=0.002$ ) and achieved higher marbling scores (4.44 vs. 3.31 % ether extractable fat;  $P=0.002$ ) compared to **WM**-fed calves during the first 34 d after weaning. Body weights after 34 d and 64 d remained greater for **B** ( $P=0.04$ ) and tended ( $P=0.08$ ) to be higher until 90 d. The ADG during the 90 d growing period continued to be greater ( $P<0.05$ ) for calves fed **B** (1.36 kg·d<sup>-1</sup>) compared to calves fed **WM** (1.24 kg·d<sup>-1</sup>). Calculated final live weights were only numerically greater ( $P=0.15$ ) for **B**-fed calves. By time of harvest, BW and i.m. fat differences were similar. Calves fed **B** initially deposited a greater amount of i.m. fat. However, after 217 d on a common diet these differences disappeared and steers achieved similar quality grades. These results suggest that in order to enhance i.m. fat deposition and possibly final carcass weight, it may be possible to do this early in the growth curve with grain based diets.

Key words: Early weaning, carcass, beef cattle

## Introduction

The response of early weaning of beef calves fed high concentrate diets based on corn grain has been well documented (Fluharty et al., 2000; Story et al., 2000;

Barker-Neef et al., 2001). Early-weaning has been practiced during periods of drought, in which forage availability was limited and energy requirements are greater for the cow due to lactation. Early-weaning has resulted in a greater percentage of calves that graded Choice or higher (Myers et al., 1999), heavier weight calves compared to normal weaning at 210 d of age (Fluharty et al., 2000), better feed conversions (Myers et al., 1999), resulting in a lower cost of gain (Barker-Neef et al., 2001), as well as a younger age at slaughter (Peterson et al., 1987; Makarechian et al., 1988).

Barley contains lesser amounts of dietary energy compared to corn because of increased protein and fiber content. Barley also exhibits a greater extent of ruminal starch digestion (Theurer, 1986; McCarthy et al., 1989; Overton et al., 1995) and any similarities in performance between the two grains is likely due to the more complete starch digestion from a higher microbial protein production (Alberta Feedlot Management Guide, 2000).

Wheat middlings comprise a major portion of the by-product from flour milled in the United States. The nutrient content of wheat middlings can be highly variable (Dalke et al., 1997), but wheat middlings in high concentrate diets may improve nutrient availability when fed at restricted levels (Hermesmeier et al., 2002).

The objectives of this experiment were to determine the effects of feeding a high starch (light test weight barley-based) diet vs. a high fiber (wheat midds-based) diet on carcass characteristics of early weaned calves. We hypothesized that differences in animal performance and carcass characteristics may exist between diets.

## Materials and Methods

This study was conducted at the E.L. Peterson Ranch in Judith Gap, Montana. Steer and heifer calves were born between February and April and nursed their dams while grazing native range. Forty-seven calves of similar genetics were weaned on May 29, 2002 from cows that were labeled as culls by the ranch. The calves were weaned at approximately 74 ± 16 d of age. Calves were randomly assigned to one of two treatments testing the effects of high starch (**B**; 17.25 kg·bu<sup>-1</sup>) vs. high fiber (**WM**) diets on ADG and carcass characteristics. Treatment effects were measured over a 90 d backgrounding period. Two pens per treatment were allocated and steers and heifers were mixed within pen. There were two pens of **B** calves (pen 1; n = 9 steers, 3 heifers; pen 2; n = 8 steers, 3

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heifers) and two pens of early-weaned calves fed WM (pen 1; n = 9 steers, 3 heifers; pen 2; n = 9 steers, 3 heifers).

**Growing Phase** Calves were given ad libitum access to their respective diets (Table 1), were fed at approximately 0700 h daily. Feed samples were collected every 7 d, dried in a forced-air oven (60°C) for 72 h, ground in a Wiley mill to pass a 1-mm screen and composited (DM basis). Feed samples were analyzed for DM and N according to AOAC (2000) methods, and Ca and P were determined using inductively coupled argon plasma methods (Fassel 1978).

Steer calves were implanted with Ralgro (36 mg zeranol) at approximately 50 d of age (branding) and again at approximately 155 d of age (preconditioning). At the time of early-weaning all calves were weighed, and ultrasound was used to determine s.c. and i.m. fat and longissimus muscle area at the 12<sup>th</sup> rib. Ultrasound measurements were recorded at four time points at approximately 28 d intervals. Measurements were taken beginning with the day of weaning and then 34, 64, and 90 d post-weaning. Real time ultrasound measurements (RTU) were conducted using a Classic Medical Scanner 200 SLC veterinary ultrasound system (Classic Medical, Tequesta, FL) attached with an ASP-18 probe; 18 cm, 3.5-MHz. Digitized images were interpreted using Scanner 200 SLC (Classic Medical, Tequesta, FL) image analysis software. Subcutaneous fat thickness was measured at the 12<sup>th</sup> to 13<sup>th</sup> rib interface over the longissimus muscle. The perimeter of the longissimus muscle was traced from the digitized image and muscle area was computed by the software. Real time ultrasound images for s.c. and i.m. fat and longissimus muscle area were taken and interpreted by a single technician at all four time points. All individual weights were taken without withdrawal from feed and water. Only steers were included in the analysis because all heifer calves were retained as replacements, thus carcass data could not be collected.

Steers were shipped to a commercial feedyard (High Gain Feeders, Cozad, NE) 61 d following the end of data collection and were fed a finishing diet for  $156 \pm 5$  d, (Table 2). Steers were processed at a commercial packing plant. Carcass data were collected 24 to 40 h postmortem by a USDA grader.

**Statistical Analysis** Data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) for a completely randomized design. The model included effects due to diet, (B vs. WM), birth date, weaning status, days on feed, harvest date, and yield grade. Effects due to time (performance from 75 d of age to 165 d of age), the weaning status x time, longissimus muscle area x time, s.c. and i.m. fat x time interactions were analyzed using the MIXED procedures of SAS (Littell et al., 1998). Carcass marbling scores were calculated to estimate a percent of ether extractable fat (Savell et al., 1986). Differences in means were determined using the LSD procedures of SAS (SAS Inst., 2003). Animal was the experimental unit and differences were found to be significant at  $P < 0.05$ .

## Results and Discussion

There was no morbidity or mortality from the time of weaning until the calves were sent to the commercial feedlot. Period 1 began at weaning and lasted for 34 d. There were no differences in initial body weights, daily gains from birth to weaning, longissimus muscle area, or s.c. or i.m. fat ( $P > 0.05$ ).

During this first period B calves achieved higher weight gain and marbling scores (4.44 vs. 3.31 %EEF;  $P = 0.002$ ) compared to WM calves (Figures 1&2). These results agree with Prior (1983) who suggested that higher starch diets for cattle will increase marbling score. Our observations are also consistent with Cianzio et al., (1982) who found that lower s.c. fat does not necessarily predict reduced marbling scores, and that both hypertrophy and hyperplasia may be occurring post-weaning (Cianzio et al., 1985). As marbling score increased over the first period, s.c. fat remained similar for B vs. WM-fed calves (0.15 vs. 0.15 cm,  $P = 0.89$ ). After 64 d post-weaning B calves exhibited a significantly greater amount of s.c. fat compared to WM-fed calves (0.51 vs. 0.15 cm,  $P < 0.01$ ), with no differences being detected at 90 d post weaning or at harvest. Although statistical differences were not measured for s.c. fat throughout other periods of the feeding phase or i.m. fat at harvest, numerical differences did occur. Interestingly, it appeared that B-fed calves required increasing levels of energy as suggested by the initial increase in i.m. fat with no increase in the calculated estimate, % EEF beyond 34 d post-weaning. This speculation is supported by Fluharty et al., (2000) as carcass fat percentage was numerically greater for early-weaned calves fed 90 and 100% concentrate diets vs. those fed 60% concentrate diets. Although no differences existed between dietary treatments in the present study, calculated final yield grades were similar to those reported by Fluharty, et al., (2000) for early-weaned calves fed 60% concentrate diets (3.32 vs. 3.5).

Barley calves gained faster ( $1.29 \text{ kg} \cdot \text{d}^{-1}$  vs.  $0.99 \text{ kg} \cdot \text{d}^{-1}$ ;  $P = 0.002$ ) during the first period and consequently BW at 34 d and 64 d post-weaning were significantly greater ( $P = 0.04$ ) and were numerically greater ( $P = 0.08$ ) until 90 d post weaning (34 d = 171 kg vs. 154 kg; 64 d = 203 kg vs. 184 kg; 90 d = 250 kg vs. 233 kg; Figure 1).

Gains for the entire 90 d growing period were significant (B =  $1.36 \text{ kg} \cdot \text{d}^{-1}$  vs. WM =  $1.24 \text{ kg} \cdot \text{d}^{-1}$ ;  $P = 0.045$ ). The B calves did deposit more i.m. fat from the time of weaning until 34 d post-weaning and retained this until transported to the finishing lot (4.44 vs. 3.31; % ether extractable fat). Calculated final live weights were only numerically greater for B compared to WM. However, by time of harvest, WM calves caught up and differences were nonsignificant (Figure 2). Dalke et al., (1997) suggested that WM could only replace up to 5% of dry rolled corn in high concentrate diets. The WM-fed calves appeared to have exhibited compensatory gain following an extended backgrounding period (151 d, first 90 d measured), thus these data imply that WM were as effective as light test weight barley in terms of providing similar performance.

## Implications

These data do not explain why B calves initially deposited a greater amount of i.m. fat even though diets had similar NE content, yet harvested with similar quality grades as WM calves. It would appear that in order to optimize the advantages of early weaning, calves should be fed increasing amounts of energy in order to maximize rates and efficiencies of gain, attain higher quality grades and be harvested at a younger age. Despite similarities in final weights and carcass characteristics, numerical differences in weight gain, carcass traits, and growth curves suggest that further research be focused on feeding regimens to determine if diet manipulation early in the growth curve can positively enhance carcass composition.

## Literature Cited

- Alberta Feedlot Mgmt Guide. 2000. Section 1. Nutrition and Management. 2<sup>nd</sup> ed. 1A1:1-1B1-11.
- AOAC. 2000. Official Methods of Analysis. 17<sup>th</sup> ed. Assoc. of Official Analytical Chemists, Washington, DC.
- Arthington, J. D., and R. S. Kalmbacher. 2003. Effect of early weaning on the performance of three-year-old, first-calf beef heifers and calves reared in the subtropics. *J. Anim. Sci.* 81:1136-1141.
- Barker-Neef, J. M., D. D. Buskirk, J. R. Black, M. E. Doumit, and S. R. Rust. 2001. Biological and economic performance of early-weaned Angus steers. *J. Anim. Sci.* 79:2762-2769.
- Cianzio, D. S., D. G. Topel, G. B. Whitehurst, D. C. Beitz and H. L. Self. 1982. Adipose tissue growth in cattle representing two frame sizes: Distribution among depots. *J. Anim. Sci.* 55:305-312.
- Cianzio, D. S., D. G. Topel, G. B. Whitehurst, D. C. Beitz and H. L. Self. 1985. Adipose tissue growth and cellularity: Changes in bovine adipocyte size and number. *J. Anim. Sci.* 60:970-976.
- Dalke, B. S., R. N. Sonon, Jr., M. A. Young, G. L. Huck, K. K. Kreikemeier, and K. K. Bolsen. 1997. Wheat middlings in high-concentrate diets: Feedlot performance, carcass characteristics, nutrient digestibilities, passage rates, and ruminal metabolism in finishing steers. *J. Anim. Sci.* 75:2561-2566.
- Fassell, V. A. 1978. Quantitative elemental analyses by plasma emission spectroscopy. *Science*. 202:183.
- Fluharty, F. L., S. C. Loerch, T. B. Turner, S. J. Moeller, and G. D. Lowe. 2000. Effects of weaning age and diet on growth and carcass characteristics in steers. *J. Anim. Sci.* 78:1759-1767.
- Hermesmeier, G. N., L. L. Berger, N. R. Merchen, and T. G. Nash. 2002. Effects of restricted and ad libitum intake of diets containing wheat middlings on site and extent of digestion in steers. *J. Anim. Sci.* 80:812-817.
- Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76:1216-1231.
- Makarechian, M., H. M. Kubisch, and M. A. Price. 1988. Effects of date of weaning on subsequent performance of beef cows and their female calves. *Can. J. Anim. Sci.* 68:1035-1040.
- McCarthy, R. D., Jr., T. H. Klusmeyer, J. L. Vicini, J. H. Clark, and D. R. Nelson. 1989. Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. *J. Dairy Sci.* 72:2002-2016.
- Myers, S. E., D. B. Faulkner, F. A. Ireland, L. L. Berger, and D. F. Parrett. 1999. Production systems comparing early weaning to normal weaning with or without creep feeding for beef steers. *J. Anim. Sci.* 77:300-310.
- Overton, T. R., M. R. Cameron, J. P. Elliott, J. H. Clark, and D. R. Nelson. 1995. Ruminal fermentation and passage of nutrients to the duodenum of lactating cows fed mixtures of corn and barley. *J. Dairy Sci.* 78:1981-1998.
- Peterson, G. A., T. B. Turner, K. M. Irvin, M. E. Davis, H. W. Newland, and W. R. Harvey. 1987. Cow and calf performance and economic considerations of early weaning of fall-born beef calves. *J. Anim. Sci.* 64:15-22.
- Prior, R. L. 1983. Lipogenesis and adipose tissue cellularity in steers switched from alfalfa hay to high concentrate diets. *J. Anim. Sci.* 56:483-492.
- Savell, J. W., H. R. Cross, and G. C. Smith. 1986. Percentage ether extractable fat and moisture content of beef longissimus muscle as related to USDA marbling score. *J. Food Sci.* 51:838.
- Story, C. E., R. J. Rasby, R. T. Clark, and C. T. Milton. 2000. Age of calf at weaning of spring-calving beef cows and the effect on cow and calf performance and production economics. *J. Anim. Sci.* 78:1403-1413.
- Theurer, C. B. 1986. Grain processing effects on starch utilization by ruminants. *J. Anim. Sci.* 1649-1662.

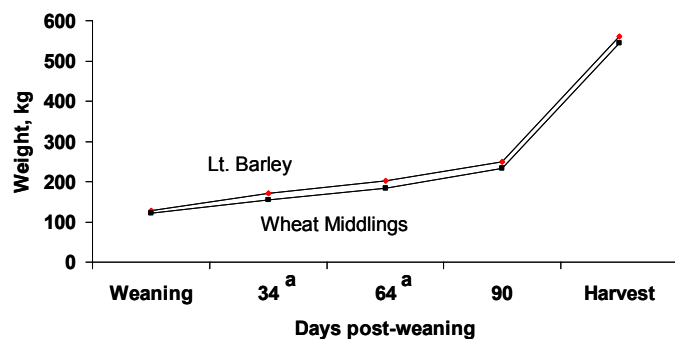
**Table 1. Ingredient and nutrient composition of growing diets fed to early-weaned calves immediately placed in the drylot (% of DM)**

Item	Lt. Barley	Wheat Middlings
Ingredient, % DM basis		
Lt. Barley (17.25 kg·bu <sup>-1</sup> )	57.7	--
Wheat Middlings	--	57.7
Barley Hay	15.4	15.4
Grass Hay	15.4	15.4
Canola Meal	3.8	3.8
Wean Pellet	7.7	7.7
Nutrient Analysis		
CP, %	15.5	15.8
Ca, %	1.32	1.42
P, %	0.64	0.37
NE <sub>m</sub> , Mcal·kg <sup>-1</sup>	1.83	1.78
NE <sub>g</sub> , Mca·kg <sup>-1</sup>	1.19	1.17

**Table 2. Ration composition of the finishing diet (as fed) (High Gain feeders, Cozad, NE)**

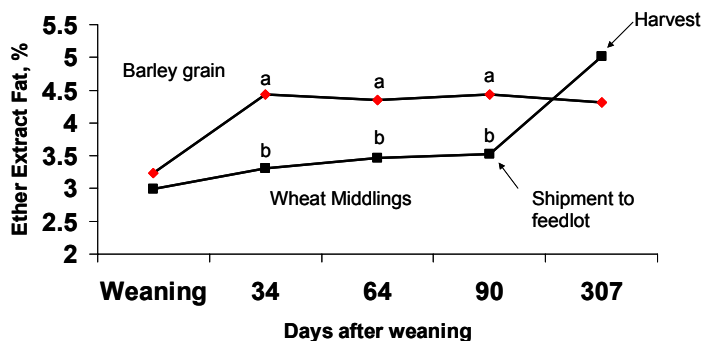
Item	%, As fed
Flaked Corn	60.19
Alfalfa	7.29
Rolled Corn	12.26
Tallow	2.31
Liquid Supplement	6.32
Dry Supplement	1.50
Corn Steep	10.13

**Figure 1.**  
**Change in BW for early weaned steers fed**  
**Lt. Barley or Wheat Middlings**



<sup>a</sup>Differences in BW post-weaning ( $P < 0.05$ )

**Figure 2.**  
**Effect of Barley Grain vs Wheat Midds on**  
**Changes in Intramuscular Fat of Early**  
**Weaned Calves**



<sup>ab</sup>Means without a common superscript letter differ ( $P < 0.05$ )

## **FREQUENT RINSING AND CLEANING OF DRINKING WATER VESSELS IMPROVED THE PERFORMANCE OF HUTCH-RAISED HOLSTEIN CALVES**

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**ABSTRACT:** It is a common observation on commercial dairies that the water vessels of hutch-raised calves are not cleaned and rinsed with enough frequency. Due to the relationship between dry matter intake and water intake, low water quality in the hutch could reduce feed intake and daily gain. The objective of this study was to compare the performance of hutch-raised calves when water vessels were cleaned and rinsed with decreasing frequency. For three consecutive years, 24 Holstein bull calves (2 - 7 d of age) were purchased from a commercial dairy. Calves were purchased in four sets of six calves each in September, December, March and June of each year. Calves remained in the hutches for 60-d during which time they received milk replacer twice daily. A concentrate mix was available at all time. Of the six calves in each set two had their water vessels cleaned and rinsed daily, two were cleaned each 7-d, and two were cleaned each 14-d. Average daily gain (ADG) of the calves were measured during the pre-weaning period while in the hutches and during the post-weaning period when the sets of calves were quartered together until they reached sale weight (227 kg). The frequency of cleaning and rinsing of water vessels had a major impact on ADG of calves while in the hutches: daily, 0.71 kg/d; 7 d, 0.67 kg/d; 14 d, 0.63 kg/d. All means were different, ( $P < 0.05$ ). These differences in ADG carried over through the post-weaning period: daily, 1.42 kg/d; 7-d, 1.37 kg/d; 14-d, 1.32 kg/d. The daily and 14-d treatment means were different ( $P < 0.05$ ). The 7-d treatment mean was intermediate. Thus, a minor management practice such as frequently cleaning and rinsing of water vessels in calf hutches can have a major impact on the ADG of calves.

**Key Words:** Calf performance, Drinking Water, Quality

### **Introduction**

Those involved in livestock production have often observed the relationship between drinking water intake, dry matter intake, and performance. Recent studies and popular press articles have reported negative production responses and/or compromised health status when water high in dissolved solids or pollutants was used for livestock production. Patterson et al. (2003) reported poor performance and an increased incidence of polioencephalomalacia when high sulfate water was used for feedlot cattle. Similarly, Vetter (2003) reported poor range cattle performance when the concentration of dissolved solids and microbial pollutants in drinking water was increased due to drought conditions. Hutches

commonly used for the rearing of Holstein calves have individual drinking water vessels. It is a common observation that these drinking water vessels are infrequently rinsed and cleaned. It was the objective of this study to determine if the frequency of rinsing and cleaning of drinking water vessels in calf hutches would affect the performance of the calves.

### **Materials and Methods**

Twenty-four Holstein bull calves ages two to seven days were purchased from a commercial dairy for each of three years (72 calves). Calves were purchased each year in four sets of six calves each the first week of September, December, March, and June. Calves were reared in individual polyethylene hutches for 60 days after purchase. Commercial milk replacer was fed as recommended twice per day at 0500 and 1700. Each hutch was equipped with two feeding vessels, one for feed and one for water. A concentrate mixture (Table 1) and water were available to the calves at all times. Two calves in each group of six were randomly assigned to one of three drinking water treatments: (1) cleaned and rinsed daily, (2) cleaned and rinsed each 7-d, (3) cleaned and rinsed each 14-d. Any drinking water vessel contaminated with fecal material was cleaned and rinsed immediately. Otherwise cleaning and rinsing of drinking water vessels was as assigned above. Hutches were heavily bedded with wood shaving initially. Small amounts of shavings were added thereafter to keep hutches clean.

All calves were weaned after 60-d in the hutches. One week prior to weaning calves precondition to weaning. Calves were castrated using the elastrator system, hot-iron dehorned, and vaccinated for Clostridial and respiratory diseases. These vaccinations were boosted as recommended by manufacturer. After weaning the six calves in each group were placed in a common pen and remained together as a group until sold at 150 to 170-d of age. During this post-weaning period calves were offered a choice of alfalfa hay (18.0% CP, 48% NDF, 60% TDN) and a concentrate mix (Table 2). Drinking water was offered through a polyethylene tank that was cleaned and rinsed on a weekly basis. Calves were weighed as they were placed in the hutches, at weaning and when they were sold.

Data were analyzed using the Proc Mixed procedure in SAS (SAS Inst. Inc., 1996, Cary, NC) with average daily

gain as the dependent variable and treatment, season and treatment by season interaction as the independent variables. Year effect was not included as part of the model. Multiple comparisons were made with P-values adjusted using Tukeys procedure. A  $P < 0.05$  was considered significant.

## Results and Discussion

The effect of frequency of cleaning and rinsing water vessels on daily gain of calves from birth to weaning while in hutches is presented in Table 3. Although the differences in ADG are not of great magnitude, the frequency of cleaning of drinking water vessels in calf rearing hutches had a consistent affect on daily gains. Daily rinsing/cleaning resulted in a 4.5% and 9.4% improvement in ADG compared to rinsing/cleaning at 7-d and 14-d intervals, respectively. The longer the interval between rinsing/cleaning resulted in lower ADG. It would not be unreasonable to assume that if the intervals between rinsing/cleaning used in this study had been longer the effect on ADG would have been of greater magnitude.

Season also affected the ADG of the calves. Calves started in the fall and spring exhibited higher ADG during the hutch-rearing period than those started during the summer or winter. Lower ADG during the winter can be explained by higher maintenance energy costs associated with cold weather. High-quality drinking water would likely be of most importance during the hot summer months. Drinking water in vessels in hutches would be more likely to become contaminated with algae, etc., during the summer months if left in the vessels for extended periods without rinsing/cleaning. Thus the lower ADG exhibited by calves started in the summer may have been due at least in part to drinking water quality.

Drinking water management and ADG during the 60-d hutch-rearing period carried over into the 100 to 110-d post-weaning feeding period (Table 4). During this period calves were placed in a common pen with ad libitum alfalfa hay and concentrate mix (Table 2). Calves drank from a common water tank that was rinsed/cleaned on a weekly basis.

Even though management of all calves was identical during the post-weaning period, calves that had drinking water vessels rinsed and cleaned daily during the 60-d hutch rearing period gained weight at a more rapid rate (7.5%) than those who's waterers were rinsed and cleaned each 14-d. There was no difference in the ADG of calves that had waterer's service daily or each 7-d. Seasonal effects were also apparent during the post-weaning period. Calves that were started during the summer gained weight at a less rapid rate than those started in the fall (89.7%) or spring (90.8%). Performance of calves started during the summer and winter was similar. Water quality during hot summer months where water was

allowed to stagnate in drinking water vessels had long-term negative effects on calf performance.

Performance of the calves' birth to sale weight (160 to 170-d of age) is summarized in Table 5. Daily rinsing/cleaning of drinking water vessels during the 60-d hutch-rearing period resulted in a 7.5% increase in ADG during the entire 160 to 170-d feeding period compared to rinsing/cleaning drinking water vessels each 14-d. There was no difference in the ADG of calves having the drinking water vessels rinsed/cleaned daily or each 7-d. Thus, a simple management practice such as frequent rinsing/cleaning of drinking water vessels during the hutch-rearing phase of dairy calf production can have positive effects of performance during an extended period during the production calendar. Calves started during the summer season were the most sensitive to frequency of rinsing/cleaning drinking water vessels. Calves started during the summer and had drinking water vessels rinsed/cleaned each 14-d gained weight at a rate only 80% that of calves started in the fall or spring with drinking water vessels rinsed/cleaned daily.

## Implications

Poor management of drinking water in hutch-raised dairy calves where drinking water is offered in vessels such as bucket, etc., results in reduced ADG. Rinsing/cleaning drinking water vessels daily versus each 14-d during a 60-d hutch-rearing period resulted in a 7.5% improvement in ADG extending through an entire 160 to 170-d feeding period. Effects of drinking water quality are more pronounced in calves started during hot summer months.

## Literature Cited

- Patterson, H.H., P.S. Johnson and W.B. Epperson. 2003. Effects of total dissolved solids and sulfates in drinking water for growing steers. *Proc. West. Sect. J. Anim. Sci.* 54:378-381.
- Vetter, S.D. 2003. Stockwater quality a concern during drought. *Western Livestock Journal*, September 8, 2003. p.9.

Table 1. Ingredient and Nutrient Composition of Concentrate Mix Offered Calves While in Hutches

Ingredient	% As-Fed
Dry-rolled barley	81.77
Soybean meal (44%)	8.25
Corn silage	6.22
Crushed limestone	1.60
Yeast culture	0.70
Mineral premix <sup>a</sup>	0.46
Vitamin premix <sup>b</sup>	0.40
Salt	0.31
Dicalcium phosphate	0.27
Rumensin 80 <sup>c</sup>	0.03
Nutrient	Concentration in DM
Crude protein, %	16.00
NE <sub>m</sub> Mcal/kg	1.98
NE <sub>g</sub> Mcal/kg	1.34
Calcium, %	0.78
Phosphorus, %	0.45
Vitamin A, kiu/kg	5.18
Vitamin D, kiu/kg	0.52
Vitamin E, iu/kg	31.06
Dry matter, %	85.00

<sup>a</sup>Zinc, 6000 mg/kg; manganese, 5000 mg/kg; copper, 2000 mg/kg; iodine, 200 mg/kg; selenium, 50 mg/kg; cobalt, 50 mg/kg

<sup>b</sup>Vitamin A, 1100 kiu/kg; vitamin D, 110 kiu/kg; vitamin E, 6600 iu/kg

<sup>c</sup>Monensin, 176 g/kg

Table 2. Ingredient and Nutrient Composition of Concentrate Mix Offered to Calves Post-weaning

Ingredient	% As-Fed
Dry-rolled barley	77.12
Corn silage	15.27
Soybean meal (44%)	3.55
Crushed limestone	1.44
Yeast culture	0.70
Mineral premix <sup>a</sup>	0.48
Vitamin premix <sup>b</sup>	0.48
Dynamate <sup>c</sup>	0.36
Salt	0.31
Dicalcium phosphate	0.27
Rumensin 80 <sup>d</sup>	0.03
Nutrient	Concentration in DM
Crude protein, %	14.00
NE <sub>m</sub> Mcal/kg	1.95
NE <sub>g</sub> Mcal/kg	1.32
Calcium, %	0.75
Phosphorus, %	0.42
Vitamin A, kiu/kg	6.60
Vitamin D, kiu/kg	0.66
Vitamin E, iu/kg	39.60
Dry matter, %	80.00

<sup>a</sup>Zinc, 6000 mg/kg; manganese, 5000 mg/kg; copper, 2000 mg/kg; iodine, 200 mg/kg; selenium, 50 mg/kg; cobalt, 50 mg/kg

<sup>b</sup>Vitamin A, 1100 kiu/kg; vitamin D, 110 kiu/kg; vitamin E, 6600 iu/kg

<sup>c</sup>Magnesium, 11%; potassium, 18%; sulfur, 22%

<sup>d</sup>Monensin, 176 g/kg

Table 3. Average Daily Gain of Holstein bull calves from birth to weaning (60 days) as affected by frequency of rinsing and cleaning drinking water vessels

Season	Frequency of cleaning and rinsing drinking water vessels			
	Daily	7-days	14-days	Mean
----- Daily gain, kg -----				
Summer	0.65	0.64	0.61	0.63 <sup>z</sup>
Fall	0.75	0.70	0.65	0.70 <sup>x</sup>
Winter	0.68	0.65	0.64	0.66 <sup>yz</sup>
Spring	0.75	0.70	0.64	0.70 <sup>xy</sup>
Mean	0.70 <sup>a</sup>	0.67 <sup>b</sup>	0.64 <sup>c</sup>	

<sup>abc</sup>Column effects, frequency of cleaning drinking water vessels. Means in the same row with different superscripts differ,  $P < 0.05$

<sup>xyz</sup>Row effects, season. Means in the same column with different superscripts differ,  $P < 0.05$

Table 4. Average daily gain of Holstein calves from weaning (60 days) until sale weight (160-170 days) as affected by frequency of rinsing and cleaning of drinking water vessels during the 60-day hutch-rearing period

Season	Frequency of cleaning/rinsing drinking water vessels during the 60-day hutch rearing period			
	Daily	7-days	14-days	Mean
----- Daily gain, kg -----				
Summer	1.37	1.24	1.17	1.26 <sup>z</sup>
Fall	1.50	1.45	1.41	1.45 <sup>x</sup>
Winter	1.35	1.39	1.36	1.36 <sup>y</sup>
Spring	1.46	1.40	1.33	1.40 <sup>xy</sup>
Mean	1.42 <sup>a</sup>	1.37 <sup>ab</sup>	1.32 <sup>b</sup>	

<sup>ab</sup>Column effects, frequency of cleaning drinking water vessels during the 60-day hutch-rearing period. Means in the same row with different superscripts differ,  $P < 0.05$

<sup>xyz</sup>Row effects, season. Means in the same column with different superscripts differ,  $P < 0.05$

Table 5. Average daily gain of Holstein calves from birth to sale weight (160-170 days of age) as affected by frequency of rinsing and cleaning drinking water vessels during the 60-day hutch-rearing period

Season	Frequency of cleaning and rinsing drinking water vessels			
	Daily	7-days	14-days	Mean
----- Daily gain, kg -----				
Summer	1.07	1.02	0.97	1.02 <sup>z</sup>
Fall	1.22	1.17	1.13	1.17 <sup>x</sup>
Winter	1.09	1.10	1.09	1.09 <sup>y</sup>
Spring	1.20	1.15	1.08	1.15 <sup>x</sup>
Mean	1.15 <sup>a</sup>	1.11 <sup>a</sup>	1.07 <sup>b</sup>	

<sup>ab</sup>Column effects, frequency of cleaning drinking water vessels. Means in the same row with different superscripts differ,  $P < 0.05$

<sup>xyz</sup>Row effects, season. Means in the same column with different superscripts differ,  $P < 0.05$

# EFFECTS OF IMPLANTS ON LIVE PERFORMANCE, CARCASS CHARACTERISTICS, AND SERUM CONCENTRATIONS OF GLUCOSE AND NEFA FOR LONG-FED HOLSTEIN STEERS.

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**ABSTRACT:** Our objective was to evaluate the effects of increasingly aggressive implant programs for long-fed Holstein steers on performance, carcass characteristics, and serum metabolite concentrations. Nineteen Holstein steers (initial BW = 182 kg) were randomly assigned to one of four implant treatment regimens including control (no implant; n = 5); 36 mg zeranol (Ralgro) on d 0, 20 mg estradiol benzoate plus 200 mg progesterone (Synovex S) on d 84 and 168 (RSS; n = 5); Ralgro on d 0, Synovex S on d 84, and 28 mg estradiol benzoate plus 200 mg trenbolone acetate (Synovex Plus) on d 168 (RSP; n = 5); and Ralgro on d 0, Synovex Plus on d 84 and 168 (RPP; n = 4). Steers were individually fed an 85% concentrate steam-flaked corn based diet for 259 d. As expected, implanted steers had greater ADG ( $P < 0.01$ ), increased DMI ( $P < 0.01$ ), but no differences in G:F ( $P = 0.40$ ). As a result, implanted steers had heavier final BW ( $P < 0.01$ ), heavier HCW ( $P < 0.01$ ), and larger LM area ( $P < 0.01$ ). In addition, implanted steers had a slightly darker coloration ( $P < 0.08$ ) vs. control steers. No differences among treatments were observed in dressing percentage ( $P > 0.52$ ), kidney pelvic and heart fat ( $P > 0.20$ ), back fat ( $P > 0.54$ ), yield grade ( $P > 0.46$ ), marbling score ( $P > 0.35$ ), texture ( $P > 0.27$ ), firmness ( $P > 0.34$ ), or Warner-Bratzler shear force values ( $P > 0.32$ ). Averaged across sampling d, serum concentrations of glucose ( $P > 0.23$ ), NEFA ( $P > 0.13$ ) and serum urea nitrogen (SUN:  $P > 0.21$ ) did not differ among treatments. Overall, the implant programs evaluated enhanced performance, carcass weight and LM area, did not affect adipose deposition, and thus, did not adversely affect carcass characteristics in long-fed Holstein steers. No responses in serum concentrations of glucose, NEFA, or SUN were detected.

**Key Words:** Carcass Characteristics, Holstein Steers, Implants, NEFA, Glucose, Serum Urea Nitrogen

## Introduction

Numbers of Holstein steers fed out for beef in feedlots today are greater than ever before in Arizona and the southwest United States. Their benefits in uniformity, live performance, and carcass value has out-weighed their deficits in carcass characteristics and maintenance costs. It is widely recognized that carcasses from Holstein steers have smaller LM area and an altered shape compared with carcasses from traditional beef breeds. Research is now focusing on management opportunities to reduce these

deficits; thereby, enhancing the productivity of Holsteins in the beef industry. One management option available is anabolic implants. Most of the previous research with implants has focused on conventional beef breeds. Using anabolic implants increases performance, LM area, and hot carcass weight (Scheffler et al., 2003, Perry et al., 1991). However little research has focused on the affects of different implant strategies on Holstein steers. Therefore the focus of this study was to examine the effects of progressively intensive implant programs on performance, carcass characteristics, serum metabolites and the tenderness of the final product in Holstein steers.

## Materials and Methods

*Cattle Management and Experimental Treatments.* Nineteen Holstein steers ( $190 \pm 22$  kg) were purchased from an order buyer and delivered to the University of Arizona Feedlot (Tucson) in March 2003. Upon arrival, steers were treated for internal and external parasites (Ivomec; Merial, GA), vaccinated with infectious bovine rhinotracheitis, parainfluenza<sub>3</sub>, bovine viral diarrhea, bovine respiratory syncytial virus plus pasteurella haemolytica (Pyramid 4 + Presponse SQ, Fort Dodge Animal Health, Fort Dodge, IA) and a clostridial preparation (Ultra Choice 8; Pfizer Animal Health, PA), dehorned as necessary, and tagged for individual identification. Steers were allowed a 14-d adaptation period and worked up to an 86% concentrate diet (Table 1). Steers were randomly assigned to one of four treatments including: no implant (**CON**, n = 5); implanted with 36 mg of zeranol (Ralgro; Schering-Plough Animal Health, Union, NJ) on d 0 followed by an implant of 20 mg of estradiol benzoate + 200 mg of progesterone (Synovex-S; Ft. Dodge Animal Health) d 84 and 168 (**RSS**, n = 5); implanted with Ralgro on d 0 followed by an implant of Synovex-S on d 84 and 28 mg estradiol benzoate + 100 mg trenbolone acetate (Synovex Plus; Ft. Dodge Animal Health) on d 168 (**RSP**, n = 5); or an implant with Ralgro on d 0 followed by an implant with Synovex-Plus on d 84 and 168 (**RPP**, n = 4). Steers were then randomly allocated to individual, partially shaded, soil-surfaced pens (6 x 2.5 m) with an individual water source and feed bunk. Steers were monitored for daily feed intake with orts and feed samples collected weekly. Feed and ort samples were then subjected to DM analysis (oven drying at 55°C until no further weight loss), and feed samples were ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 1 mm

screen and subjected to CP (% N  $\times$  6.25; LECO Corporation, St. Joseph, MI 49085), ADF (ANKOM Technol. Corp., Fairport, NY) and ash (combusted 6-h in muffle furnace at 500°C). Steers were weighed and blood was collected prior to feeding via jugular puncture every 28 d. Venous blood was centrifuged at 1,000  $\times$  g for 20-min and serum stored (-20 °C) for later analysis. Serum urea nitrogen (SUN) concentration (TECO Diagnostics, Anaheim, CA 92807), glucose (Glucose C2, Wako Chemicals, Richmond, VA), and NEFA (NEFA C, Wako Chemicals, Richmond, VA) were determined using colorimetric methods.

**Carcass Data Collection.** On d 259 steers were humanely harvested via exsanguination at the University of Arizona Meat Laboratory and blood samples were collected. Hot carcass weight (HCW) was recorded and LM area, backfat thickness, kidney pelvic and heart fat % (KPH), firmness, color, texture and yield grade and marbling score, were collected (USDA, 1997). Carcasses were processed and 2.54 cm steaks were cut for Warner-Bratzler Shear Force value analysis. Steaks were labeled and allowed to thaw to room temperature. Steaks were cooked on an open-hearth grill until internal temperature reached 35°C then turned and allowed to continue cooking until internal temperature reached 70°C. Steaks were allowed to cool to room temperature and eight, 2.54 cm cores were removed and tested for shear force values. High and low scores were eliminated and the remaining six were used to calculate a mean value.

**Statistical Analysis.** All data sets were statistically analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). For performance and carcass characteristics, the model included effects for treatment. Steer within treatment was used as the random effect. Serum metabolites were analyzed with repeated measures. Preplanned contrasts included 1) control vs. the average for implants, 2) RSS vs. the average of RSP and RPP, and 3) RSP vs. RPP.

## Results and Discussion

**Live Performance.** As expected, implanted cattle outperformed non-implanted controls. Final BW ( $P < 0.01$ ), ADG ( $P < 0.01$ ), and DMI ( $P < 0.01$ ) were greater for implanted steers than for control steers; however, no treatment differences ( $P > 0.54$ ) were observed between the various implant strategies (Table 2). Gain:feed did not differ between implanted and control steers or as a result of implant strategy ( $P > 0.20$ ). Implants increased ADG by approximately 23% and daily DMI by 18%. Our results are in agreement with Samber et al. (1996) on the effects of implants vs. nonimplanted steers. However, we were somewhat surprised that the combination implants did not improve performance over the progesterone-estradiol implant. Montgomery et al. (2001) reported that combination implant programs increased feeding performance greater than estrogen based implants.

**Carcass Characteristics.** All steers harvested fell into the “A” maturity category and there were no instances of dark cutters observed. No differences ( $P > 0.10$ ) were observed between treatments for KPH, backfat thickness, yield grade, dressing percentage, texture or firmness scores (Table 2). Likewise, no differences were observed for marbling score ( $P > 0.18$ ), as a result of implanting or implant strategy. Darker coloration scores ( $P < 0.08$ ) were observed for implanted steers compared with control steers; however, no differences were observed as a result of implant strategy ( $P > 0.29$ ). No differences ( $P > 0.33$ ) were observed in Warner-Bratzler Shear Force values for implanted steers compared to control steers or as a result of implant strategy. Nichols et al. (1996) reviewed 19 publications and found no deleterious effects on Warner-Bratzler Shear Force values between implanted and nonimplanted cattle when multiple treatments were examined. However, our results are in contrast to previous studies, which focused on the adverse effects of implants on meat tenderness (Samber et al., 1996, Roeber et al., 2000). These authors reported that anabolic growth promotants have unfavorable effects on meat tenderness, shear force values, and beef grade factors. Hot carcass weight ( $P < 0.01$ ) and LM area ( $P < 0.01$ ) were increased in implanted steers compared to controls, but no differences ( $P > 0.57$ ) were observed as a result of implant strategy. This is supported by previous studies, which concluded that implants have a marked enhancement on carcass weight and LM area (Hermesmeier et al., 2000, Roeber et al., 2000).

**Serum Metabolite.** No differences ( $P > 0.13$ ; Table 2) were observed for SUN, glucose, or NEFA concentrations in implanted steers compared to control steers or as a result of implant strategy. The inter- and intra-assay coefficients were 4.8, 4.9; 11.2, 11.2; and 10.2, 10.2 for glucose, NEFA, and SUN, respectively. It is not surprising that NEFA and SUN levels were similar. Animals were in a well-fed state depositing both adipose and muscle tissues. However, we hypothesized there may be differences in NEFA and SUN because of altered rates of protein metabolism and possible lipid metabolism between the various implant strategies used in our experiment.

In conclusion implants increased live animal performance by increasing DMI, ADG, and Final BW. Carcass characteristics were positively influenced by increasing HCW and enlarging LM area. Coloration was slightly darker, but not the extent of being labeled a dark cutter. No differences were noted for serum metabolites.

## Literature Cited

- Hermesmeier, G.N., L.L. Berger, T.G. Nash, and R.T. Brandt, Jr. 2000. Effects of energy intake, implantation, and subcutaneous fat end point on feedlot steer performance and carcass composition. *J. Anim. Sci.* 78:825-831.
- Montgomery, T.H., Dew, P.F., and M.S. Brown. 2001. Optimizing carcass value and the use of anabolic

implants in beef cattle. J. Anim. Sci. 79 (E. Suppl.):E296-E306.

Nichols, W.T., M.L. Galyean, D.U. Thomson, and J.P. Hutcheson. 2002. Review: Effects of steroid implants on the tenderness of beef. Prof. Anim. Scientist. 18:202-210.

Perry, T.C., D.G. Fox, and D.H. Beermann. 1991. Effect of an implant of trenbolone acetate and estradiol on growth, feed efficiency, and carcass composition of Holstein and beef steers. J. Anim. Sci. 69:4696-4702.

Roeber, D.L., R.C. Cannell, K.E. Belk, J.D. Tatum, and G. C. Smith. 2000. Implant strategies during

feeding: Impact on carcass grades and consumer acceptability. J. Anim. Sci. 78:1867-1874.

Samber, J.A., J.D. Tatum, M.I. Wray, W.T. Nichols, J.B. Morgan, and G.C. Smith. 1996. Implant program effects on performance and carcass quality of steer calves finishing for 212 days. J. Anim. Sci. 74:1470-1476.

Scheffler, J.M., D.D. Buskirk, S.R. Rust, J.D. Cowley, and M.E. Doumit. 2003. Effect of repeated administration of combination trenbolone acetate and estradiol implants on growth, carcass traits, and beef quality of long-fed Holstein steers. J. Anim. Sci. 81:2395-2400.

USDA. 1997. United States standards for grades of carcass beef. Agric. Mkt. Serv., USDA, Washington, D.C.

Table 1. Ingredient and chemical composition of diet

Ingredient	Diet Composition (%DM)
Corn, steam flaked	67.54
Sorghum sudan grass hay	14.0
Soybean meal	6.25
Molasses	5.0
Tallow	4.0
Urea	0.75
Finishing supplement <sup>a</sup>	2.5
Chemical Composition	
Dry matter	87.7
CP	15.42
ADF	7.91
Ash	14.0

<sup>a</sup> Mineral supplement composition (DM basis): limestone, 47.059%; dicalcium phosphate, 1.036%; potassium chloride, 8.000%; magnesium oxide, 3.448%; ammonium sulfate, 6.667%; salt, 12.000%; cobalt carbonate, 0.002%; copper sulfate, 0.157%; iron sulfate, 0.133%; calcium iodate, 0.003%; manganese sulfate, 0.500%; selenium premix (0.16%), 0.125%; zinc sulfate, 0.845%; vitamin A (30,000 IU/g), 0.264%; vitamin E (500 IU/g) 0.540%; Rumensin-80, 0.675%; Tylan 40, 0.450%; ground corn, 18.096%.

Table 2. Effects of implant program on live performance and carcass characteristics of long-fed Holstein steers

Item	Treatments <sup>a</sup>				SEM	Contrast <sup>b</sup>		
	CON	RSS	RSP	RPP		1	2	3
Initial BW, kg	184	188	189	191	6.18	0.41	0.86	0.88
Final BW, kg	509	585	584	576	12.77	0.01	0.71	0.64
ADG, kg/d								
0 to end	1.29	1.57	1.57	1.53	0.05	0.01	0.63	0.58
DMI, kg/d								
0 to end	8.06	9.62	9.35	9.64	0.35	0.01	0.75	0.55
Gain:feed								
0 to end	0.158	0.164	0.168	0.159	0.004	0.35	0.95	0.20
Carcass characteristics								
Hot carcass								
wt, kg	292	340	337	334	7.5	0.01	0.64	0.72
Dressing, %	57.5	58.0	58.1	58.0	0.01	0.52	0.96	0.95
Kidney, pelvis,								
& heart fat, %	5.1	5.1	4.2	4.4	0.39	0.20	0.11	0.73
Backfat	0.66	0.89	0.66	0.70	0.14	0.53	0.19	0.84
LM area, sq cm	64.4	76.1	73.9	76.1	2.8	0.01	0.75	0.57
Marbling score <sup>c</sup>	554	644	540	643	54.0	0.35	0.40	0.18
Yield grade	3.4	3.5	3.2	3.1	0.25	0.46	0.18	0.80
Color	5.0	5.8	5.4	5.5	0.29	0.08	0.30	0.80
Texture	5.4	5.8	5.8	5.5	0.25	0.27	0.60	0.39
Firmness	5.8	5.8	5.6	5.3	0.24	0.34	0.19	0.30
Shear Force, kg	1.98	2.14	2.25	2.52	0.31	0.33	0.48	0.52
Serum metabolites								
Glucose	99.86	95.14	101.33	99.61	3.8	0.77	0.23	0.74
NEFA	268.5	274.2	312.6	312.17	21.5	0.19	0.14	0.99
Serum urea								
Nitrogen	5.16	5.13	5.30	4.90	0.23	0.84	0.92	0.21

<sup>a</sup>CON = no implant; RSS = Ralgro on d 0, Synovex-S on d 84 and Synovex S on d 168; RSP= Ralgro on d 0, Synovex-S on d 84 and Synovex Plus on d 168; RPP = Ralgro on d 0, Synovex-Plus on d 84 and Synovex-Plus on d 168.

<sup>b</sup>Contrast 1 = CON vs implants; 2 = RSS vs. the average of RSP and RPP; and 3 = RSP vs. RPP.

<sup>c</sup>Marbling score 500 = small; 600 = modest; 700 = moderate

## EFFECT OF EARLY AND DELAYED IMPLANT APPLICATION ON PERFORMANCE, CARCASS TRAITS, AND MEAT CUTS YIELDING OF HOLSTEIN STEERS

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**ABSTRACT:** An experiment was conducted to evaluate the effect of early (EI) and delayed (DI) implant application on growth performance, carcass traits and meat cuts yielding of growing Holstein steers. Ninety-five Holstein steers (146 kg av. BW) fed in a commercial feed yard were used. All steers received the same commercial growing-finishing diets. This trial was conducted in the Mexicali valley, B.C., México, where commercial livestock is slaughtered at lightweight (480 kg). Treatments were: T1) No implant application, control group; T2) steers implanted at d1, and re-implanted at d84 and d168, EI; T3) steers implanted at d70 and d140, DI. Steers were first implanted with Revalor G® (40 mg trenbolone acetate/8 mg estradiol), and re-implanted with Implemax® (140 mg trenbolone acetate/ 28 mg estradiol). At the end of the feeding period, all steers were sacrificed at a commercial slaughter house, where carcass traits were evaluated. Non implanted calves had a 17.5% lower ADG than implanted ( $P<.01$ ). There was no difference ( $P>.10$ ) in total ADG between EI and DI steers. Holstein steers that received a more intensive implant program during the finishing phase (EI) had a larger reduction in the ADG as compared with the DI group. Delayed implantation of steers had lower marbling score ( $P<.01$ ), pelvic fat ( $P<.10$ ) and fat thickness ( $P<.10$ ), as compared with the NI and EI steers. However the rib eye area was bigger 16.6% in implanted as compared to NI steers. The weight of meat cuts was adjusted to hot carcass weight. No differences were observed in the total yielding cuts between treatments ( $P>.10$ ). These data indicate that early implant application of Holstein steers weighting 140 kg BW did not enhance growth performance, carcass traits and meat yielding compared with delay implant application. These, also suggest that early implantation does not bring any benefit to Holstein feeders.

Key word: Holstein steers, Implant, Carcass traits

### Introduction

The use of trenbolone acetate (TBA) in combination with estradiol ( $E_2$ ) may enhance ADG, and feed efficiency. However, the combination of implants may have also deleterious effects on marbling (Foutz et al., 1997, but several studies have shown no effect of implant combination on quality grade (Hunt et al., 1991; Gerken et al., 1995).

Holstein steers, typically, are repeatedly implanted with anabolic agents. But it is not clear if numerous implant applications or if implants administered early in life decrease the effectiveness of implants applied at later stages on growth performance. Also, it is not clear whether the growth benefits obtained from implant applications compromise the carcass

quality. There is a scarcity of information showing the effect of repeated implant applications of TBA/ $E_2$  on retail yield. Hence the objective of this study was to determine the effect of implant strategy on animal growth, carcass characteristics, and retail yielding of calf-fed Holstein steers.

### Materials and Methods

Ninety calf-fed Holstein steers were used to evaluate the effects of implant strategies on growth performance, carcass characteristics and retail yield. The trial was conducted in a commercial feedlot in Mexicali, Baja California, México. Upon arrival calves were castrated, vaccinated for bovine rinotraqueitis, viral diarrhea, parainfluenza IBR-PI<sub>3</sub> (Piramide-4®, Ford Dodge), clostridials-haemophilus (Ultrabac -7®, Pfizer), pasteurella hemolytic (One Shot®, SmithKline Beechman), treated for internal and external parasites (Dectomax®, Pfizer), injected with 500 000 UI vitamin A, and ear-tagging. At beginning (January 11, 2003), the steers were weighted and assigned to pens randomly by treatment, providing 30 steers by treatment. Pens were 421 m<sup>2</sup> with 174-m<sup>2</sup> overhead shade; equipped with automatic waterers, and 15 m fence-length line feed bunks.

The treatments were: 1) No implanted (NI); 2) Early Implanted (EI) Revalor G® (40 mg trenbolone acetate/ 8 mg estradiol, Intervet) on d - 1 and reimplanted with Implemax® (140 mg TBA/ 28 mg  $E_2$ ; Intervet ) on d-84 and d-168; 3) Delay Implanted (DI) Revalor-G on d-70 and reimplanted with Implemax on d-140. Steers were weighed at 28-d intervals. Steers were shipped for slaughter when pen, within implant treatment, achieved an estimated average net weight of 465 kg. All steers within each pen were processed on the same day. Hot carcass weight (HCW, kg) was obtained from all steers at time of slaughter. After a 24 h of carcass chilled, the following measurements were obtained: longissimus muscle area (ribeye area) taken by direct grid reading of the ribeye muscle at the twelfth rib; 2) subcutaneous thickness fat over the ribeye muscle at the twelfth rib taken at 3/4 the lateral length from the chine bone end; 3) kidney, pelvic and heart fat (KPH) as a percentage of carcass weight; 4) marbling score (USDA, 1997). After 24 h of carcass chilled the retail cuts were obtained following the commercial procedures of the Mexican market. Initial and final live weights were reduced 4% to account for fill. The trial was analyzed as a completely randomized design. Individual steers were considered as the experimental unit. Treatment means were compared using the following contrasts: 1) NI vs EI + DI; 2) EI vs DI, using SAS (SAS Inst., Inc., Cary, NC).

## Results and Discussion

The performance results obtained from this trial are presented in Table 1. Implant application improved the overall performance of Holstein steers as compared with no implanted steers. Overall weight gain increased ( $P < 0.01$ ) by 20% upon implant application. The largest responses were obtained from d 56 to d 84 (50%), d 112 to d 140 (42%), d 140 to d 164 (96%), and d 84 to d 168 (39%). On average, no response was observed from d 168 to 124. In studies ranging from 90 to 151 d in length, TBA/E2 implants improved ADG of crossbred beef cattle by 6 to 15%, respectively, compared to nonimplanted cattle (Johnson et al., 1996; Hermesmeyer et al., 2000). Early implanted steers had greater ADG than delayed implanted ( $P < 0.05$ ) during the first 84 d of the trial. This response is attributed to the fact that DI steers hardly had 14 d of implant application. No difference ( $P > 0.10$ ) in ADG between EI and DI was observed from d 28 to 84, but from d 1 to 84, EI steers had a higher ( $P < 0.05$ ) ADG (8.6%) than DI steers. However, from d 84 to 168 and from 168 to 224, the ADG was 18.6 and 25.7% higher ( $P < 0.01$ ), respectively, in DI than EI steers. Overall, no differences ( $P > 0.10$ ) were observed in ADG between EI and DI steers from d 1 to 224.

The HCW was similar ( $P > 0.10$ ) between treatments (Table 2) given that steers were slaughtered to similar final weight, but NI steers remained 24 more days in feedlot. Dressing percentage did not differ among treatments ( $P > 0.10$ ). Perry et al. (1991) and Johnson et al. (1996) found no differences in dressing percentage and 12<sup>th</sup>-rib fat for implanted steers. Implants have been shown to decrease fat as KPH (Johnson et al., 1996); fat thickness and marbling score (Duckett et al., 1999). In this study, carcasses from NI steers were higher ( $P < 0.0$ ) in marbling score, fat thickness and KPH as compared with implanted steers. Consistently with Roeber et al. (2000) who showed that implantation with Revalor-S at d 0 and 59 of a 140-d feeding period increased ( $P < 0.01$ ) LMA, both EI and DI enlarged 19% and 14%, respectively, the ribeye area as compared with NI steers. But, no differences were observed in these variables between EI and DI.

Table 3 shows the effect of implant strategies on yield weight of meat cuts. The total weight of meat cuts as percentage of HCW of implanted steers was larger ( $P < 0.01$ ) than NI, but there were no differences between EI and DI steers. Although, delayed implantation tended to have a higher retail yield ( $P < 0.10$ ) considering the cuts that are marketed similarly in Mexico and USA.

## Implications

The data obtained from this trial suggest that implants administered to lightweight (150kg) Holstein steers may decrease the efficacy of later re-implantations to improve weight gain. The use of delayed implantation (70 d) may optimize animal performance without compromising beef quality of long-fed Holstein steers.

## Literature Cited

- Duckett, S. K., D. G. Wagner, F. N. Owens, H. G. Dolezal, and D. R. Gill. 1999. Effect of anabolic implants on beef intramuscular lipid content. *J. Anim. Sci.* 77:1100–1104.
- Foutz, C. P., H. G. Dolezal, T. L. Gardner, D. R. Gill, J. L. Hensley, and J. B. Morgan. 1997. Anabolic implant effects on steer performance, carcass traits, subprimal yields, and longissimus muscle properties. *J. Anim. Sci.* 75:1256–1265.
- Gerken, C. L., J. D. Tatum, J. B. Morgan, and G. C. Smith. 1995. Use of genetically identical (clone) steers to determine the effects of strogenic and androgenic implants on beef quality and palatability characteristics. *J. Anim. Sci.* 73:3317–3324.
- Hunt, D. W., D. M. Henricks, G. C. Skelley, and L. W. Grimes. 1991. Use of trenbolone acetate and estradiol in intact and castrate male cattle: Effects on growth, serum hormones, and carcass characteristics. *J. Anim. Sci.* 69:2452–2462.
- Hermesmeyer, G. N., L. L. Berger, T. G. Nash, and R. T. Brandt Jr. 2000. Effects of energy intake, implantation, and subcutaneous fat endpoint on feedlot steer performance and carcass composition. *J. Anim. Sci.* 78:825–831.
- Hunt, D. W., D. M. Henricks, G. C. Skelley, and L. W. Grimes. 1991. Use of trenbolone acetate and estradiol in intact and castrate male cattle: Effects on growth, serum hormones, and carcass characteristics. *J. Anim. Sci.* 69:2452–2462.
- Perry, T. C., D. G. Fox, and D. H. Beermann. 1991. Effect of an implant of trenbolone acetate and estradiol on growth, feed efficiency, and carcass composition of Holstein and beef steers. *J. Anim. Sci.* 69:4696–702.
- Roeber, D. L., R. C. Cannell, K. E. Belk, R. K. Miller, J. D. Tatum, and G. C. Smith. 2000. Implant strategies during feeding: Impact on carcass grades and consumer acceptability. *J. Anim. Sci.* 78:1867–1874.
- Scheffler, J. M., D. D. Buskirk, S. R. Rust, J. D. Cowley and M. E. Doumit. 2002. Effect of repeated administration of combination trenbolone acetate and estradiol implants on growth, carcass traits, and beef quality of long-fed Holstein steers. *J. Anim. Sci.* 80:2395–2399.
- USDA. 1997. Standards for grades of carcass beef. Agric. Marketing Service, USDA, Washington, DC.

Table 1. Effects of implant strategies on growth performance of Holstein steers

Item	Treatments			SEM
	No Implant	Early Implant	Delay Implant	
Live weight, kg <sup>a</sup>				
Initial	157.18	148	139	
Final	455.6	447.8	451.5	1.5
Days on feed	250	226	226	
Weight gain, kg/d <sup>b</sup>				
1-28 d <sup>d</sup>	1.51	1.43	1.14	.02
28 – 56 d	1.43	1.70	1.52	.03
56 – 84 d <sup>c</sup>	1.37	2.07	2.05	.03
<b>1- 84 d<sup>d</sup></b>	<b>1.34</b>	<b>1.70</b>	<b>1.57</b>	<b>.02</b>
84 – 112 d	1.71	1.57	1.81	.14
112 – 140 d <sup>e</sup>	1.15	1.56	1.71	.11
140 – 168 d <sup>c,e</sup>	.93	1.58	2.07	.03
<b>84 – 168</b>	<b>1.15</b>	<b>1.57</b>	<b>1.63</b>	<b>.03</b>
168- 196 d <sup>e</sup>	1.13	1.10	1.55	.05
196 – 224 d	1.29	1.07	1.23	.05
<b>168 – 224<sup>e</sup></b>	<b>1.21</b>	<b>1.09</b>	<b>1.37</b>	<b>.02</b>
<b>1- 224<sup>c</sup></b>	<b>1.23</b>	<b>1.45</b>	<b>1.51</b>	<b>.01</b>

<sup>a</sup> Weight reduced to account for digestive tract (4%)

<sup>b</sup> Initial weight as covariable

<sup>c</sup> No Implant vs Implant: P < .01

<sup>d</sup> Early implant vs Delay implant: P < .05

<sup>e</sup> Early implant vs Delay implant: P < .01

Table 2. Effect of implant strategies on carcass traits of Holstein steer

Item	Treatments			SEM
	No Implant	Early Implant	Delay Implant	
Hot carcass weight, kg	283.4	280.7	282.1	2.2
Dressing , %	61.05	60.61	60.13	.15
Fat thickness <sup>ab</sup> , cm	0.88	.79	.66	.03
Marbling score <sup>abc</sup>	3.85	3.21	2.79	.07
KPH <sup>ab</sup> , %	1.88	1.70	1.61	.04
Rib eye area <sup>ab</sup> , cm <sup>2</sup>	65.10	79.98	76.08	1.09

<sup>a</sup> Carcass weight as covariable

<sup>b</sup> No implant vs Implant: P < .01

<sup>c</sup> Coded: minimum traces =2, minimum slight=3, minimum small = 4

Table 3. Effect of implant strategies on yield weight of meat cuts

Item	No Implant	Early Implant	Delay Implant	SEM
<b>Yield cuts<sup>a</sup>, %</b>				
Sirloin <sup>b</sup>	3.22	3.47	3.66	.04
Short loin <sup>d</sup>	3.56	3.65	3.31	.07
Knuckle special <sup>c,e</sup>	3.91	3.84	3.65	.02
Gooseneck round <sup>c</sup>	7.20	7.27	7.17	.02
Inside round	5.73	5.79	5.66	.04
Rib <sup>b,d</sup>	5.64	5.73	6.48	.04
Tenderloin	1.79	1.80	1.69	.01
Flank steak <sup>b,d</sup>	.90	.72	.83	.01
Outside skirt <sup>b</sup>	2.77	2.86	2.94	.02
Triangle	.77	.80	.87	.01
Plate	7.48	7.72	7.81	.05
Neck boneless	3.60	3.76	3.61	.03
Chuck tender	1.08	1.07	1.08	.01
Brisket	3.59	3.60	3.76	.03
<b>Total<sup>b</sup></b>	<b>51.24</b>	<b>52.08</b>	<b>52.51</b>	<b>.05</b>
Bone <sup>d</sup>	6.83	6.80	6.21	.10

<sup>a</sup> Expressed as carcass weight percentage

<sup>b</sup> No Implant vs Implant: P < .01

<sup>c</sup> No Implant vs Implant: P < .05

<sup>d</sup> Early implant vs Delay implant: P < .01

<sup>e</sup> Early implant vs Delay implant: P < .05

# COOKING DOES NOT AFFECT FATTY ACID COMPOSITION OF BEEF *LONGISSIMUS* MUSCLE

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**ABSTRACT:** *Longissimus* muscle (LM) from five forage-fed beef cattle were used to determine the effect of flame-broiling on fatty acid composition of meat. Samples of LM were taken adjacent to the following vertebrae: thoracic 4, thoracic 12, and lumbar 6. For each sample, the entire LM cross section (2.54 cm thick) was trimmed of external fat and separated from the bone. Each steak was cut in half with one half used for cooking. Steaks were flame-broiled to achieve an internal temperature of 65°C. Raw and cooked steaks were placed into whirl-pack bags and frozen before being freeze-dried. Freeze-dried raw and cooked samples were ground and 125 mg of material was subjected to direct fatty acid methyl ester preparation using methanolic KOH, and analyzed by GLC. Data were analyzed as a 3 × 2 factorial with LM location as the first treatment factor and raw vs cooked as the second treatment factor. Flame-broiling resulted in 63.1% moisture loss ( $P < 0.0001$ ). Therefore, fatty acid data were corrected for differences in moisture content. No location × cooking treatment interactions were detected ( $P \geq 0.14$ ). Total fatty acid concentration was not affected by location ( $P = 0.79$ ) or by cooking ( $P = 0.57$ ). Weight percentages of 18:1*cis*-9 and 18:2*cis*-9, *trans*-11 decreased ( $P \leq 0.05$ ) from 0.5 to 0.4% and 39.5 to 38.0% in raw vs cooked steak, respectively. Weight percentages of 18:1*trans*-11 (2.2 vs 1.6 %), 17:0 (0.9 vs 0.7%), and 15:0 (0.5 vs 0.4%) were greater ( $P \leq 0.03$ ) in cooked vs raw steaks. Weight percentage of 18:1*trans*-9 tended to be greater ( $P = 0.06$ ) in raw (0.2%) compared to cooked (0.1%) steaks. Weight percentages of other fatty acids were not affected ( $P = 0.21$  to 0.94). We conclude that neither location of the LM nor cooking, once corrected for moisture loss, affected total fatty acid concentration and only slight alterations occur in fatty acid composition by flame-broiling beef LM steaks.

**Key words:** Fatty acid, *Longissimus* muscle, Beef

## Introduction

Beef consumption in the United States contributes significantly to total caloric, protein, and lipid intake (Slover et al., 1987). Boiling, broiling, oven-roasting, and microwave cooking could induce significant changes in content and composition of lipids of beef steaks. Concentrations of most nutrients, including lipids, increase after cooking because of moisture loss (Badiani et al., 2002).

Extensive data on fatty acid content and composition of beef products are available from both research publications and food composition tables (Moss et al., 1980). However, little attention has been paid to possible changes in fatty acid composition of beef after cooking. The objective of the present study was to determine how flame-broiling affects

the fatty acid content and composition of the bovine *longissimus* muscle.

## Materials and Methods

*Longissimus* muscle from five forage-fed Angus × Gelbvieh rotationally crossbred beef cattle were used to determine the effect of flame-broiling on fatty acid content and composition of meat. Cattle were from 3 to 5 yr of age and hot carcass weight ranged from 304 to 528 kg

Samples of LM were taken adjacent the following vertebrae: thoracic 4, thoracic 12, and lumbar 6. For each sample, the entire LM cross section (2.54 cm thick) was trimmed of external fat and separated from the bone. Each steak was cut in half with one half used for cooking and the other half left uncooked. Steaks were flame-broiled to achieve an internal temperature of 65°C (Koch hand thermometer, Kansas City, MO). Raw and cooked steak samples were weighed, placed into whirl-pack bags (Nasco, Modesto, CA), and frozen before being freeze-dried (Genesis freeze Dryer, Virtis Co., Gardiner, NY). Moisture content was determined (AOAC, 1990) before being ground in a home-style electric coffee grinder (Sunbeam Products, Inc., Hattiesburg, MS).

Fatty acid methyl esters were prepared by direct transesterification with methanolic KOH of duplicate 125-mg samples of raw and cooked meat (Murrieta et al., 2003). Fatty acid composition was analyzed by capillary GLC (Agilent 6890, Agilent Technologies, Wilmington, DE). Peaks were separated with a 100-m fused silica capillary column (SP-2560, 0.25-mm i.d. and 0.2-μm film thickness, Supelco, Bellefonte, PA). Injector and detector temperatures were 250°C. Column oven temperature was maintained at 175°C for 40 min, and then increased to 240°C at 10°C/min. Peaks were integrated using ChemStation software (Agilent Technologies, version A.09.03). Identification of fatty acids was based on retention times of reference fatty acid methyl ester standards (Nu-Chek Prep. Inc., Elysian, MN and Matreya, Inc. Pleasant Gap, PA). Fatty acids were quantified by using glyceryl tridecanoate (tri-13:0) as internal standard.

Data were analyzed as a 3 × 2 factorial using the GLM procedure of SAS (SAS Institute, Cary, NC) with LM location as the first treatment factor and raw vs cooked as the second treatment factor. After a significant F-test, least-squared means were compared using the LSMEANS option of SAS.

## Results and Discussions

Flame-broiling caused 63.1% moisture loss ( $P < 0.0001$ ) of the LM steaks. Consequently, fatty acid concentrations were greater ( $P = 0.007$ ) in cooked

compared to raw meat. Baldiani et al. (2002) reported decreased moisture content of single-muscle cuts after being subjected to different cooking methods, which resulted in a significant increase in fat and cholesterol content of cooked compared with raw meat. To account for variation in water content, fatty acid data of the present study were corrected for moisture content, and are presented on a dry-weight basis (Table 1).

No location  $\times$  treatment interaction was detected ( $P \geq 0.14$ ) for fatty acid composition or content. Hence, the main effects of fatty acid composition and content of raw and cooked LM steaks will be presented. Although there was no location effect ( $P \geq 0.13$ ) for most fatty acids reported, LM steaks cut adjacent to lumbar 6 tended ( $P = 0.09$ ) to have greater 18:1*trans-11* than the steaks cut adjacent to thoracic 4 or 12.

Total fatty acid concentration was not affected by cooking ( $P \geq 0.57$ ) or location ( $P \geq 0.79$ ). This finding agrees with Bragagnolo and Rodriguez (2003) who reported similar mean total fatty acids concentrations of raw and cooked beef steaks.

For raw and cooked LM steaks, the most abundant fatty acids, based on weight percentages, were 16:0, 18:0, 16:1, 18:1*cis-9*, 18:1*trans-11*, 18:2*cis-9,trans-12*, and 18:2*cis-9,trans-11*. The fatty acid profile of beef is attributed to several factors, including diet. For example, Shantha et al. (1994) reported that beef from cattle fed high-forage diets had 90% of total CLA as the *cis-9, trans-11* isomer. Moreover, Shantha et al. (1997) reported that the concentration of CLA from steers fed diets based on pasture grass averaged 7.4 mg/g of fat. Results reported by Rule et al. (2002) showed that weight percentages of 16:0 and 18:0 tended to be greater in *longissimus dorsi* of range beef cows and feedlot steers compared to *semiteminosus* or *supraspinatus*. Furthermore, the weight percentage of 18:2*cis-9,trans-11* was higher in range beef cows than in feedlot steers. Meat from cattle finished on pasture with a grain supplement containing soybean oil had the highest total CLA content in both raw and cooked *longissimus lombo*, while ribeye steaks from cattle finished on a feedlot diet had the lowest total CLA content for raw and cooked steaks when expressed as mg/g of fat (Lorenzen et al., 2004). While results from Shantha et al. (1994) have shown that cooking method did not alter CLA content of beef steaks, Ha et al., (1989) suggested that cooking meat increased the amount of total CLA. In the current study, the weight percentage of CLA was greater ( $P = 0.05$ ) in raw compared to cooked LM steaks, perhaps due to oxidative changes.

Duckett and Wagner (1998) reported that cooking ribeye steaks decreased the percentages of 18:1*cis-9*, 18:1*cis-9,12*, and 18:3*cis-9,12,15*, as well as increased the percentage of 18:0. Baldiani et al. (2002) reported similar weight percentages for 16:0 and 18:0 in cooked beef steaks. In the present study, cooking decreased ( $P \leq 0.05$ ) the weight percentage of 18:1*cis-9*, but did not affect the weight percentage of 18:0, 18:1*cis-9,12*, and 18:3*cis-9,12,15*. Weight percentages of 15:0, 17:0, and 18:1*trans-11* were greater ( $P \leq 0.03$ ) in cooked than in raw steaks. Weight percentage of 18:1*trans-9* tended to be greater ( $P = 0.06$ ) in raw compared to cooked steaks.

## Implication

Although there were slight alterations in fatty acid composition, total fatty acid concentration, once corrected for moisture, was not affected by either location of the LM or flame-broiling of the beef steaks. We can imply that fatty acid profile of beef that is flame-broiled will be essentially maintained through the cooking process.

## Literature Cited

- AOAC. 1990. Official Methods of Analysis (13<sup>th</sup> Ed.). Association of official Analytical Chemists, Washington, DC.
- Badiani A, S. Stipa, F. Bitossi, P. P. Gatta, G. Vignola, and R. Chizzolini. 2002. Lipid composition, retention and oxidation in fresh and completely trimmed beef muscles as affected by common culinary practices. Meat Sci. 62:169-186.
- Bragagnolo, N. and D. B. Rodriguez-Amaya. 2003. New data on the total lipid, cholesterol and fatty acid composition of raw and grilled beef *longissimus dorsi*. Archivos Latinoamericanos de Nutricion. 53: 312-319.
- Duckett S. K. and D. G. Wagner. 1998. Effect of cooking on fatty acid composition of beef intramuscular lipid. J. Food Compos. Anal. 11: 357-362.
- Ha Y. L., J. Storkson, M. W. Pariza. 1989. Newly recognized anticarcinogenic fatty acids. Identification and quantification in natural and processed cheeses. J. of Agric Food Chem. 37:75-81.
- Lorenzen C. L., J. W. Golden, F. A. Martz, I. U. Gruen, J. R. Gerrish, and K. C. Moore. 2004, conjugated linoleic acid content of ribeye steaks from beef finished on pasture. Abst. #114 presented at the Midwestern Section ASAS and Midwest Branch ADSA 2004 Meeting, Des Moines, IA.
- Moss M., K. Ono, R. Cross, J. Holden, and K. Steward. 1980. Nutrition composition of cooked beef. Proc. 40<sup>th</sup> Annual Meeting of Institute of Food Technologists. New Orleans, LA. Pp:153-156.
- Murrieta C. M., B. W. Hess, D. C. Rule. 2003. Comparison of acidic and alkaline catalysts for preparation of fatty acid methyl esters from ovine muscle with emphasis on conjugated linoleic acid. Meat Sci. 65:523-529.
- Rule D. C., K. S. Broughton, S. M. Shellito, and G. Maiorano. 2002. Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. J. Anim. Sci. 80:1202-1211.
- Shantha C. N., C. D. Andrea, E. D. Decker. 1994. Evaluation of Conjugated Linoleic Acid Concentrations in Cooked Beef. J Agric. Food Chem. 42:1757.
- Shantha C. N., W. G. Moody, and Z. Tabeidi. 1997. A research note: Conjugated linoleic acid concentration in semimembranous muscle of grass- and grain-fed and zeranol-implanted beef cattle. J. Muscle Food 8:105.
- Slover H. T., E. Lanza, R. H. Thompson Jr., C. S. Davis, and G. V. Meola. 1987. Lipids in raw and cooked beef. J. Food Compos. Anal. 1: 26-37.

Table 1. Fatty acid composition of forage-fed beef *longissimus* muscle (DM basis)

Item	Locations on vertebrae			Treatment		P-values		
	Thoracic 4	Thoracic 12	Lumbar 6	Cooked	Raw	Location	Treatment	Treatment × location
Fatty acid <sup>a</sup> , mg/g	272.5±22.9	268.4±25.3	289.0±21.3	284.4±22.1	268.9±15.3	0.79	0.57	0.19
	-----wt %-----							
14:0	2.7±0.1	2.9±0.1	2.9±0.1	2.9±0.1	2.7±0.1	0.18	0.09	0.99
14:1	0.4±0.04	0.5±0.04	0.4±0.04	0.4±0.04	0.4±0.03	0.29	0.71	0.45
15:0	0.4±0.03	0.4±0.03	0.5±0.03	0.5±0.03	0.4±0.02	0.34	0.002	0.32
15:1	0.2±0.02	0.2±0.02	0.2±0.02	0.2±0.02	0.2±0.01	0.73	0.94	0.31
16:0	30.1±0.5	30.8±0.6	30.3±0.5	30.1±0.5	30.8±0.3	0.67	0.27	0.27
16:1	3.0±0.2	3.4±0.2	2.9±0.2	3.0±0.2	3.2±0.1	0.18	0.53	0.78
17:0	0.8±0.1	0.7±0.1	0.8±0.1	0.9±0.1	0.7±0.04	0.32	0.03	0.14
17:1	0.5±0.03	0.5±0.04	0.4±0.03	0.4±0.03	0.5±0.02	0.13	0.21	0.24
18:0	16.4±0.7	14.7±0.8	16.5±0.7	16.3±0.7	15.5±0.5	0.19	0.35	0.64
18:1 <i>cis</i> -9	38.7±0.6	39.5±0.6	38.0±0.6	38.0±0.6	39.5±0.4	0.20	0.03	0.51
18:1 <i>trans</i> -9	0.2±0.03	0.2±0.04	0.2±0.03	0.1±0.03	0.2±0.02	0.74	0.06	0.39
18:1 <i>trans</i> -10	0.3±0.03	0.3±0.03	0.3±0.03	0.3±0.03	0.3±0.02	0.36	0.38	0.83
18:1 <i>trans</i> -11	1.8±0.1	1.8±0.1	2.1±0.1	2.2±0.1	1.6±0.1	0.09	<0.0001	0.25
18:2 <i>cis</i> -9,12	1.4±0.1	1.2±0.1	1.2±0.1	1.3±0.1	1.3±0.1	0.34	0.93	0.31
18:2 <i>cis</i> -9, <i>trans</i> -11	0.5±0.04	0.4±0.04	0.4±0.04	0.4±0.04	0.5±0.02	0.43	0.05	0.24
18:3 <i>cis</i> -9,12,15	0.2±0.02	0.2±0.02	0.2±0.02	0.2±0.02	0.2±0.01	0.47	0.70	0.27
20:4 <i>cis</i> -5,8,11,14	0.3±0.04	0.3±0.04	0.3±0.04	0.3±0.04	0.3±0.02	0.29	0.28	0.72
22:5 <i>cis</i> -7,10,13,16,19	0.2±0.02	0.2±0.02	0.1±0.02	0.1±0.01	0.2±0.01	0.23	0.41	0.72

<sup>a</sup> Fatty acids are denoted as number of carbon atoms:number of carbon-carbon double bonds.

## GROWTH IMPLANTS AFFECT TENDERNESS OF BEEF STEAKS.

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**ABSTRACT:** Two experiments were designed to evaluate the effect of growth implants on the carcass characteristics, and tenderness of cattle with different genetic growth potentials. The first experiment evaluated Angus steers (128) from sires with high EPD's for retail product yield (n=64) or marbling (n=64). Implant treatment (with, without) were imposed randomly within sire groups. Steers were harvested following normal industry practices when ultrasound measure determined that 75% of the steers were USDA Choice. Carcass data was collected. Loins (IMPS 180) were collected from each carcass. Loins were cut into 1.3 inch steaks and frozen after 7, 14 and 21 days. The second experiment evaluated steers and heifers of British (n=34) and Continental (n=46) breed descent. Heifers and steers from both breed combinations were assigned to implant treatments. Steers and heifers were harvested following normal industry procedures after they had been on feed for 120 days. Carcass data was collected. Loin sections (3 inches) were cut into one 1.3 in steak for tenderness analysis. Eight to ten samples for shear force evaluation were removed from each cooked steak and sheared once perpendicular to the fiber. Individual animals were utilized as the experimental unit with planned comparisons used to compare growth potential, sex or implant treatments. Using growth implants significantly reduced the tenderness of steaks regardless of the growth potential or sex of the animal. Steaks from heifers were less tender than steers and steaks from Continental breeds were less tender than those from British breeds. There was a trend ( $P=0.26$ ) suggesting that the tenderness of steaks from Continental breed steers and heifers were affected more by the use of growth implants than those from British breeds. Use of implants does decrease the tenderness and may contribute to added tenderness variability because of the different responses observed between heifers and steers, and different growth potentials.

Keywords: growth implants, beef, tenderness

### Introduction

Growth implants are routinely used to improve efficiency of meat production by improving red meat yield. Increased feed efficiency and increased longissimus muscle area have been reported with use of hormonal implants

To accomplish muscle growth, protein accretion must exceed protein breakdown. The enzyme system that is partially responsible for controlling protein accretion and

breakdown is the calpain system (Boehm et al., 2000). Testosterone increases the activity of calpastatin (inhibitor of calpain) and increases protein accretion. Use of exogenous growth hormones in implants increase growth and therefore affect the calpastatin activity. Decreased calpastatin activity postmortem has been linked to increased tenderness. Roeber et al (2000) and Platter et al (2003) reported that steaks from implanted steers had significantly higher Warner-Bratzler shear values than steaks from steers that were never implanted. Platter et al. (2003) also reported that the closer the implant was applied to slaughter, the more likely shear values were to be affected.

Work reported by Platter and co-workers (2003) utilized steers with various genetic backgrounds, but did not analyze to determine if a compounding affect on tenderness is observed when implants were administered to animals with a genetic propensity for greater growth. Late maturing, heavily muscled animals already have a larger rate of protein accretion with reduced degradation (increased calpastatin activity) than earlier maturing light muscled animals. Growth implants increase the rate of growth and may compound any tenderness problems created by growth implants. The current studies evaluate the affect of growth implants on the carcass characteristics, and tenderness of steers with different genetic potential for growth or marbling.

### Materials and Methods

#### *Experiment 1*

Steers (64) from sires with high EPD's for retail product yield (low yield grades) and high marbling (64) were assigned to an implant protocol (implanted or not implanted) on entry into the feedlot in two different years. Cattle were harvested when ultrasound indicated that the majority of the steers had reached low USDA Choice or greater.

Steers were shipped (9 h with 12 h rest period) to a commercial processing facility where they were harvested following normal industry procedures. Carcass data was collected including hot carcass weight, fat thickness, ribeye area, internal fat percentage and marbling scores by trained university personnel. Loins (IMPS 180) were collected from each carcass. These sections were transported (4°C) to Montana State University. Striploins were cut into three 3.3 cm steaks. Steaks were aged for 7, 14 or 27 days at 4°C and then frozen at -20°C until cooked for tenderness analysis.

#### *Experiment 2*

Steers and heifers of British (n=34) and Continental (n=46) breed descent were assigned to implant treatments. Implants were a combination implant containing estradiol benzoate (24

mg) and trenbolone acetate (120 mg). After steers and heifers had been on feed for 120 days, they were shipped to a commercial processing facility (8 h with 12 h rest) and harvested following normal industry procedures. After 24 h at 4°C, carcass data was collected by experienced university personnel, including hot carcass weight, fat thickness, ribeye area, internal fat percentage and marbling scores. Loin sections (7.62 cm) were removed from each carcass. The loin sections were cut into one 3.3 cm steak for tenderness analysis.

#### *Tenderness analysis*

Steaks were thawed at 4°C for 24 hours. Each steak was weighed before and after cooking to determine cook loss. Eight to ten samples (1.27 x 1.27 x 2.54 cm) for shear force evaluation were removed from each steak parallel to the fiber direction. Samples were sheared once perpendicular to the fiber direction with a TMS 30 Food Texturometer fitted with a Warner-Bratzler shear attachment. The average of the samples sheared was used for statistical analysis.

#### *Statistics*

Individual animals were used as the experimental unit in both studies. The GLM procedure of SAS was used to analyze carcass and tenderness data. Planned comparisons between implant strategy (implant versus no implant) and genetic classifications (high retail product versus high marbling) or implant strategy, sex and growth potential were done.

### **Results and Discussion**

Growth implants used in Angus steers significantly affected carcass traits and shear force values. When implants were used hot carcass weight and ribeye area increased but fat depth and internal fat was not affected. In addition, shear force values were significantly higher ( $P < 0.05$ ) for steaks that were from implanted steers (Table 1). No interaction was observed between sire type (high retail product, high marbling) and use of implant for carcass traits or shear force values.

When growth implants were administered to steers and heifers with different growth potentials, similar results were seen. Growth implants increased hot carcass weight and LD area, decreased internal fat and yield grade whereas fat depth was not affected. Steaks from steers and heifers that had been implanted had significantly ( $P < 0.05$ ) higher shear force values than did steaks from steers and heifers that were not implanted (Table 2).

Sex and breed also influenced some carcass traits and shear force values. Steer carcasses were heavier with larger LD area than heifer carcasses. In addition carcasses from animals of Continental descent were heavier, with larger LD area, less external fat and lower yield grade. Shear force was significantly lower ( $P < 0.05$ ) for steaks from steers than steaks from heifers. Furthermore, shear force was significantly lower ( $P < 0.05$ ) for steaks from steers and heifers of British decent

when compared to steers and heifers of Continental descent. This information along with increased hot carcass weight and LD area of Continental cattle would suggest that increased growth rate might have some impact on tenderness. However, heifers normally grow slower than do steers thus steaks from heifers should be more tender. However the data reported herein does not support this assumption. Also, no significant interaction was seen between growth implant and breed or between growth implant and sex. Growth implants administered to continental steers and heifers did however, have a much greater numeric increase in shear force values than when administered to steers and heifers of British breed decent (Table 2).

### **References**

- Boehm, M.L., T.L. Kendall, V.F. Thompson, and D. E. Goll. 1998. Changes in calpains and calpastatin during postmortem storage of bovine muscle. *J. Anim. Sci.* 76:2415-2434.
- Platter, W. J., J. D. Tatum, K. E. Belk, J. A. Scanga, and G. C. Smith. 2003. Effects of repetitive use of hormonal implants on beef carcass quality, tenderness, and consumer ratings of beef palatability *J. Anim. Sci.* 81: 984-996.
- Roeber, D.L., R.C. Cannell, K.E Belk, R.K. Miller, J.D. Tatum, and G.C. Smith, 2000. Implant strategies during feeding: Impact on carcass grades and consumer acceptability. *J. Anim. Sci.* 78:1867-1874.

Table 1. Effect of sire type and implant strategy on carcass characteristics of Angus steers

		Hot carcass, kg	LD area, cm <sup>2</sup>	Fat depth, cm	KPH, %	Yield Grade	Marbling <sup>a</sup>
Sire type <sup>b</sup>	Marbling	324.8	71.5	1.3	2.0	3.5	535
	Retail product	322.3	72.7	1.2	2.0	3.3	484
P =		0.8693	0.5163	0.1500	0.6716	0.0859	0.0002
Implant	With	334.7	74.7	1.3	1.9	3.4	490
	Without	312.2	70.0	1.3	2.1	3.4	580
P =		<0.0001	<0.0001	0.0904	0.0011	0.7839	<0.0001
Sire type × Implant	Marbling with	345.0	76.1	1.4	2.0	3.5	485
	Retail product with	342.2	78.5	1.3	2.0	3.2	459
	Marbling without	325.5	70.3	1.4	2.1	3.6	589
	Retail product without	311.1	70.3	1.2	2.1	3.4	552
P =		0.2127	0.4119	0.3858	0.3577	0.9850	0.7959

<sup>a</sup>200 to 299 = Traces; 300-399 = Slight; 400-499 = Small; 500-599 Modest.

<sup>b</sup>Sires were selected for high expected progeny differences for retail product yield or marbling.

Table 2 Effect of sire type, implant strategy and ageing on the tenderness of beef top loin steaks.

		WBS, kg
Sire type <sup>a</sup>	Marbling	5.5
	Retail product	5.4
	P – value	0.9283
Implant	With	5.9
	Without	6.5
	P – value	<0.0001
Ageing	7	6.2
	14	5.8
	21	5.8
	P – value	0.3302
Sire × Implant	Marbling with	6.0
	Retail product with	5.8
	Marbling without	5.0
	Retail product without	5.1
	P – value	0.6385

<sup>a</sup>Sires were selected for high expected progeny differences for retail product yield or marbling.

Table 3 Effect of breed type, sex and growth implants on carcass traits and tenderness.

		Hot carcass, kg	LD area, cm <sup>2</sup>	Fat depth, cm	KPH, %	Yield Grade	Marblin g <sup>a</sup>	WBS, kg
Breed type <sup>b</sup>	Continental	294.8	75.5	0.8	1.8	2.2	389	7.9
	British	320.8	82.6	0.9	1.9	2.5	388	6.7
	P – value	<0.0001	0.0001	0.0001	0.4687	0.0002	0.9812	0.0060
Sex	Steer	310.9	78.7	0.8	1.9	2.4	384	6.9
	Heifer	293.9	76.8	0.9	1.9	2.4	393	7.7
	P – value	0.0002	0.0378	0.378	0.5946	0.8244	0.2746	0.064
Implant	With	314.5	81.3	0.9	1.0	2.3	381	7.9
	Without	290.4	74.2	0.9	2.0	2.5	396	6.8
	P – value	<0.0001	<0.0001	0.5978	0.0005	0.0011	0.0556	0.0081
Breed × Sex	Cont. Steer	330.5	83.2	0.7	1.9	2.2	367	7.2
	Brit. Steer	303.0	76.1	0.9	1.9	2.4	388	6.7
	Cont. Heifer	310.0	82.6	0.7	2.0	2.2	394	8.6
	Brit Heifer	286.1	74.5	1.0	1.9	2.5	411	6.8
	P – value	0.8418	0.0544	0.2576	0.8483	0.1847	0.5017	0.1230
Breed × Implant	Continental with	332.6	81.1	0.7	1.8	2.1	371	8.7
	British with	306.3	79.5	0.9	1.7	2.4	381	7.0
	Continental without	308.2	79.4	0.7	2.0	2.4	390	7.1
	British without	282.8	72.0	0.9	2.0	2.6	418	6.4
	P – value	0.8058	0.9281	0.7290	0.8373	0.7910	0.3974	0.2613
Sex × Implant	Steer with	332.2	84.5	0.9	1.7	2.3	365	7.7
	Steer without	289.7	72.9	0.8	2.0	2.5	397	6.2
	Heifer with	296.7	78.7	0.9	1.8	2.3	391	8.0
	Heifer without	291.0	75.5	1.0	1.9	2.5	421	7.3
	P – value	0.0062	0.0086	0.0979	0.0887	0.5302	0.9066	0.3334

<sup>a</sup>200 to 299 = Traces; 300-399 = Slight; 400-499 = Small.<sup>b</sup>Breed types were characterized by what the sire was known to be. Continental descent cattle were from Simmental sires whereas British descent cattle were from Angus and Hereford sires.

## Optimization of Conditions for Leukotriene B<sub>4</sub> Synthesis by Neutrophils or Heterophils Isolated from Peripheral Blood of Monogastric Animals

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**ABSTRACT:** Inflammation is the body's response to internal or external injury and is characterized by pain, swelling, redness and heat. Eicosanoids are lipid mediators of inflammation. Neutrophils and heterophils are involved in inflammation through leukotriene (LT) production. The predominant proinflammatory eicosanoid released is LTB<sub>4</sub> which serves as a biological marker of inflammation. Measuring LTB<sub>4</sub> synthesis will be useful in determining the role of dietary nutrients in modulating the inflammatory response. The purpose of this study was to optimize the conditions for LTB<sub>4</sub> production by heterophils from chickens, and polymorphonuclear leukocytes (PMNs) from horses and dogs. Optimal production of LTB<sub>4</sub> from equine and canine PMNs and chicken heterophils was characterized in terms of incubation time (0, 2.5, 5, 10, 15, 20 min), temperature (25, 37°C), and calcium ionophore A23187 concentration (0, 1, 10, 20 µM). In all species 10 min incubation time, 37°C, and 10 µM ionophore concentration resulted in substantial LTB<sub>4</sub> production. At 37°C and 10 µM ionophore concentration, time was varied from 0 to 20 min, and at 37°C and 10 min incubation time, ionophore concentration was varied from 0 to 20 µM. In all species, incubation at 37°C allowed optimal LTB<sub>4</sub> synthesis compared to 25°C (P<0.05). Production of LTB<sub>4</sub> was maximum when neutrophils were stimulated with 20 µM calcium ionophore in all species (P<0.05). Incubation times greater than 2.5 min did not further increase production of LTB<sub>4</sub> in chicken and horses (P<0.05). However, in dogs incubation at 2.5 and 10 min produced the highest

concentration of LTB<sub>4</sub> (P<0.05). The amount of LTB<sub>4</sub> produced for each species using optimal conditions was 111.8 ± 0.42 ng in chickens, 95.4 ± 0.42 ng in equines, and 108.0 ± 19.5 ng in canines /5 × 10<sup>6</sup> cells, mean ± SEM. These results indicate that heterophils from chickens and PMNs from horses and dogs are capable of producing LTB<sub>4</sub> and optimum conditions for LTB<sub>4</sub> production are similar in all three species.

**Keywords:** Inflammation, Leukotrienes, Neutrophils.

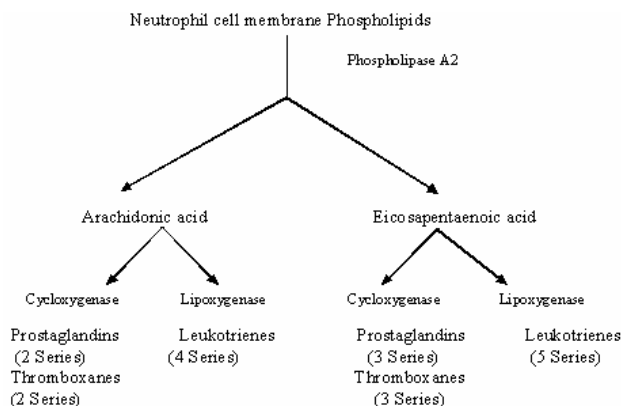
### Introduction

Inflammation is characterized by redness, heat, pain, swelling and loss of function. Important cells involved in the inflammatory process are lymphocytes and neutrophils. Neutrophils are also known as heterophils in poultry. Major functions of neutrophils are to release mediators of inflammation such as oxygen radicals, and enzymes such as proteases, phospholipases, collagenases, and lysozyme. All these are involved in killing of pathogens inside phagolysosomes, but can also be used in extracellular clearing of pathogens.

Neutrophil migration is an important step in inflammation. Many kinds of chemotactic factors for neutrophils have been reported, including LTB<sub>4</sub>. LTB<sub>4</sub> is a potent chemotactic agent produced by almost all types of immune cells, but mainly by PMNs after activation of the 5-lipoxygenase pathway. Arachidonic acid (AA) in cell membrane phospholipids is cleaved by phospholipase A<sub>2</sub> and acted on by 5-lipoxygenase to form LTA<sub>4</sub>.

Neutrophils contain LTA<sub>4</sub>-hydrolases, which convert the 5-lipoxygenase product LTA<sub>4</sub> into LTB<sub>4</sub> through addition of a triene epoxide. The important eicosanoids formed from AA, as well as eicosapentaenoic acid (EPA), are outlined in Figure 1. LTA<sub>4</sub> can also be converted into LTB<sub>4</sub> outside the cell by adjacent cells. The major action of LTB<sub>4</sub> in the body is on leukocytes (mainly neutrophils) where in nanomolar concentrations it elicits chemotaxis, adherence and aggregation. As one of the most potent chemoattractants discovered to date, LTB<sub>4</sub> modulates immune responses and participates in host defense against infection and injury. LTB<sub>4</sub> can be thought of as an eicosanoid involved in developing and maintaining inflammatory reactions. LTB<sub>4</sub> is also involved in certain pathological conditions because of overproduction.

The purpose of this study was to optimize the assay conditions for LTB<sub>4</sub> production by heterophils from chickens and PMNs from horses and dogs under a variety of experimental conditions.



**Figure 1.** Release of arachidonic acid and eicosapentaenoic acid from membrane phospholipids and their metabolism to eicosanoids.

## Materials and Methods

**Blood collection.** Whole blood was collected from chickens, dogs, and a horse. Blood was collected from the jugular vein into plastic tubes containing 2% EDTA (Sigma).

**Isolation of Neutrophils.** Briefly, whole blood was layered over Histopaque 1119 and then centrifuged for 30 min at 700x g. After removing the PMN band, RBCs that remained were lysed with buffered 0.83% NH<sub>4</sub>Cl until contaminating RBCs were no longer visible. After this, PMNs were washed twice with HBSS without CaCl<sub>2</sub> (Gibco). Cells were resuspended in HBSS with 0.8 mM CaCl<sub>2</sub>. An aliquot of the cell suspension was used to count the cells with a Coulter counter.

**Stimulation of Neutrophils for LTB<sub>4</sub> Production.** Aliquots of 5x10<sup>6</sup> cells were transferred to plastic tubes and HBSS with 0.8 mM CaCl<sub>2</sub> was added to achieve a final volume of 475 µl. Before stimulation, aliquots of cells were pre-incubated at 37°C for 10 minutes. To initiate leukotriene production, 25 µl of calcium ionophore A23187 (Sigma) was added to each tube. Control tubes received 25 µl of 0.2% DMSO in HBSS without CaCl<sub>2</sub>. Control and calcium ionophore stimulated cells were incubated for 10 min at 37°C in a shaking water bath. After incubation, 2 ml of ice-cold methanol was added to each tube to terminate the reaction. Cells were chilled for 20 minutes on ice. Finally, tubes were centrifuged for 5 min at 1000x g at 4°C to pellet cellular debris. Supernatants were transferred to another tube and were stored at -80°C until subsequent LTB<sub>4</sub> analysis.

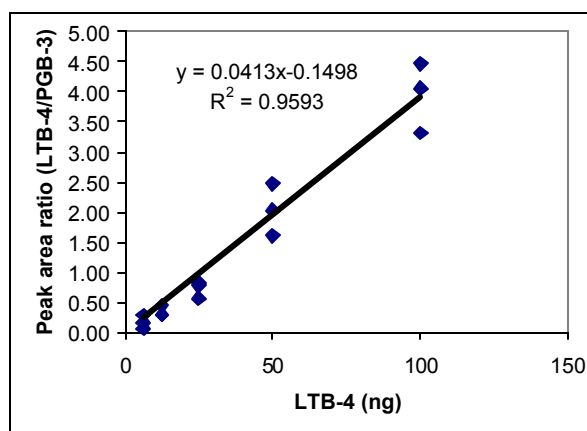
**Extraction and Separation of LTB<sub>4</sub>.** LTB<sub>4</sub> was extracted and separated using a modified version of the methods described by Terano, *et. al.*, and Vaughn, *et. al.*, (1994). Briefly, stored supernatants were centrifuged for 15 min at 400x g. Supernatants were transferred to 15 ml graded conical tubes containing 100 ng of PGB<sub>3</sub> as an internal standard. Freshly prepared citrate buffer was added to bring the volume up to ~14.5 ml.

A 12-ml syringe was attached to a C-18 solid phase extraction cartridge and 5 ml of HPLC-grade methanol was passed through the cartridge for pre wetting the cartridge. Samples were then loaded onto the cartridge by gravity flow and leukotrienes were absorbed onto the extraction column. The cartridges were rinsed with 5 ml of double deionised H<sub>2</sub>O followed by 5 ml of HPLC-grade hexane. Leukotrienes were

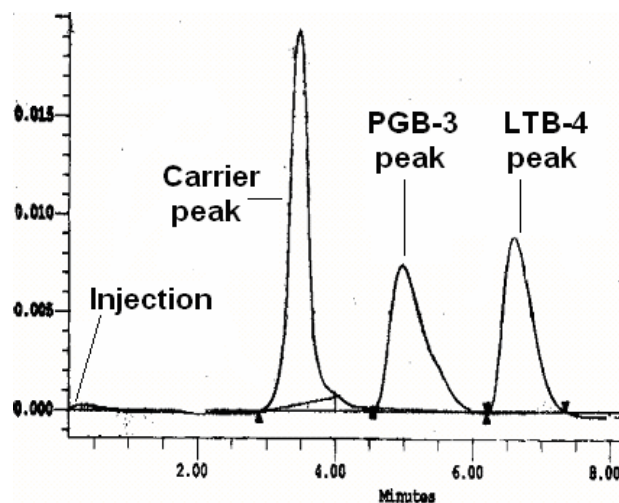
then eluted from the column, using gravity flow, with 5 ml of a 90:10 mixture of methanol and ddH<sub>2</sub>O into 5 ml plastic test tubes. The eluted samples were placed in a 30°C water bath and solvent was evaporated under a stream of N<sub>2</sub>. After evaporation, the residues were reconstituted in 125 µl of mobile phase made up of methanol: water: glacial acetic acid (75:25:01) and pH was adjusted to 5.7 with NH<sub>4</sub>OH. Samples were capped with N<sub>2</sub> and stored at -80°C until leukotrienes were separated by HPLC.

**Quantitation of Leukotrienes by RP-HPLC.** LTB<sub>4</sub> was separated by HPLC using a C-18 reverse-phase column fitted with a pre-column C-18 guard column. The mobile phase was methanol:water:glacial acetic acid (75:25:01), with pH adjusted to 5.7 with NH<sub>4</sub>OH. The flow rate of the pump was set at 0.7 ml/min and the variable wavelength UV detector was set at 270 nm.

A standard calibration curve for LTB<sub>4</sub> was made by adding 100 ng of PGB<sub>3</sub> as an internal standard to samples containing 6.25 to 100 ng of LTB<sub>4</sub>. The standard solutions were extracted as above and LTB<sub>4</sub> was detected by HPLC. The peak area ratio for LTB<sub>4</sub>/PGB<sub>3</sub> was calculated and plotted against the concentration of LTB<sub>4</sub> (Figures 2 and 3). The concentrations of leukotriene in test samples were calculated with reference to the standard curve.



**Figure 2.** Standard curve for LTB<sub>4</sub> using PGB<sub>3</sub> as internal standard, after extraction and RP- HPLC.

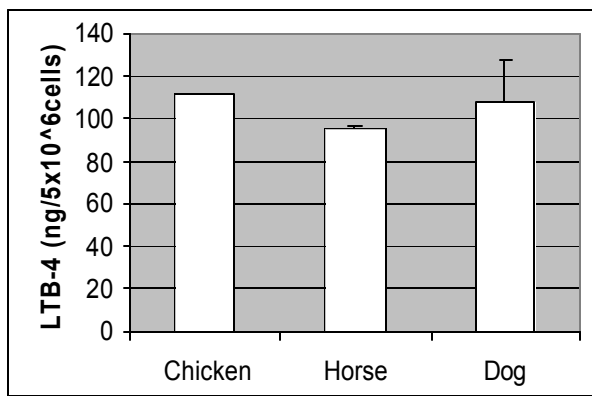


**Figure 3.** Reverse-phase HPLC separation of a mixture containing 100 ng of PGB<sub>3</sub> and LTB<sub>4</sub>. The HPLC conditions are described in the text.

## Results and Discussion

Chicken heterophils showed maximal production of LTB<sub>4</sub> upon stimulation with 20 µM calcium ionophore. This represented a 100 fold increase over unstimulated cells. Equine PMNs stimulated with calcium ionophore A23187 at 20 µM concentration showed similar results. Similarly, canine neutrophils showed highest production of LTB<sub>4</sub> after calcium ionophore A23187 stimulation at 20 µM. In all species, 10 min incubation time, 37°C, and 20 µM calcium ionophore concentration resulted in optimum LTB<sub>4</sub> production ( $P < 0.05$ ). A comparison of LTB<sub>4</sub> produced from these three species under these conditions is summarized in Figure 4.

The results of this study indicate that regardless of species of origin, PMNs and heterophils readily synthesize and release large quantities of LTB<sub>4</sub> following stimulation with low micromolar concentrations of calcium ionophore A23187. These results are similar to what has previously been reported in the dog (Amalsadvala, *et. al.*, 1992). A dose-response increase in calcium ionophore A23187 concentration resulted in a dose-response increase in 5-lipoxygenase activity as reflected by increased LTB<sub>4</sub> concentrations, which were 100 fold higher than in nonstimulated PMNs.



**Figure 4.** Effect of calcium ionophore on LTB<sub>4</sub> synthesis in three species. Neutrophils were incubated with calcium ionophore A23187 at 20  $\mu$ M for 10 min and 37°C. The values are mean  $\pm$  SEM.

Activation of 5-lipoxygenase activity was also maximal at 37°C.

The data obtained from the time-response experiments indicate that the time for activation of phospholipase A<sub>2</sub> with subsequent liberation of AA and further metabolism by 5-lipoxygenase and LTA<sub>4</sub>-hydrolase to produce LTB<sub>4</sub> occurs very quickly (within 10 minutes).

### Implications

Optimization of health and productivity is important in companion as well as production animals. Eicosanoids such as LTB<sub>4</sub> are involved in inflammatory responses and affect progression of disease in addition to productivity. Methods for assaying leukotrienes

will help animal scientists in understanding the role of pathogens and nutrients in disease prevention (Wander *et. al.*, 1997). Further research on the role of dietary nutrients in modulating leukotriene production is warranted.

### Literature Cited

- Amalsadvala, T. M., and D.M.Vaughn.  
1992. Characterization of leukotriene B<sub>4</sub> synthesis in canine polymorphonuclear leukocytes. *Prostaglandins Leukot. and Essent. Fatty Acids.* 45:283–288.
- Terano, T., J. A. Salmon, and S. Moncada.  
1984. Effect of orally administered eicosapentaenoic acid (EPA) on the formation of leukotriene B<sub>4</sub> and leukotriene B<sub>5</sub> by rat leukocytes. *Biochem. Pharmacol.* 33(19):3071–3076.
- Vaughn, D. M., G. A. Reinhardt, S. F. Swaim, S. D. Lauten, C. A. Garner, M. K. Bourdeaux, J. S. Spano, C. E. Hoffman, and B. Conner. 1994. Evaluation of effects of dietary n-6 to n-3 fatty acid ratios on leukotriene B synthesis in dog skin and neutrophils. *Veterinary Dermatology.* 5(4):163-173.
- Wander, R.C., J. A. Hall, J. L. Gradin, S. Du, and D. E. Jewell. 1997. The ratio of dietary (n-6) to (n-3) fatty acids influences immune system function, eicosanoid metabolism, lipid peroxidation and vitamin E status in aged dogs. *J. Nutr.* 127:1198-1205.

## EFFECTS OF INCORPORATING AN ANTIBIOTIC "AVILAMYCIN" AND A PROBIOTIC "ACTIVIS" IN BROILER DIETS

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**ABSTRACT:** A study on the effects of incorporating additives into the diets of growing broilers was conducted over a period of 50 days. Four groups of 100 birds each were divided into four treatments with 4 replication (25 x 4). Animals were 1 day old at the beginning of the experiment. Each group received one of 4 diets: A) maize + soybean; B) maize + soybean + avilamycin; C) maize + soybean + activis; and D) maize + soybean + avilamycin + activis.

Body weight (kg), food conversion index (g food intake/ g weight gain), daily gain (g/d/bird), dry and organic matter digestibilities (%), nitrogen retention (%), carcass yield (%), abdominal fat%, and gizzard weight (g) were determined by growth period for the four diets.

Broilers receiving diet D had the lowest ( $p < 0.05$ ) daily gain (42.6) and the highest ( $p < 0.05$ ) feed conversion index (2.43) among all groups. The heaviest gizzard (42.2) and the lowest ( $p < 0.05$ ) abdominal fat% (1.84) were observed for the C and B diets, respectively. Organic matter (73.61) and dry matter (67.45) digestibilities and nitrogen retention (60.93) were the highest for the C diet.

It appears that activis might replace avilamycin in broiler diets. These two additives should however not be given together to chicks.

**Key Words :** Broiler, Antibiotic, Probiotic

### Introduction

The use of antibiotics in animal nutrition to enhance growth performances has been practised for long time (Pontes and Castello, 1995, Boyd, 1994). The search for additives that improve growth of animals and lower risks from side effects on consumer health have been investigated (Wiedmer and Hadorn, 1999). Probiotics (direct-fed microbials) have been proven effective in broiler nutrition and might replace antibiotics in diet formulation (Buenrostro and Kratzer., 1983; Watkins and Kratzer, 1984; Goodling *et al.*, 1987; Baba *et al.*, 1991).

The objective of this study was to investigate the effectiveness of incorporating activis into broiler diets as a replacement for avilamycin.

### Materiel and methods

**Animals and diets:** This study was conducted at the firm of ESA Mateur on 400 birds of the Arbor-Acres breed. Environmental and habitat conditions for the breed were

respected. Four groups of 100 birds each were divided into four treatments with 4 replication. Each experimental unit included 25 birds.

Animals were 1 day old at the beginning of the experiment. Each group received one of 4 diets: A) maize + soybean; B) maize + soybean + avilamycin; C) maize + soybean + activis; and D) maize + soybean + avilamycin + activis. Contents of diets on maize and soybean were adjusted to follow birds requirements in three periods: starter, growth, and finisher diets. In addition to maize and soybean, rations were supplemented with minerals and vitamins according to the breed requirements.

Incorporation of avilamycin and activis varied with the age of animals (Table 1). Activis was derived by natural fermentation of cereals and is composed of *Saccharomyces cerevisiae*, *Aspergillum orizae*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus brevis*.

**Measures and diet effects:** Feed intake was measured on a daily basis while weights of birds were determined every week. Food conversion index (g food intake / g weight gain), body weight (kg), and daily gain (g/d/bird) were calculated every week on all experimental units. Birds were weighed individually throughout the whole experiment.

At the end of the experiment, 20 birds per diet were slaughtered to measure carcass yield (%), abdominal fat%, and gizzard weight. Measures of digestibility (%) were collected on 36 birds (9 birds per diet) at 36 days of age for dry and organic matters. Nitrogen retention (%) was also determined. Dry and organic matters and nitrogen were determined by AOAC methods (1990).

Deaths were registered as they occurred by diet and by growth period.

The effects of diets (A, B, C, and D) on growth performances, food conversion index, carcass yields, and dry and organic matter digestibilities were tested by a one way ANOVA (SAS, 1989). Mean diet effects were then compared by LSD.

### Results

Effects of diets on daily gain and food conversion index are summarised in Table 2. Estimated daily gains ranged from 13 g in the first week to 70 g in the fifth week for the B diet. Daily gains of birds receiving diets

including either avilamycin or activis were comparable after the first three weeks of age. Microorganisms (*Saccharomyces cerevisiae*, *Aspergillum orizae*, *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus brevis*) in activis and its other contents seem to favour growth of young chicks (1-21 days of age) compared to avilamycin (44.0 g/b/d vs 40.8 g/b/d). A similar result was reported by Gary *et al.* (1994) working on growing poult fed two diets: control and with *Saccharomyces cerevisiae* diets. The lowest daily gains were observed for the diet including both the antibiotic and the probiotic over the whole experimental period (Table 2). This result was clear in the aggregate daily gain (42.6 g/d) for the 50 days period. Birds receiving the control diet had growth performances comparable to birds receiving either of the two additives. Gary *et al.* (1994) found no significant differences between the effects of a control diet and a diet that included *Saccharomyces cerevisiae* on weight gain and feed consumption of birds aged 21 to 35 days.

Food conversion index tended to increase with the age of birds. This index ranged from 1.17 to 3.87 and was comparable for the four diets until the end of the 5<sup>th</sup> week of age. From day 36 to the end of the experiment, food conversion index was the highest ( $p<0.05$ ) for the D diet supplemented by both activis and avilamycin.

Studies (Buenrostro and Kratzer, 1983; Watkins and Kratzer, 1984; Roth and Kirchgesser, 1986; Radecki *et al.*, 1992, Ignacio, 1995) on the effects of incorporating probiotics in the rations of broilers reported improvements in growth and reduction of food conversion index in the 1 to 28 d, 1 to 35 d, and 1 to 49 d phases in growth experiments.

Means of dry and organic matter digestibilities are given in Table 3. These digestibilities ranged from 62.18 to 67.45% and from 68.84 to 73.51% for dry and organic matter, respectively. Both dry and organic matter digestibilities were the highest ( $p<0.05$ ) for the diet including the activis among all diets. Incorporating avilamycin in the diet did not improve dry and organic matter digestibilities compared to the control diet. The diet D, including both the probiotic and the antibiotic had intermediary measures of digestibility.

Nitrogen retention was clearly the highest for the regime including activis (60.9%) among all diets. That of the diet including the antibiotic was the lowest (48.1%). While the control and D diets had intermediary retention values. This result implies that activis might have improved nitrogen retention of growing broilers. Nahashon *et al.* (1994) reported that feeding 1,100 ppm *Lactobacillus* diets to Loghorn layers increases nitrogen retention.

Body weight of birds at slaughter varied from 2.4 to 2.8 kg (Table 3). Carcass yield was comparable among all diets (Table 3) and was around 70 to 74% with a tendency of higher yields for the control and activis diets. The probiotic diet resulted in the lowest abdominal fat% (1.84%). While the highest abdominal fat% was 2.38% measured on birds receiving the control diet. The

B and D diets were associated with intermediary abdominal fat%.

The gizzard weight varied ( $p<0.05$ ) also with the diet. It ranged from 36.2 g to 42.2 g for the antibiotic (B) and activis (C) diets, respectively.

Deaths of birds collected during the whole period were identical for the four diets. They were around 2.5%

## Discussion

This study on replacement of avilamycin by activis revealed that growth performances might be improved by the use of the probiotic in the broiler diets. This improvement is essentially felt in the early in the growth period when chicks begin to develop their lean tissues. In addition, food conversion index tended to lower when activis was incorporated into the ration. The antibiotic however seems to be more efficient for growth in the finishing phase (37-50 days). Wiedmer and Hadorn (1999) reported small but non-significant improvement in animal weights, feed conversion rate, and litter quality of Ross Hybrid chicks fed diets supplemented with either a probiotic or an antibiotic compared to a control diet up to 41 days.

Activis increases digestive utilisation of feed and improves growth performances of broilers by stimulating enzymatic activities such as protease and consequently improve digestibility of dry and organic matters in the diets. Microorganisms and activis constituents may act to reduce abdominal fat and favour carcass lean tissue (Radecki *et al.*, 1992) and gizzard weight. Gary *et al.* (1994) advanced that the ileal sections of male poult fed with 0.02% *Saccharomyces cerevisiae* var. *boulardii* supplemented diet showed a decrease in the number of goblet cells per mm of villus height and in crypt depth. Energy conserved by the reduced turn over rate of epithelial cells may be converted to lean tissue mass (Radecki *et al.* 1992). Activis includes lactic acid and has a low pH (3). It therefore may inhibit the proliferation of pathogenic bacteria. Line *et al.* (1995a) found that *Saccharomyces cerevisiae* var. *boulardii* has the ability to reduce populations of *Salmonella typhimurium* and *Campylobacter jejuni*. In another study, the same authors (Line *et al.* 1995b) reported that isolated *Lactobacillus* spp (Crave, 1995) from intestine of chicks tracts inhibit adhesion of *Salmonella typhimurium* to intestinal mucus of chicks. Furthermore, avilamycin action is antagonistic to activis effects. Avilamycin may limit probiotic microorganisms' actions and consequently decreases chicken growth performances.

Birds in the control diet performed similarly to birds receiving activis. This might be explained by the good and controlled conditions where the experiment was conducted. Control birds, on the other hand, had the highest abdominal fat%.

## Implications

Consumers are reluctant regarding the usage of antibiotics in animal nutrition because of their probable undesirable side effects on health. These additives might be replaced by probiotics in non-ruminant nutrition. Activis seems to improve growth, carcass yield and favor development of lean tissues. Effects of this probiotic might be explained by its content on yeast and *Lactobacillus*. These microorganisms are associated with the improvement dry and organic matter digestibilities and the increase of nitrogen retention rate.

Combining activis and avilamycin in broiler diets would probably have depressive effects on broilers' performances.

## Literature Cited

1. AOAC. 1990. Official methods of analysis (15<sup>th</sup> Ed). Association of Official Analytical Chemists, Arlington, VA, USA.
2. Baba, E., S. Nagaishi, T. Fukuta, and A. Arakawa. 1991. The role of intestinal microflora on the prevention of *salmonella* colonisation in gnotobiotic chickens. J. Poultry Sci. 70:1902-1907.
3. Boyd, H. L. 1994. Good manufacturing practices. J. Poultry Sci. 73:1419-1422.
4. Buenrostro, J. L. and F. H. Kratzer. 1983. Effects of Lactobacilli inoculation and antibiotic feeding of chickens on available of dietary biotin. J. Poultry Sci. 62:2022-2029.
5. Craven, S. E. 1995. Inhibition of mannose-resistant adhesion of *Salmonella thyphimurium* to chicken cecal mucus by *Lactobacillus spp.* Poultry Sci. 74 (Supplement1): 162.
6. Gary, L. B., T. F. Savage, and K. I. Timm. 1994. The effects of supplementing diets with *Saccharomyces cerevisiae* var. *Boulardii* on male poult performance and ideal morphology. J. Poultry Sci. 73:1766-1773.
7. Goodling, A. A., G. J. Cerniglia, and J. A. Hebert. 1987. Production performance of white Leghorn layers fed *Lactobacillus* fermentation products. J. Poultry Sci. 66:480-486.
8. Ignacio, E. D. 1995. Evaluation of the effect of yeast culture on the growth performance of broiler chickens. J. Poultry Sci. 74 (Supplement 1):196 (Abstr.).
9. Line, E. J., N. J. Stern, N. A. Cox and J.S. Bailey. 1995a. Treatment of feed with *Saccharomyces boulardii* for reduction of *Salmonella* populations in broiler chickens. J. Poultry Sci. 74 (Supplement 1): 49(Abstr.).
10. Line, E. J., N. J. Stern, N. A. Cox and J.S. Bailey. 1995b. *Saccharomyces* treatment to diminish *Campylobacter* and *Salmonella* in chickens. J. Poultry Sci. 74 (Supplement 1):201(Abstr.).
11. Nahashon, S. N., H. S. Nakaue, and L. W. Mirosh. 1994. Production variables and nutrient retention in single comb white leghorn laying pullets fed diets supplemented with direct-fed microbials. J. Poultry Sci. 73:1699-1711.
12. Pontes, P. M. and J. A. Castello. 1995. Alimentacion de las aves. Grinver – Arts graphiques, S. A., Barcelona, Spain.
13. Radecki, S. V., P. K. Ku, M. R. Bennink, M. T. Yokoyama, and E. R. Miller. 1992. Effect of dietary copper on intestinal mucosa enzyme activity, morphology, and turn over rates in weanling pigs. J. Animal Sci. 70:1424-1431.
14. Roth, F.X. and M. Kirchgessner. 1986. Zur nutritiven Wirksamkeit von *Streptococcus faecium* (Stamm M 74) in der Kükenmast. Arch. Geflügelk. 50:225-228.
15. SAS Institue. 1989. SAS/STAT User's Guide. Version 6, Fourth Edition. Vol. 2. Cary, NC.
16. Watkins, B. and F. H. Kratzer. 1984. Drinking water treatment with a commercial preparation of a concentrated *Lactobacillus* culture for broiler chickens. J. Poultry Sci. 63:1671-1673.
17. Wiedmer H. and R. Hadorn. 1999. Les effets de différents additifs alternatifs dans les aliments d'engraissement comparés avec l'effet d'un antibiotique et un témoin zero. 3<sup>e</sup> Journées de la recherche avicole, pp. 109-111, Saint-Malo, France.

Table 1. Contents of diets on avilamycin and activis by growth period.

Additive	Age in days		
	1-5	6-15	16-49
Antibiotic (g/kg diet)	5	5	10
Probiotic (g/kg diet)	5	3	1

Table 2. Means\* of daily gain (g/d/bird) and food conversion index (%) by growth period (in days) for the four diets A, B, C, and D.

Item	Diet	Growth period in days							
		1-8	9-15	16-22	23-29	30-36	37-43	44-50	1-50
Daily gain(g/b/d)	A	14.1a	26.5a	43.2a	57.1a	71.3a	67.0a	68.0a	49.7a
	B	13.1b	26.0a	40.8b	55.8a	70.0a	63.2a	62.1b	47.1a
	C	14.0a	26.5a	44.0a	55.6a	67.4b	62.5a	59.5b	46.9a
	D	13.5a	25.3a	39.4b	54.5a	65.0b	52.0b	50.9c	42.6b
n		400	397	395	394	392	391	389	389
SE		0.21	0.54	0.78	1.00	1.46	1.72	2.36	0.78
Food conversion index (%)	A	1.13a	1.42a	1.77a	2.13a	2.39a	2.52a	3.36a	2.21a
	B	1.13a	1.33a	1.85a	2.41b	2.24a	2.64a	2.38b	2.27a
	C	1.17a	1.62a	1.76a	2.27a	2.03a	2.88a	3.33a	2.24a
	D	1.17a	1.45a	1.82a	2.21a	2.46a	3.87b	3.56a	2.43b
n		400	397	395	394	392	391	389	389
SE		0.02	0.04	0.06	0.06	0.06	0.06	0.02	0.04

A: maize + soybean; B: maize + soybean + avilamycin; C: maize + soybean + activis; and D: maize + soybean + avilamycin + activis.

\*Means with different superscripts for the same variable in the same column are significantly different ( $p < 0.05$ ).

\*n: Total number of observations.

Table 3. Means\* of dry and organic matter digestibilities, nitrogen retention, and main carcass measures.

Item	Diet				n	SE
	A	B	C	D		
Body weight (kg)	2.799 <sup>a</sup>	2.752 <sup>a</sup>	2.762 <sup>a</sup>	2.425 <sup>b</sup>	80	0.03
Carcass yield(%)	73.87 <sup>a</sup>	70.84 <sup>a</sup>	73.10 <sup>a</sup>	71.42 <sup>a</sup>	80	0.56
Abdominal fat%	2.38 <sup>a</sup>	2.17 <sup>a</sup>	1.84 <sup>b</sup>	1.93 <sup>b</sup>	80	0.07
Gizzard weight (g)	40.2a	36.2c	42.2a	38.3b	80	0.75
Digestibility (%):						
Dry matter	64.83a	62.18b	67.45a	64.48b	36	0.75
Organic matter	70.76a	68.84b	73.51c	71.41a	36	0.61
Nitrogen retention (%)	50.67a	48.1a	60.93b	52.1c	36	1.10

A: maize + soybean; B: maize + soybean + avilamycin; C: maize + soybean + activis; and D: maize + soybean + avilamycin + activis.

\*Means with different superscripts for the same variable in the same line are significantly different ( $p < 0.05$ ).

\*n: Total number of observations.

## ALFALFA HAY STORAGE IN THE SONORAN DESERT

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**ABSTRACT:** The irrigated Sonoran Desert of southeastern California and southwestern Arizona is the hottest habitable part of the US. Hay stored for prolonged periods of time may increase in NDF and ADF content. Stored hay that overheats may have decreased DM and CP digestibility. In June, 1998, at the University of California Desert Research and Extension Center we baled alfalfa (*Medicago sativa*) hay and stored the hay for 21 weeks under three storage methods: T1) stored uncovered, T2) stored under a roof, and T3) covered with a plastic tarp. At the end of the storage period we took the hay to the agriculture school of the Autonomous University of Baja California and fed the hay to three 150 kg fistulated (rumen, ileum, and duodenum) Holstein steers. After 11 weeks of storage, bale temperatures were 42.2, 36.9, and 41.7° for T1, T2, and T3 hays; respectively. After 11 weeks of storage, ADIN/DM% increased from 0.10 to 0.51, 0.51, and 0.56% for T1, T2, and T3; respectively. ADIN/N increased from 2.59% to 12.79, 13.56, and 15.56% for T1, T2, and T3, respectively; suggesting the formation of Maillard products. Total tract DM digestion for T1, T2, and T3 was 59.5, 66.2, and 64.7% and for CP was 76.7, 81.2, and 80.6; respectively. Crude protein total tract digestibility was greater ( $P < 0.05$ ) for T2 and T3 than for T1. Similarly, DM digestibility for T2 and T3 was greater ( $P < 0.05$ ) than for T1. We conclude that unprotected alfalfa hay stored during the summer in the Sonoran desert may become heat damaged and thereby decrease in overall digestibility. Under the conditions of this study, both T2 and T3 treated hay had higher ( $P < 0.05$ ) overall digestibility values than T1 hay.

Key Words: alfalfa, Maillard products, heat-damage

### Introduction

Hays stored for prolonged periods of time lose DM and have increases in NDF% and ADF% (Rotz and Muck, 1994) and have reduced DM and CP digestion (Thomas et al., 1982). Hays stored at temperatures  $> 37.8^{\circ}$  may form Maillard products (Pitt, 1990). Maillard products in heat damaged hay may be quantified by measuring Kjeldahl N in ADF; ADIN (Goering et al., 1972). Either ADIN (Van Soest and Mason, 1991) formation or ADIN/N% increase (Thomas et al., 1982) may be used as measures of reduced CP digestibility and decreased livestock performance. Guerrero and Winans (1997) reported that alfalfa hay stored during the summer in the irrigated Sonoran Desert

increased in NDF%, ADF%, and ADIN/N%. The Sonoran Desert extends from Mexico into southeastern California and southwestern Arizona and is the hottest habitable part of the USA. During summer in the Sonoran Desert, temperatures  $> 43.3^{\circ}$  are common and temperatures  $> 48.9^{\circ}$  have been recorded. Due to the lack of rainfall, baled alfalfa hay commonly is stored unprotected. To quantify the effects of summertime heat on alfalfa hay quality we stored alfalfa under three different methods for 21 weeks during the summer of 1998 and measured chemical attributes of the hay during summer storage. We also fed the summer stored hay to Holstein steers to measure digestibility attributes.

### Materials and Methods

Alfalfa hay was baled on June 2, 1998 at the University of California Desert Research and Extension Center (UCDREC), located about 11 km east of El Centro, and for 21 weeks subject to three storage treatments: T1) stored uncovered, T2) stored under a roof, and T3) covered with a plastic tarp. Each treatment consisted of about two tons of baled hay. Every two weeks during the 21week storage period, four hay samples per treatment were taken using a hay coring device (Penn State Forage Sampler; NASCO, Modesto, CA). When hay was sampled at 1500, bale moisture and temperature were recorded (Delmhorst Instrument Co.; Towaco, NJ). Hay samples were dried for 72 h in an air conditioned room under forced air, ground using a 1 mm screen, stored in air-tight plastic bags and stored in an air conditioned room at a constant  $22^{\circ}$ . Hay samples were subjected to the following analyses: DM, Kjeldahl N (AOAC, 1980), NDF, ADF (Goering and Van Soest, 1970), and ADIN (Goering et al., 1972).

At the end of the hay storage period, the experimental hay was fed to 3 fistulated (rumen, ileum, and duodenum; Zinn and Plasencia, 1993) 150 kg Holstein steers at the Autonomous University of Baja California agriculture school, south of Mexicali. Fistulated steers were offered daily, at 0700 and 1900, *ad libitum* access to experimental hay over three rotational 14 d periods; a 10 d adaptation period and 4 d of sampling. Fifteen g of chromic oxide per d were administered to each steer in ground feed. Ileal and duodenal digesta were collected twice daily during the 4 d collection period and placed into 500 ml containers. On day 14, 500 ml of ruminal digesta was placed into plastic containers. Digesta samples were lyophilized, ground, and

stored. Fecal samples were placed in a forced air oven for 72 h at 50°, ground, and stored. Digesta and fecal samples were subjected to the following analyses: DM, Kjeldahl N (AOAC, 1980), Cr (Hill and Anderson, 1958), NDF, and ADF (Goering and Van Soest, 1970). Chemical attributes of stored hay were compared with paired t-tests (Steel and Torrie, 1960) and chemical attributes of digesta samples were analyzed as a 3 X 3 Latin square (Hicks, 1973).

## Results and Discussion

Mean monthly maximum temperatures from June to October, 1998, at UCDREC were 35.7, 41.7, 41.7, 37.5, and 31.2°; compared to long term mean monthly maximum temperatures of 41.1, 42.8, 41.7, 39.6, and 34.4° (Imperial Irrigation District, 1995); respectively, a cool summer, by Sonoran Desert standards. An electrical malfunction in the storage facility where September to October hay samples were stored resulted in mold formation and the loss of these samples. In June, hay was initially stored at 14% moisture; by late August the hays in T1, T2, and T3 had dried to 6, 8, and 7%; respectively (Table 1). Guerrero and Winans (1997) reported similar drying rates for alfalfa hay in similar locations. At baling, bale temperature of experimental hay was 26.5°. After 11 weeks of storage in the Sonoran Desert, bale temperatures were 42.2, 36.9, and 41.7° for T1, T2, and T3 hays; respectively. Hay stored at temperatures > 35° for prolonged periods decrease in nutritive value (Goering et al., 1973, Yu and Veira, 1977).

Initial NDF content of experimental hay was 33.98%. Eleven weeks later, NDF content for T1, T2, and T3 hays was 32.68, 36.89, 34.50%; respectively. Guerrero and Winans (1997) reported that after 11 weeks of storage in the Sonoran Desert, NDF increased from 1 to 2.5%. Rotz and Muck (1994) reported that after prolonged storage of hay, hay desiccation and loss of soluble carbohydrates result in NDF increases. Initial ADF content of experimental hay was 24.00%. Eleven weeks later, ADF content for T1, T2, and T3 hays was 19.63, 26.74, and 23.81%; respectively. Guerrero and Winans (1997) reported that after 11 weeks of storage in the Sonoran Desert, ADF increased from 1 to 2%. Rotz and Muck (1994) reported that as hay dries, ADF% increases.

After 11 weeks of storage, ADIN/DM% increased from 0.10 to 0.51, 0.51, and 0.56% for T1, T2, and T3; respectively. Alfalfa silage heated artificially for 48 h at 54.4° increased to 0.80% ADIN (Yu and Veira, 1977). Guerrero and Winans (1997) reported ADIN formation in alfalfa hay in the Sonoran Desert at 11 weeks of 0.50%. ADIN/N increased from 2.59% to 12.79, 13.56, and 15.56% for T1, T2, and T3, respectively; suggesting the formation of Maillard products (Thomas et al., 1982). Guerrero and Winans (1997), after 11 weeks of hay storage in the irrigated Sonoran Desert, had ADIN/N of 13, 17, and 17% for similar treatments. The summer when Guerrero and Winans (1997) did their baled hay storage trail, the summer of 1993, temperatures were 45.6, 45.0,

47.8, 44.4, and 40.6°, from June through October; respectively, warmer than the temperatures of this trial. Goering et al. (1972) suggested that forages greater than 14% ADIN/N may be considered heat damaged.

Ruminal digestion of DM for T1, T2, and T3 was 49.5, 59.2, and 53.4%; respectively (Table 2). Ruminal digestion of CP for T1, T2, and T3 was 46.9, 58.0, and 55.0%; respectively. Total tract DM digestion for T1, T2, and T3 was 59.5, 66.2, and 64.7%; NDF was 39.2, 53.2, and 47.2%; ADF was 33.0, 47.5, and 37.9%; and for CP was 76.7, 81.2, and 80.6; respectively. Crude protein total tract digestibility was greater ( $P < 0.05$ ) for T2 and T3 than for T1. Similarly, DM digestibility for T2 and T3 was greater ( $P < 0.05$ ) than for T1. From initial baling conditions (Table 1), all hays increased in bale temperature. In late August 1998, T2 bales (36.9°) were not as warm as T1 (42.2°) or T3 (41.7°) bales, although T3 bales were protected from direct sunlight by the tarp. Several authors have reported that heat-damaged hay decreases in overall digestibility (Goering et al., 1972; Goering et al., 1973, Yu and Veira, 1977; Thomas et al., 1982). We conclude that unprotected alfalfa hay stored during the summer in the Sonoran desert may become heat damaged and thereby decrease in overall digestibility. Under the conditions of this study, both T2 and T3 treated hay had higher ( $P < 0.05$ ) overall digestibility values than T1 hay.

## Implications

In the irrigated Sonoran Desert, during summer, the protection of hay bales from excessive heat is recommendable. Either the use of hay barns or plastic tarps may protect summer stored alfalfa hay in the irrigated Sonoran Desert from decreases in hay nutritive value.

## Literature Cited

- Assoc. of Official Analytical Chemists. 1980. Official methods of analysis (13<sup>th</sup> ed.). AOAC. Washington, D. C.
- Goering, H. K., C. H. Gordon, R. W. Hemken, D. R. Waldo, P. J. Van Soest, and L. W. Smith. 1972. Analytical estimates of nitrogen digestibility in heat damaged forages. *J. Dairy Sci.* 55:1275-1280.
- Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis. *USDA-ARS Agric. Handb.* 379.
- Goering, H. K., P. J. Van Soest, and R. W. Hemken. 1973. Relative susceptibility of forages to heat damage as affected by moisture, temperature, and pH. *J. Dairy Sci.* 56:137-143.
- Guerrero, J. N., and S. S. Winans. 1997. Summertime storage of alfalfa hay in the irrigated Sonoran Desert affects hay quality. *J. Prod. Agric.* 10:495-501.
- Hicks, C. R. 1973. *Fundamental concepts in the design of experiments.* Holt, Rinehart, and Winston. NY.
- Imperial Irrigation District. 1995. Weather data. Imperial,

- CA.
- Hill, F. N., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. *J. Nutr.* 64:587-603.
- Pitt, R. E. 1990. Silage and hay preservation. NRAES-5. NE Reg. Agric. Eng. Serv. Ithaca, NY.
- Rotz, C. A., and R. E. Muck. 1994. Changes in forage quality during harvest and storage. p. 828-868. *In* G. C. Fahey et al. (ed.) Forage quality, evaluation, and utilization. ASA, CSSA, and SSSA. Madison, WI.
- Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill. NY.
- Thomas, J. W., Y. Yu, T. Middleton, and C. Stallings. 1982. Estimations of protein damage. p. 81-98. *In* F. N. Owens (ed.) Protein requirements for cattle. Oklahoma State Univ. MP 109.
- Van Soest, P. J., and V. C. Mason. 1991. The influence of the Maillard reaction upon the nutritive value of fibrous feeds. *Anim. Feed Sci. Technol.* 32:45-53.
- Yu, Y., and D. M. Veira. 1977. Effect of artificial heating of alfalfa haylage on chemical composition and sheep performance. *J. Anim. Sci.* 44:1112-1118.
- Zinn, R. A., and A. Plasencia. 1993. Interaction of whole cottonseed and supplemental fat on digestive function in cattle. *J. Anim. Sci.* 71:11-17.

Table 1. Mean chemical attributes of alfalfa hay stored from June to November, 1998, in the irrigated Sonoran Desert.

	Initial	T1 <sup>†</sup>	August 25, 1998		
	Conditions <sup>*</sup>		T2	T3	SE
DM%	86.0	94.0	92.0	93.0	0.76
ADIN, %DM	0.10	0.51	0.51	0.56	0.02
N,%DM	3.90	3.95	3.76	3.72	0.54
ADIN/N,%	2.59	12.79	13.56	15.56	0.64
NDF%	33.98	32.68	36.89	34.50	1.73
ADF%	24.00	19.63	26.74	23.81	2.54
Bale temperature <sup>‡</sup>	26.50	42.20	36.90	41.70	2.69

<sup>\*</sup> hay baled June 2, 1998

<sup>†</sup> T1 = stored outside uncovered, T2 = stored outside under a roof and protected from sunlight; and T3 = stored outside covered with plastic tarp.

<sup>‡</sup> bale temperatures at 1500.

Table 2. Mean digestibility of nutrients of 150 kg Holstein steers consuming alfalfa hay stored from June to November, 1998, in the irrigated Sonoran Desert.

	Treatment <sup>*</sup>			SE
	1	2	3	
Consumption, kg/d				
DM	4.07	4.11	3.60	0.14
NDF	1.30	1.42	1.25	0.08
ADF	0.92 <sup>b</sup>	1.08 <sup>ab</sup>	0.88 <sup>b</sup>	0.11
CP	0.89 <sup>a</sup>	0.81 <sup>b</sup>	0.84 <sup>ab</sup>	0.03
Ruminal digestion, % DM consumption				
DM	49.5 <sup>b</sup>	59.2 <sup>a</sup>	53.4 <sup>ab</sup>	2.1
NDF	40.7 <sup>ab</sup>	48.0 <sup>a</sup>	32.4 <sup>b</sup>	6.0
ADF	35.7 <sup>ab</sup>	45.8 <sup>a</sup>	25.5 <sup>b</sup>	7.0
CP	46.9 <sup>b</sup>	58.0 <sup>a</sup>	55.0 <sup>a</sup>	2.6
Total digestion, % of consumption				
DM	59.5 <sup>c</sup>	66.2 <sup>a</sup>	64.7 <sup>b</sup>	0.7
NDF	39.2 <sup>b</sup>	53.2 <sup>a</sup>	47.2 <sup>ab</sup>	4.2
ADF	33.0 <sup>c</sup>	47.5 <sup>a</sup>	37.9 <sup>b</sup>	2.0
CP	76.7 <sup>b</sup>	81.2 <sup>a</sup>	80.6 <sup>a</sup>	0.3

<sup>\*</sup> 1 = stored outside uncovered, 2 = stored outside under a roof and protected from sunlight; and 3 = stored outside covered with plastic tarp.

<sup>a</sup> means within row with different superscripts differ, (P < 0.05) LSD.

# EFFECT OF SPECIES AND VARIETAL TYPE ON YIELD AND NUTRITIONAL QUALITY OF SMALL GRAIN FORAGE<sup>1</sup>

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**ABSTRACT:** Small grains are popular annual forages in the Great Plains. Oat (*Avena sativa*.) is the most popular, cool-season annual forage grown in the Northern Plains. However, barley (*Hordeum vulgare* L.) has been shown to produce equal or greater amounts of superior quality forage when compared to oat in sub-humid regions. The effects of forage species (oat [OAT] or barley [BAR]) and type (forage or grain) on forage yield and quality were evaluated in each of two years. Ten varieties were evaluated in 2002 (5 OAT, 3 forage barley [BRF] and 2 grain barley [BFG]) and 12 in 2003 (6 OAT, 2 BRF and 4 BRG). In 2002, CP concentration (13.5 and 12.0%;  $P = .02$ ) was greater in BRG than BRF. Concentrations of ADF, NDF, TDN (estimated from ADF) and IVDMD and yields of DM, CP or IVDMD did not differ between barley types ( $P > .15$ ). Concentrations of CP (12.7 and 11.5%;  $P = .01$ ) and IVDMD (66.6 and 58.6%;  $P = .01$ ) and yields of DM (2749 and 1854 kg/ha;  $P < .01$ ), CP (355 and 209 kg/ha;  $P < .01$ ) and IVDMD (1856 and 1069 kg/ha;  $P < .01$ ) were greater in BAR than OAT. Concentrations of ADF, NDF and TDN did not differ ( $P > .5$ ) between BAR and OAT. In 2003, forage type did not affect ( $P > .14$ ) any yield or quality parameter. Concentrations of ADF (40.7 and 42.3%;  $P = .01$ ), NDF (60.5 and 63.9%;  $P < .01$ ) and TDN (57.2 and 56.0%;  $P = .01$ ) were reduced, and IVDMD (59.7 and 55.1%;  $P < .01$ ), increased in BAR compared to OAT. CP concentration and yields of DM, CP and IVDMD were not affected ( $P > .3$ ) by forage species. In both years, the ratio of IVDMD to TDN did not differ between forage types ( $P > .7$ ), but was greater in BAR than OAT (1.01 and .93;  $P < .01$ ). These data suggest that forage type barley varieties are not superior to grain type varieties in forage production. However, barley forage is of superior quality, and may produce as much or more forage, when compared to oat in the Northern Plains.

Key Words: Oat, Barley, Annual Forage

## Introduction

Small grains are popular annual forages in the Northern Great Plains. While oat is the most popular, cool-season annual forage, barley produces higher-quality forage

when compared to oat in more humid regions (Cherney and Martin, 1982). Barley forage contained greater concentrations of DDM and CP and lower concentrations of ADF. The CP concentration of barley and barley-pea (*Pisum sativum*) forage was superior to that of oat and oat-pea forage in southwestern North Dakota (Carr et al., 1998).

Barley forage yield has been equal to or superior to oat forage, whether grown alone (Cherney and Martin, 1982) or with pea as a companion crop for alfalfa (*Medicago sativa* L.) establishment (Chapko et al., 1991). However, barley forage yield has been inconsistent when compared with oat in Northern Great Plains.

Additional research is needed to determine the forage potential of barley and oat in the more arid regions of the Northern Great Plains. The objective of this experiment was to compare the effects of forage species (barley or oat) and varietal type (forage or grain) on forage yield and quality in southwestern North Dakota.

## Materials and Methods

In each of two years, oat (OAT) and barley (BAR) varieties were seeded into replicated plots ( $n = 3$ ) in a randomized complete block design in a producer field southwest of Beach, North Dakota. In year 1, five grain OAT (Derby, Killdeer, Otana, Paul and Whitestone), two grain barley (BRG; Conlon and Robust) and three forage barley (BRF; Haybet, Horsford and Westford) varieties were evaluated. In year 2, six OAT (Derby, Hy-Test, Killdeer, Morton, Paul and Whitestone), four BRG (Conlon, Drummond, Logan and Robust) and two BRF (Haybet and Washford) were evaluated.

Two harvest dates were used each year in an attempt to harvest forage in individual plots at the soft dough stage of development. At harvest, actual stage of development was recorded and forage sampled from the interior rows of the entire plot using a mechanical forage harvester. Plot width, length and weight of wet forage sample were recorded and a subsample of forage retained for DM and nutrient analysis.

Forage subsamples were dried at 55°C, ground and split into two portions. One portion was submitted to a commercial laboratory for determination of DM, CP, ADF and NDF. In year 1, samples were submitted to Servi-Tech Laboratories (Hastings, NE) for analysis using NIR. In year 2, samples were submitted to Dairyland Labs (Sauk Rapids, MN) for analysis using wet chemistry methodologies. Both laboratories also reported TDN concentration and relative feed value (RFV) calculated from analyzed fiber concentrations. In both years, the remaining portion of each

<sup>1</sup> Funds for nutrient analysis were provided through a grant from the North Dakota State Board of Agricultural Research and Education. Mention of a proprietary product does not constitute a guarantee or warranty of the product by ND SBARE, ND AES or the authors and does not imply its approval to the exclusion of other products that may also be suitable.

sample was submitted to the Nutrition Laboratory in the Department of Animal and Range Sciences at North Dakota State University for IVDMD analysis.

Dry matter yield was calculated as wet weight multiplied by DM concentration. Yields of CP and IVDMD were calculated as DM yield multiplied by CP and IVDMD concentrations, respectively.

Data from each year were analyzed separately using a general linear model procedure (PROC GLM, SAS Inst., Cary, NC) and a randomized complete block design. The model included effects of block, treatment (OAT, BRG and BRF) and variety within treatment. Significant treatment effects were described using two orthogonal contrasts (OAT vs BAR and BRG vs BRF).

## Results

In year 1 (Table 1), stage at harvest was not different between BAR and OAT ( $P = 0.74$ ). While CP ( $P = 0.01$ ) and IVDMD ( $P < 0.01$ ) concentrations and yields of DM ( $P < 0.01$ ), CP ( $P < 0.01$ ) and IVDMD ( $P < 0.01$ ) were greater in BAR, concentrations of ADF ( $P = 0.50$ ), NDF ( $P = .84$ ) and TDN ( $P = .51$ ) did not differ between forage species. Although BRF was slightly more mature at harvest ( $P = 0.03$ ), CP concentrations ( $P = 0.02$ ) were reduced in BRF compared to BRG. Other quality and yield measurements were not different ( $P \geq 0.19$ ) between BRG and BRF.

In year 2 (Table 2), BAR was more mature at harvest ( $P < 0.01$ ), had lower ADF ( $P = 0.01$ ) and NDF ( $P < 0.01$ ) concentrations and had greater TDN ( $P = 0.01$ ) and IVDMD ( $P < 0.01$ ) concentrations compared to OAT. Concentrations of CP ( $P = .91$ ) and yields of DM ( $P = .48$ ), CP ( $P = .58$ ) and IVDMD ( $P = .33$ ) did not differ between BAR and OAT. With the exception of stage at harvest ( $BRG > BRF$ ;  $P = .01$ ), there were no differences ( $P \geq 0.14$ ) between BRG and BRF.

In both years (Tables 1 and 2), the ratio of IVDMD to TDN did not differ between BRG and BRF ( $P > .70$ ). However, the ratio ( $P < 0.01$ ) was greater in BAR compared to OAT in both years and averaged 1.01 and 0.93 for BAR and OAT, respectively.

## Discussion

Although increased forage production would be a possible result of the development of forage type cultivars, these data would not suggest an advantage in yield or quality characteristics to forage type barley cultivars when compared to grain type cultivars.

Barley forage yield has been reported to be equal to or superior to oat forage when grown in a sub-humid environment (Cherney and Martin, 1982; Chapko et al., 1991). This is in agreement with this study where barley produced more forage in the first year and similar amounts of forage in the second year. However, it is in contrast to Carr et al. (2002) where oat consistently yielded more forage than barley.

Barley has been shown to produce higher quality forage than oat when grown in a sub-humid environment

(Cherney and Martin, 1982). Better quality was suggested based upon greater concentrations of DDM and CP and lower concentrations of ADF in barley forage. Carr et al. (1998) also reported higher CP concentrations in barley forage in southwestern North Dakota. Differences in CP, ADF and NDF concentrations between barley and oat forage are less clear in this study. Barley forage produced a greater CP concentration in the first year and lower fiber concentrations in the second year. Whereas in the opposing year, concentrations were determined to be not different.

Concentrations of ADF and NDF are routinely used to estimate energy concentration (TDN) and potential intake of DDM (RFV) in forages (NFTA, 2004). As was the case in this study, differences in TDN and RFV would be expected to mirror consistent differences in forage fiber concentrations.

Irrespective of the differences in ADF and TDN concentrations, BAR consistently produced greater IVDMD concentrations and IVDMD to TDN ratios. This would imply that the relationship of forage digestibility to fiber concentration is not consistent among barley and oat forages.

## Implications

These data suggest that forage-type barley varieties are not superior to grain-type varieties in forage production in the Northern Great Plains. Nonetheless, barley forage is higher quality, and has the potential to produce as much or more forage, when compared directly to oat forage.

## Literature Cited

- Carr, P.M., G.B. Martin, J.S. Caton and W.W. Poland. 1998. Forage and nitrogen yield of barley-pea and oat-pea intercrops. *Agron. J.* 90:79-84.
- Carr, P.M., R.D. Horsley and W.W. Poland. 2002. Barley and oat as dryland forage in the Northern Great Plains. 2003 Annual Report. Dickinson Research Extension Center, North Dakota State University. (<http://www.ag.ndsu.nodak.edu/dickinson/research/2002/agron02e.htm>; accessed April 3, 2004)
- Chapko, L.B., M.A. Brinkman and K.A. Albrecht. 1991. Oat, oat-pea, barley and barley-pea for forage yield, forage quality and alfalfa establishment. *J. Prod. Agric.* 4(4):486-491.
- Cherney, J.H. and G.C. Marten. 1982. Small grain crop forage potential: I. Biological and chemical determinants of quality, and yield. *Crop Sci.* 22:227-231.
- NFTA. 2004. Forage analysis procedures. Appendix A – calculated values. National Forage Testing Association. ([http://www.foragetesting.org/lab\\_procedure/appendixa.html](http://www.foragetesting.org/lab_procedure/appendixa.html); accessed March 5, 2004)

Table 1. Effect of species and varietal type on nutrient quality and yield of small grain forage in year 1.

Item	Oat	Barley		SE <sup>b</sup>	Probability <sup>a</sup>	
	Grain	Grain	Forage		OAT vs BAR	BRG vs BRF
Stage <sup>c</sup>	5.7	6.0	5.3	.19	.74	.03
Concentration, %DM						
CP	11.5	13.5	12.0	.39	.01	.02
ADF	31.2	30.2	31.1	.81	.50	.44
NDF	52.1	52.5	52.1	.79	.84	.70
RFV <sup>d</sup>	115.7	116.0	116.3	2.5	.86	.94
TDN	67.4	68.4	67.5	.74	.51	.43
IVDMD	58.6	67.2	66.0	.62	<.01	.19
IVDMD/TDN	87.0	98.3	97.8	1.1	<.01	.76
Yield, kg/ha						
DM	1854	1662	2836	219	<.01	.65
CP	209	358	351	31	<.01	.88
IVDMD	1069	1796	1916	144	<.01	.63

<sup>a</sup> Probability of a significant treatment comparison between either oat and barley (OAT and BAR, respectively) or barley grain and forage types (BRG and BRF, respectively).

<sup>b</sup> Average standard error.

<sup>c</sup> Stage of development at harvest (3 = flower, 4 = watery ripe, 5 = milk, 6 = soft dough and 7 = hard dough).

<sup>d</sup> Relative feed value.

Table 2. Effect of species and varietal type on nutrient quality and yield of small grain forage in year 2.

Item	Oat	Barley		SE <sup>b</sup>	Probability <sup>a</sup>	
	Grain	Grain	Forage		OAT vs BAR	BRG vs BRF
Stage <sup>c</sup>	5.0	6.0	5.8	.05	<.01	.01
Concentration, %DM						
CP	7.5	7.3	7.6	.23	.91	.51
ADF	42.3	40.0	41.3	.49	.01	.14
NDF	63.9	60.0	61.0	.68	<.01	.42
RFV <sup>d</sup>	81.7	89.8	87.0	1.4	<.01	.14
TDN	56.0	57.7	56.7	.38	.01	.26
IVDMD	55.1	60.2	59.3	.59	<.01	.42
IVDMD/TDN	98.5	104.3	104.7	1.2	<.01	.84
Yield, kg/ha						
DM	3665	3382	3687	140	.48	.23
CP	273	249	279	14	.58	.22
IVDMD	2016	2036	2190	83	.33	.29

<sup>a</sup> Probability of a significant treatment comparison between either oat and barley (OAT and BAR, respectively) or barley grain and forage types (BRG and BRF, respectively).

<sup>b</sup> Average standard error.

<sup>c</sup> Stage of development at harvest (3 = flower, 4 = watery ripe, 5 = milk, 6 = soft dough and 7 = hard dough).

<sup>d</sup> Relative feed value.

**RELATIONSHIP OF IVDMD TO ADF IN OAT (*AVENA SATIVA*)  
AND BARLEY (*HORDEUM VULGARE* L.) FORAGE<sup>1</sup>**

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**ABSTRACT:** Estimation of energy concentration and digestibility of forages from relationships with ADF concentration is common in nutrient analysis. Although a constant relationship is assumed across small grain forage, recent research suggests that the relationship between digestibility and ADF may differ between oat (OAT) and barley (BAR) forage. Three sample sets of OAT and BAR forage were collected over two years, pooled across sample sets and used to test the hypothesis that the relationship between digestibility and ADF concentration differs among OAT and BAR forage. The first sample set included 17 samples collected directly from producers. The other two sample sets were from replicated trials designed to test the effects of species (OAT or BAR) and varietal type on forage yield and quality. Eighty-three samples were sent to one of two forage testing laboratories to obtain ADF, and estimated TDN, concentration using standard methodologies. IVDMD concentration was determined in the Nutrition Laboratory of NDSU. Although each of the commercial laboratories used a different equation to estimate TDN from ADF in small grain forage, the overall relationship was highly correlated ( $R^2 = .995$ ;  $TDN = 99.15 - 1.022 \cdot ADF$ ). IVDMD was also correlated with ADF ( $R^2 = .720$ ); however, the intercept ( $P < .01$ ) and slope ( $P < .01$ ) of the relationship differed by species. IVDMD in OAT was estimated by the equation  $[IVDMD = 72.27 - .399 \cdot ADF]$  and in BAR by the equation  $[IVDMD = 86.02 - .644 \cdot ADF]$ . Digestibility of BAR was 14% greater than OAT at 30% ADF and 5% greater at 45% ADF. Plotting the ratio of IVDMD to TDN suggests that standard equations for estimating TDN in small grain forage overestimates BAR digestibility below 34% ADF and OAT digestibility below 44% ADF. These data demonstrate that the digestibility, and thus energy concentration, of oat and barley forage are linearly related to ADF concentration. However, this relationship differs from standardized equations in use in the Northern Plains and differs between oat and barley forages. Using IVDMD at a common ADF concentration as a quality criteria, barley forage is of superior quality when compared to oat forage.

**Key Words:** Oat, Barley, Annual Forage

<sup>1</sup> Funds for nutrient analysis were provided through a grant from the North Dakota State Board of Agricultural Research and Education. Mention of a proprietary product does not constitute a guarantee or warranty of the product by ND SBARE, ND AES or the authors and does not imply its approval to the exclusion of other products that may also be suitable.

**Introduction**

Small grains are popular annual forages in the Northern Great Plains. While oat is the most popular, cool-season annual forage grown in the region, barley produces higher-quality forage when grown in more humid environments (Cherney and Martin, 1982). Barley forage contained greater concentrations of DDM and CP and lower concentrations of ADF.

An accurate estimate of diet digestibility and energy concentration is necessary to optimize the use of forages in livestock diet formulation and economic evaluation (Wiess, 1994). Given the constraints of determining diet digestibility directly, empirical relationships are commonly used to estimate forage digestibility. Estimation of energy concentration and forage digestibility from relationships with ADF concentration is common in routine forage analysis (NFTA, 2004).

Although a common relationship between energy and ADF concentrations in small grain forage is often assumed (NFTA, 2004), the possibility of a differential response among barley and oat forage has been suggested (Poland et al., 2004). The objective of this experiment was to compare the relationship between forage digestibility and ADF in barley and oat forage grown in the Northern Great Plains.

**Materials and Methods**

Samples from three data sets were used to test the hypothesis that constant relationship exists between concentrations of IVDMD and ADF in oat and barley forage. The first sample set included 17 samples (6 barley and 11 oat) collected from beef cattle producers in Golden Valley County, North Dakota. The second and third data sets were collected from a replicated small grain forage varietal trial. In each of two year (data sets 2 and 3, respectively; Poland et al., 2004), oat and barley varieties were seeded into replicated plots ( $n = 3$ ) in a randomized complete block design in a producer field southwest of Beach, North Dakota. In year 1, five grain oat (Derby, Killdeer, Otana, Paul and Whitestone), two grain barley (Conlon and Robust) and three forage barley (Haybet, Horsford and Westford) varieties were evaluated. In year 2, six oat (Derby, Hy-Test, Killdeer, Morton, Paul and Whitestone), four grain barley (Conlon, Drummond, Logan and Robust) and two forage barley (Haybet and Washford) were evaluated. A total of 83 samples were available for forage analysis.

When necessary forage samples were dried at 55°C prior to grinding and splitting into two portions. One portion was submitted to a commercial laboratory for determination of DM and ADF concentration. In data sets 1 and 2, samples were submitted to Servi-Tech Laboratories (Hastings, NE) for analysis using NIR. In data set 3, samples were submitted to Dairyland Labs (Sauk Rapids, MN) for analysis using wet chemistry methodologies. Both laboratories also reported TDN concentration calculated from ADF concentrations. In all data sets, the remaining portion of each sample was submitted to the Nutrition Laboratory in the Department of Animal and Range Sciences at North Dakota State University for IVDMD analysis.

Linear regression was used to test for significant relationships among ADF, TDN and IVDMD concentrations.

## Results and Discussion

Data set and forage species means and SD for ADF, TDN and IVDMD concentrations and the ratio of IVDMD to TDN are reported in Table 1.

The commercial laboratories used different equations for estimating TDN concentration from ADF concentration (Figure 1). NFTA (2004) provides a listing of commonly used equations for predicting TDN concentration in commercial testing laboratories. The equation used in data sets 1 and 2 most closely resembles an average of the two Mertens grass equations (data not shown). The equation used in data set 3 was a “prediction equation from Midwest” for legume and grass forage relating DDM to ADF and attributed to Rohweder et al. Regardless of this discrepancy, the overall relationship (Figure 1) between TDN and ADF concentrations across all samples was highly significant with minimal, albeit systematic, bias ( $R^2 = .9936$ ; residuals range within  $\pm 1.8\%$ ).

Invitro DDM concentration was also linearly related to ADF concentration ( $R^2 = 0.72$ ; Figure 2). The intercept ( $P < .01$ ) and slope ( $P < .01$ ) of this relationship also differed with forage species. In both cases, the parameter estimates were greater for barley forage. The IVDMD concentration in barley forage was greater than in oat forage across the range of ADF concentrations

included in this study (25 to 45% ADF). The digestibility of barley forage was 14% greater at 30% ADF and 5% greater at 45% ADF. This would be consistent with Cherney and Martin (1982) who reported greater DDM in barley forage without the caveat of simultaneously having lower ADF concentrations.

The ratio of IVDMD to TDN is plotted against ADF concentration in Figure 3. Reflecting the difference between forage species in IVDMD concentration, this ratio was also linearly related ( $R^2 = .68$ ) to ADF concentration with differing intercepts ( $P < .01$ ) and slopes ( $P = .06$ ). Solving for the ADF concentration where IVDMD and TDN are equal (ratio = 100) suggests that current equations overpredict the energy concentration of barley forage below 33.7% ADF and of oat forage below 43.8% ADF.

## Implications

These data demonstrate that the digestibility, and thus energy concentration, of oat and barley forage are linearly related to ADF concentration. However, this relationship differs from standardized equations in use in the Northern Plains and differs between oat and barley forages. Using IVDMD at a common ADF concentration as a quality criterion, barley forage is of superior quality when compared to oat forage.

## Literature Cited

- NFTA. 2004. Forage analysis procedures. Appendix A – calculated values. National Forage Testing Association. ([http://www.foragetesting.org/lab\\_procedure/appendixa.html](http://www.foragetesting.org/lab_procedure/appendixa.html); accessed March 5, 2004)
- Poland, W., H. Peterson, R. Ashley and L. Tisor. 2004. Effect of species and varietal type on yield and nutritional quality of small grain forage. Proceedings, Western Section, Amer. Soc. Anim. Sci. 55 (submitted).
- Weiss, W.P. 1994. Estimation of digestibility of forages by laboratory methods. In: Forage Quality, Evaluation and Utilization (G.C. Fahey, ed.). ASA, CSSA and SSSA, Madison, WI.

Table 1. Means and SD for concentrations of ADF, TDN and IVDMD and the ratio of IVDMD to TDN in barley and oat forage.

		ADF		TDN		IVDMD		IVDMD/TDN	
Data set	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Set 1									
Barley	6	28.0	5.45	70.4	4.95	67.7	4.80	96.3	4.69
Oat	11	33.6	2.25	65.2	2.05	60.1	2.62	92.1	3.48
Set 2									
Barley	15	30.6	1.53	68.0	1.37	66.5	1.63	97.9	2.71
Oat	15	31.4	2.75	67.3	2.52	59.4	3.82	88.3	6.09
Set 3									
Barley	18	40.5	2.30	57.4	1.79	59.9	2.01	104.4	2.47
Oat	18	42.3	1.43	56.0	1.11	55.1	2.52	98.5	5.11

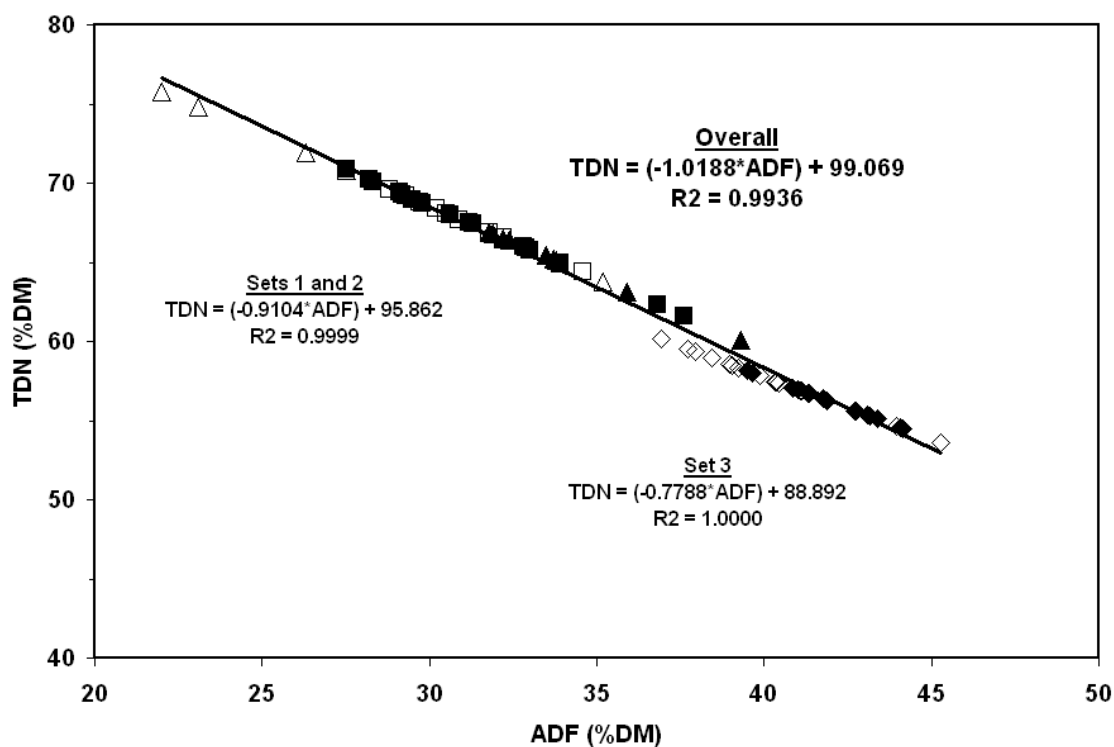


Figure 1. Effect of increasing ADF concentration on TDN concentration in barley and oat forage. Barley and oat forage are depicted as either open or closed symbols, respectively. Triangle, square and diamond shaped symbols represent data for sets 1, 2 and 3, respectively. Samples from data sets 1 and 2 were analyzed in the same commercial laboratory, while samples from data set 3 were analyzed in a separate commercial laboratory.

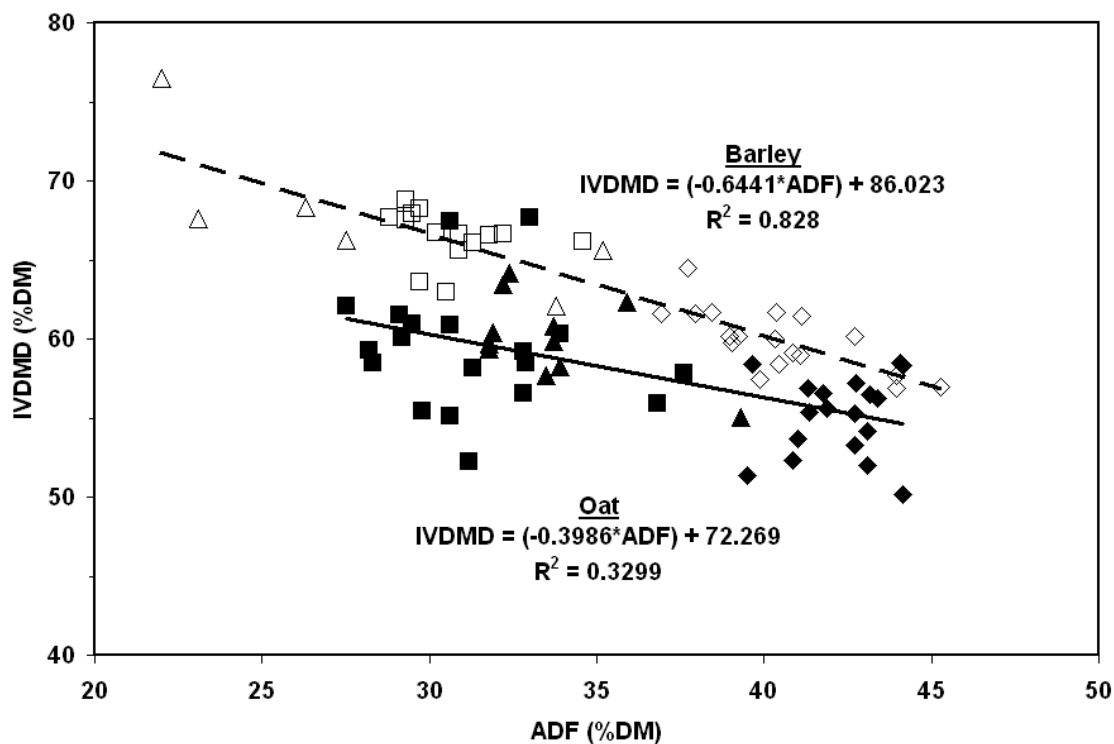


Figure 2. Effect of increasing ADF concentration on IVDMD concentration in barley and oat forage. Barley and oat forage are depicted as either open or closed symbols, respectively. Triangle, square and diamond shaped symbols represent data for sets 1, 2 and 3, respectively.

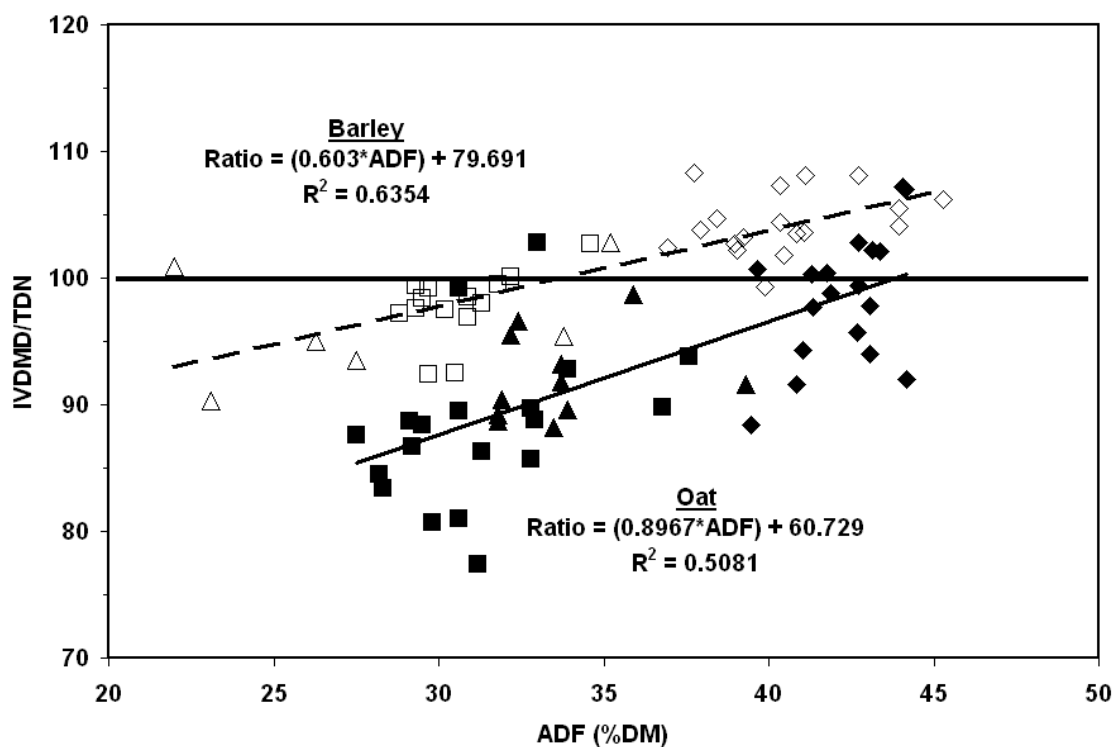


Figure 3. Effect of increasing ADF concentration on the ratio of IVDMD to TDN concentration in barley and oat forage. Barley and oat forage are depicted as either open or closed symbols, respectively. Triangle, square and diamond shaped symbols represent data for sets 1, 2 and 3, respectively.

**THE LONG-TERM EFFECTS OF CATTLE AND(OR) BIG GAME HERBIVORY AND LOGGING ON THE SUBSEQUENT DIET QUALITY OF STEERS GRAZING GRAND FIR (*ABIES GRANDIS*) HABITATS IN NORTHEASTERN OREGON**

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**ABSTRACT:** Grazing cattle on forested rangelands is a common practice in North America. However, little is known about the effects of overstory canopy cover and previous grazing on the subsequent diet quality of cattle. Therefore, the objective of this study was to document the effects of logging and herbivory on the diet quality of steers grazing grand fir (*Abies grandis*) habitats. Three randomly selected sites were established in 1986. Treatments were arranged in a split-plot design with a 3x3 factorial arrangement of treatments contrasting logging (whole-plot): 1) no logging, 2) crown thinning, 3) clearcut; and grazing (sub-plot): 1) cattle and big game grazing (grazed), 2) big game grazing (cattle exclusion), and 3) exclusion of cattle and big game grazing (total exclusion). Diet quality was determined using four ruminally cannulated steers in June and August of 2001 and 2002. Within each pasture, steers were allowed to graze for 20 min. In general, crude protein and NDF content were modestly influenced by grazing or logging treatments. Crude protein content of steer diets differed between years and months, with June having greater ( $P<0.05$ ) crude protein than August. However, an interaction ( $P<0.05$ ) occurred between grazing and logging treatments. Crude protein in grazed pastures was less than total exclusions within clearcuts, and clearcuts were greater than no logging in grazed pastures, but no logging was greater in CP than clearcuts within total exclusions. Also, NDF content of diets was different ( $P<0.05$ ) among months and years, with June having less NDF than August in 2001, and not different in 2002. Percent NDF in June was less than August within clearcuts and no logging, and not different in thinned. This study suggests that timing of grazing had a greater influence on diet quality than did previous grazing and(or) logging.

Key words: herbivory, logging, diet quality

### **Introduction**

Grazing cattle and logging are common practices associated with forested rangelands in North America. These areas comprise a significant portion of the public lands in the west and are productive in producing habitat and forage for livestock and wildlife, as well as wood products for human use.

However, over the past 100 years many areas with the potential for high forage production have low outputs due to dense canopy cover (Hedrick et al 1969). Therefore, it may be necessary to open the canopy to return the understory productivity of these lands.

Logging forested rangelands sets back succession and, in most cases, increases understory forage production. This results in an increased number of plant communities which cattle/wildlife may forage and obtain a higher quality of diet and subsequently increase gains. Typically, cattle select a diet that is predominantly grass with limited forbs and shrubs (Holechek et al. 1982, Mitchell and Rodgers 1985). However, cattle diets do vary throughout the grazing season, with woody vegetation becoming a greater part of the diet as the grazing season progressed (Holechek et al. 1982, Mitchell and Rodgers 1985, Darambazar 2003).

Few studies have evaluated diet quality upon forested rangelands over the grazing season, whereas more is known about changing body condition and weight change over this same period. Holechek et al, (1981, 1987), Vavra (1984), and Walburger (2000) have all documented that cattle gain less in the late summer and fall when compared to late spring and early summer.

The effects of logging and previous grazing (wild and(or) domestic ungulates) on diet quality have not been documented. Therefore, the objectives of this study were to determine how logging, previous herbivory and season of use affect the quality of diets obtained from forested rangelands.

### **Materials and Methods**

The study area is located at the Eastern Oregon Agriculture Research Center's Hall Ranch, which is approximately 16 km east of the city of Union in the Wallowa Mountains of northeastern Oregon. Elevation ranges from 1050 to 1250 m and annual precipitation averages 60 cm with about 65 percent coming in the winter, whereas summers are usually dry. Cattle have been grazing the area since mid-1880. Elk (*Cervus elaphus*) and mule deer (*Odocoileus hemionus*) are indigenous to the area and can be found throughout the year; however, heaviest use occurs in the spring and the fall.

The study was conducted as a replicated split-plot design. Three *Abies grandis* / *Pachistima myrsintes* (grand fir) habitat types, 22.5 ha each in size, were randomly selected within areas of relatively homogeneous stand structure. Each site (Figure 1) had three logging treatments applied: 1) clear cut, 2) crown thinning and 3) uncut (Control). Crown thinning consisted of removing co-dominant and some dominant trees. Logging began in 1985 and was completed in 1986. The grand fir clearcuts were replanted in the spring of 1988 with Ponderosa pine,

Douglas fir (*Pseudotsuga menziesii*) and western larch (*Larix occidentalis*).

The following grazing treatments were applied within all logging treatments: 1) grazing by cattle and big game (to achieve 60 percent utilization), 2) big game grazing only (Cattle enclosure), and 3) exclusion of cattle and big game grazing (Total Enclosure). Sixty percent utilization is considered heavy relative to current recommendations (Holechek 1995), but was used because it was considered a typical utilization level for industrial forests. Cattle and Total Enclosures were approximately 0.5 ha, whereas grazed pastures were approximately 6.5 ha. Fencing was not completed until 1994; therefore, all pastures were grazed during this period. From 1995 to 2000, pastures were grazed in a deferred rotation system with pastures grazed from mid-July to mid-August in odd years and mid-August to beginning of October for even years. However, in 2001 and 2002 pasture were grazed in conjunction with allotment pasture following diet collections.

Diet quality was determined by using four ruminally cannulated steers in June and August of 2001 and 2002. Prior to the grazing bout, steers were ruminally evacuated, on site, into plastic tubs and rumen walls were rinsed to remove as much material as possible. Steers were allowed to graze for 20 min. and extrusa samples were then removed. Following collection of extrusa samples, original rumen contents were replaced. Subsequent extrusa samples were completely dried at 60 °C in a forced air oven and were ground through a Wiley Mill (Thomas Scientific, Swedesboro, NJ) using a 1 mm screen. Composite samples were created for each experimental unit by combining 50 g sub-sample from each steer. Composite samples were analyzed for CP (AOAC, 1990) and NDF (ANKOM Technology Corporation Fairport, NY). Crude Protein and NDF percents are reported on an organic matter basis.

All data were analyzed as a split-plot within a randomized complete block design with three replications using MIXED procedures in SAS (SAS Inst. Inc., Cary, NC) with the block (site replication) effect considered random. The whole-plot experimental unit was logging treatment and the sub-plot experimental unit was grazing within logging treatments. All handling of steers followed protocols established by Oregon State University's Institutional Animal Care and Use Committee.

## Results and Discussion

Crude protein content of steer diets declined ( $P < 0.05$ ) between sampling dates (Table 1). In addition, the CP content in 2001 displayed a greater decline as compared to the 2002 change in protein content. Holechek et al. (1981) and Walker et al. (1989) also reported declining CP content of cattle diets as the grazing season progressed.

Crude protein was also affected by logging and previous grazing (Table 2). Within clearcuts, CP percent of steer diets was greater ( $P < 0.05$ ) in total enclosures, when compared to grazed, but only tended to be greater ( $P = 0.10$ ) than the cattle enclosures. However, within no logging controls, grazed pastures had greater ( $P < 0.05$ ) CP than total enclosures and tended to be greater ( $P = 0.10$ )

than cattle enclosures. Also, total enclosures within clearcuts had greater ( $P < 0.05$ ) CP than total enclosures within no logging controls, but grazed pastures within no logging controls had greater ( $P < 0.05$ ) CP than grazed pastures within clearcuts.

Neutral detergent fiber content of steer diets increased ( $P < 0.05$ ) between sampling dates for clearcuts and no logging controls (Table 3), but NDF was not affected in thinned pastures. Year also affected NDF content among logging treatments. Within 2001, no logging control pastures had greater ( $P < 0.05$ ) NDF than clearcuts and tended to be greater ( $P < 0.10$ ) than thinned. The no logging control had greater ( $P < 0.05$ ) NDF in 2001 than in 2002.

The influence of sampling date and year on grazing treatments was not as pronounced with NDF as it was with CP (Table 4). The only difference ( $P < 0.05$ ) between sampling dates was in cattle enclosures of 2001; June had approximately 3% less NDF than August. Also, within August of 2001, cattle enclosures had greater NDF than grazed pastures. The cattle enclosures in June 2002 had greater NDF than grazed pastures.

Cattle diets and subsequent quality of diets vary throughout the grazing season. Typically, cattle consume a diet that is predominantly grass. However, Holechek et al. (1982) and Darambazar (2003) documented that cattle increase consumption of woody species as the grazing season progressed. Forbs have been reported as minor but consistent components of cattle diets (Holechek et al. 1982, Mitchell and Rodgers 1985).

Declining quality of cattle diets is a result of declining forage quality and/or declining quantity of desirable forages. Cook and Harris (1968), Skovlin (1967), Clark (2003) and Darambazar (2003) have reported that plant quality declines as the grazing season progresses, with grass quality declining the greatest (Typically use only three citations for a thought .. delete one). However, shrubs and forbs remain higher in quality and were able to maintain that quality throughout the summer.

Changes in diet composition and selectivity may act as a supplement for cattle that are consuming lower quality grasses later in the grazing season. Increases in woody vegetation and/or forb consumption might be the reason why steer diets in this experiment maintained relatively high quality for the August sampling period. However, if grazing was present prior to the August collection it is possible that the results from the grazed pastures could be considerably different.

## Implications

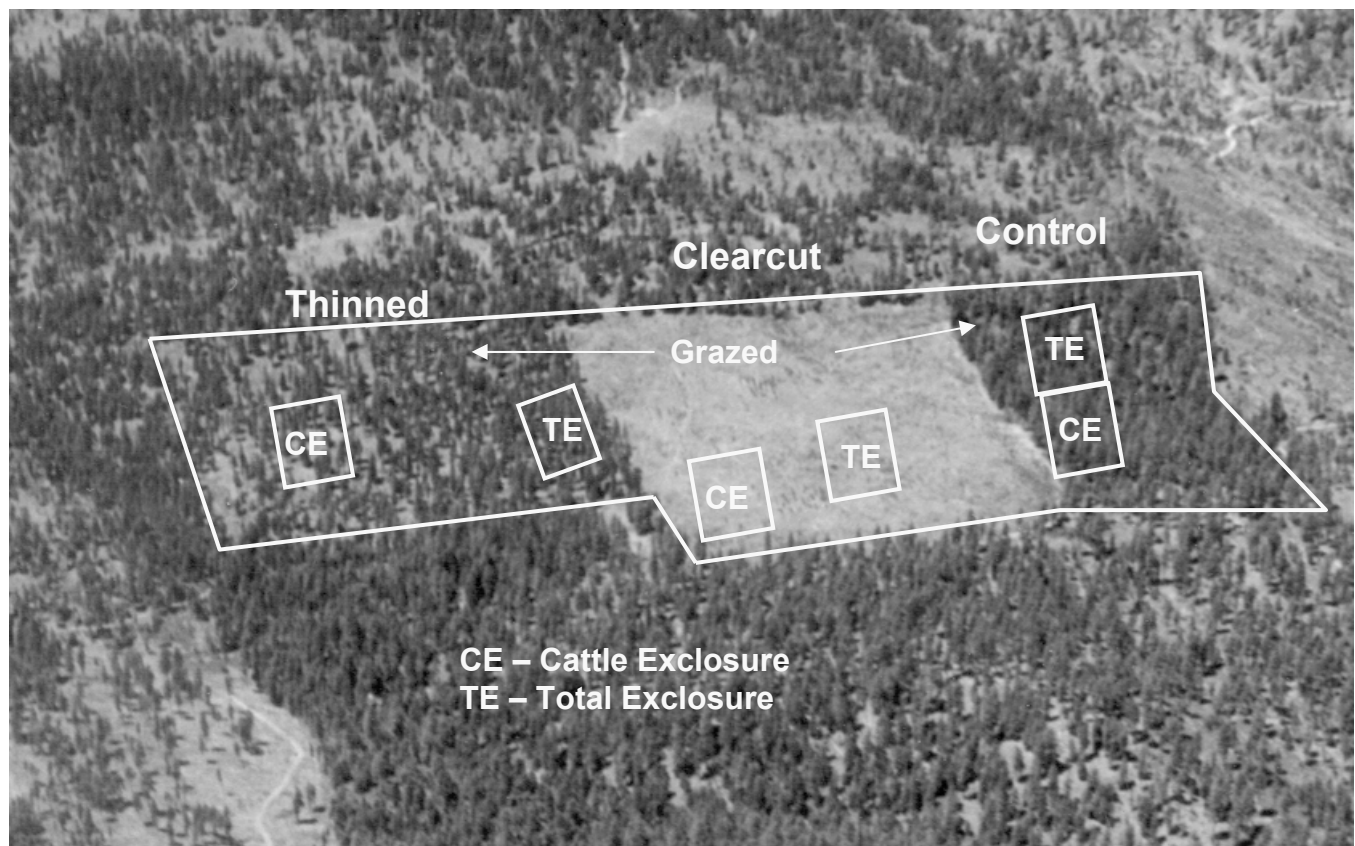
These data suggest that cattle grazing forested rangelands can select high quality diets when there is an abundance of available vegetation. In addition, logging and previous grazing only has modest effects on subsequent diets in comparison to changes associated with advancing maturity and/or season of use.

Cattle grazing forested rangelands may be able to select a diet that is of sufficient quality to allow for modest gains towards the end of summer and into the fall, even within previously logged areas. Therefore designing grazing

systems that allow for cattle to have access to these habitat types may improve red meat production on western rangelands.

### Literature Cited

- AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Offic. Anal. Chem., Arlington, VA.
- Cook, C.W. and L.E. Harris. 1968. Nutritional value of seasonal ranges. Utah Agri. Exp. Sta. Bulletin 472.
- Darambazar, E. 2003. Factors influencing diet composition of beef cattle grazing mixed conifer mountain riparian areas. M.S. Thesis. Oregon State Univ. Corvallis.
- Hedrick, D.W., B.R. Eller, J.A.B. McArthur, and R.D. Pettit. 1969. Steer grazing on mixed coniferous forest ranges in northeastern Oregon. J. Range Manage. 22: 322-325
- Holechek, J.L., M. Vavra, and J. Skovlin. 1981. Diet quality and performance of cattle on forest and grassland range. J. Anim. Sci. 53: 291-298
- Holechek, J.L., M. Vavra, J. Skovlin, and W.C. Krueger. 1982. Cattle diets in the Blue Mountains of Oregon II. Forests. J. Range Manage. 35: 239-242
- Holechek, J.L., T.J. Berry, and M. Vavra. 1987. Grazing system influences on cattle performance on mountain range. J. Range Manage. 40: 55-59
- Holechek, J.L., R.D. Pieper, and C.H. Herbel. 1995. Range Management: Practices and Principles (2<sup>nd</sup> Edition). Prentice Hall. Englewood Cliffs, New Jersey. p. 195-196
- Mitchell, J.E. and R.T. Rodgers. 1985. Food habits and distribution of cattle on a forest and pasture range in northern Idaho. J. Range Manage. 38: 214-220
- Skovlin, J. 1967. Fluctuations in forage quality on summer range in the Blue Mountains. USDA For. Serv. Res. Paper PNW-44. PNW For. and Range Exp. Sta., Portland Ore.
- Vavra, M. 1984. Livestock production possibilities on streamside meadows. Pages 35-44 in Proc. Pacific Northwest Range Manage. Short Course Range Watersheds, Riparian Zones and Economics: Interrelationships in Management and Use. Oregon State Univ. Corvallis
- Walburger K., T. DelCurto, M. Vavra, L. Bryant, and J.G. Kie. 2000. Influence of a grazing system and aspect, north vs. south, on the nutritional quality of forages, and performance and distribution of cattle grazing forested rangelands. Pages 181-184 in Proc. Western Section, Am. Soc. Anim. Sci.
- Walker, J.W., R.K. Heitschmidt, E.A. De Moraes, M.M. Kothmann, and S.L. Dowhower. 1989. Quality and botanical composition of cattle diets under rotational and continuous grazing treatments. J. Range Manage. 42: 239-242



**Fig. 1.** An aerial photo of a grand fir (*Abies grandis*) site at the Hall Ranch in northeastern Oregon demonstrating logging and grazing treatment layouts. (Photo taken in July 1996)

**Table 1.** Influence of year, grazing treatments, and sampling date on average percent CP, on an organic matter basis, within a grand fir habitat type in Eastern Oregon.

Year	Grazing Treatment	Sampling Date	
		June	August
2001*	Grazed	15.0 <sup>a</sup>	9.4 <sup>b</sup>
	Cattle Exclosure	15.0 <sup>a</sup>	9.6 <sup>b</sup>
	Total Exclosure	15.6 <sup>a</sup>	9.2 <sup>b</sup>
2002†	Grazed	14.8 <sup>a</sup>	11.0 <sup>b</sup>
	Cattle Exclosure	14.5 <sup>a</sup>	10.9 <sup>b</sup>
	Total Exclosure	14.4 <sup>a</sup>	11.6 <sup>b</sup>

\* Pooled Standard Error of mean = 0.68 (n=3)

† Pooled Standard Error of mean = 0.37 (n=3)

<sup>a,b</sup> Row values with differing superscripts are significantly different ( $P < 0.05$ )

**Table 3.** Influence of year, grazing treatments, and sampling date on average percent NDF, on an organic matter basis, within a grand fir habitat type in Eastern Oregon.

Year	Grazing Treatment	Sampling Date	
		June	August
2001*	Grazed	57.2	58.4 <sup>1</sup>
	Cattle Exclosure	57.5 <sup>a</sup>	61.3 <sup>b,2</sup>
	Total Exclosure	57.0	59.2
2002†	Grazed	56.3 <sup>1</sup>	57.2
	Cattle Exclosure	58.1 <sup>2</sup>	56.1
	Total Exclosure	57.0	58.2

\* Pooled Standard Error of mean = 1.14 (n=3)

† Pooled Standard Error of mean = 0.71 (n=3)

<sup>a,b</sup> Row values with differing superscripts are significantly different ( $P < 0.05$ )

<sup>1,2</sup> Column values, within year, with differing superscripts are significantly different ( $P < 0.05$ )

**Table 2.** Influence of logging and grazing treatments on average percent CP, on an organic matter basis, within a grand fir habitat type in Eastern Oregon.\*

Logging Treatments	Grazing treatment		
	Grazed	Cattle Exclosure	Total Exclosure
Clearcut	12.1 <sup>a,1</sup>	12.5	13.1 <sup>b,1</sup>
Thinned	12.5	12.5	12.8
Control	13.0 <sup>2</sup>	12.4	12.3 <sup>2</sup>

\* Pooled Standard Error of mean = 0.43 (n=3)

<sup>a,b</sup> Row values with differing superscripts are significantly different ( $P < 0.05$ )

<sup>1,2</sup> Column values with differing superscripts are significantly different ( $P < 0.05$ )

**Table 4.** Influence of year, sampling date, and logging treatments on average percent NDF, on an organic matter basis, within a grand fir habitat type in Eastern Oregon.

		Logging Treatments		
		Clearcut	Thinned	Control
Sampling Date*	June	57.0 <sup>1</sup>	57.6	56.9 <sup>1</sup>
	August	59.0 <sup>2</sup>	57.5	59.3 <sup>2</sup>
Year†	2001	57.6 <sup>a</sup>	57.9	59.9 <sup>b,1</sup>
	2002	58.3	57.1	56.4 <sup>2</sup>

\* Pooled Standard Error of mean = 0.77 (n=3)

† Pooled Standard Error of mean = 0.83 (n=3)

<sup>a,b</sup> Row values with differing superscripts are significantly different ( $P < 0.05$ )

<sup>1,2</sup> Column values with differing superscripts are significantly different ( $P < 0.05$ )

## THE EFFECT OF FERTILIZING WITH SODIUM SELENITE ON SELENIUM CONCENTRATION OF HAY AND DRAIN WATER AND SERUM SELENIUM CONCENTRATIONS IN BEEF HEIFERS AND CALVES

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**ABSTRACT:** The objectives of this trial were to determine the effect of fertilizing grass/alfalfa pastures with sodium selenite on selenium concentration of hay drain water and serum selenium concentrations in heifers and their calves. A 9.7 hectare grass alfalfa hay field was split into four strips and two of which were fertilized with 10 g of selenium/hectare on April 20, 2002. Hay was harvested from the field on July 1, 2002 and August 25, 2002. Drain water from the field was collected at monthly intervals from April 20, 2002 to August 20, 2002. Thirty six first calf heifers in the third trimester of gestation were randomly assigned to one of nine pens and pens were randomly assigned to one of three treatments in a completely random design. Treatments were: 1) a basal diet of selenium fertilized hay and a mineral mix containing 180 ppm selenium (+ SE hay + SE min), 2) a basal diet of selenium fertilized hay and a mineral mix containing no selenium (+ SE hay – SE min), and 3) a basal diet of non-selenium fertilized hay and a mineral mix containing 180 ppm selenium (- SE hay +SE min). Blood samples were collected via coccygeal venipuncture at the initiation of the trial and at calving. Twelve hours after birth a blood sample was collected from each calf via jugular venipuncture. Selenium fertilization increased ( $P < 0.05$ ) Se concentration in the hay 12 fold in the first cutting and four fold in the second cutting. Selenium was only detectable in the drain water one month after fertilization (0.6 ppb Se). Intake of Se was greatest ( $P < 0.05$ ) for + SE hay + SE min heifers, intermediate for – SE hay +SE min heifers, and lowest for + SE hay – SE min heifers. Serum Se concentration was greater ( $P < 0.05$ ) in +SE hay + SE min heifers than in + SE hay – SE min heifers. Serum concentration of Se was higher ( $P < 0.05$ ) in the +SE hay + SE min calves, intermediate in +SE hay –SE min calves, and lowest in –SE hay + SE min calves. Fertilization with sodium selenite can be used as an alternative method to provide Se to beef cattle.

**Key words:** Beef Cattle, Selenium, Supplementation, Fertilization

### Introduction

Since the 1950's it has been recognized that selenium is an essential nutrient (Muth, et al., 1958, Schwarz and Foltz, 1957). Diets containing less than 0.1 ppm (NRC, 1996) can lead to selenium deficiency which is associated with several

common disorders including white muscle disease, a myopathy that affects the heart and skeletal muscle, ill thrift, a condition where young animals fail to grow normally (Underwood, 1981), heinz body anemia (Morris et al., 1984) and reduced immune responses (Stabel and Spears, 1993). Excessive selenium supplementation or consumption of plants naturally high in selenium can result in toxicity (NRC, 1996). Signs of chronic selenium toxicity include lameness, anorexia, emaciation, sore feet, deformed feet, and loss of hair from the tail (Rosenfeld and Beath, 1964). The maximum tolerable level of selenium has been estimated to be 2 ppm selenium in the diet (NRC, 1980).

There are several methods available to supplement selenium in areas of selenium deficiency. These include providing selenium in mineral premixes, injecting selenium, or providing selenium in ruminal boluses that dispense over time (Wilkins and Hamilton, 1980). Current research has demonstrated that the selenium content of deficient forages can be enhanced by the addition of selenium to fertilizer (Gissel-Nielsen, 1986, Ekholm et al., 1991, and Hathaway et al., 2001). Current research has not examined the effects of providing animals with selenium fertilized forage while continuing to provide other sources of supplemental selenium. Therefore, the objectives of this study were to determine the effects of providing selenium fertilized hay to beef cattle with or without a selenium fortified mineral mix.

### Materials and Methods

A 9.7 hectare grass alfalfa mixed hay field was split into four strips and two of the strips (3.2 hectare/strip) were fertilized with 10 g of selenium/hectare in the form of sodium selenite. The remaining two strips (1.7 hectare/strip) did not receive selenium fertilization. Selenium fertilization was applied on April 20, 2002. Hay was harvested from the field on July 1, 2002 and August 25, 2002. A representative sample of the hay was obtained from each strip by coring approximately five percent of the bales. Samples were ground through a 1 mm screen and analyzed for selenium content (Brown and Watkinson, 1977). The field that was fertilized is part of a larger group of fields that have a tile drain underneath of them. The drain water from the tile drains was collected at monthly intervals from April 20, 2002 to August 20, 2002 and analyzed for selenium concentration (Brown and Watkinson, 1977).

Thirty six primiparous heifers in the third trimester of gestation were randomly assigned to one of nine pens (4 heifers/pen) and pens were randomly assigned to one of three treatments (3 pens/treatment) in a completely random design. Treatments were: 1) a basal diet of selenium fertilized hay and a mineral mix containing 180 ppm selenium in the form of sodium selenite, 2) a basal diet of selenium fertilized hay and a mineral mix containing no selenium, and 3) a basal diet of non-selenium fertilized hay and a mineral mix containing 180 ppm selenium in the form of sodium selenite. Calves born to heifers receiving mineral containing selenium received a subcutaneous injection of selenium (0.03 mg of Se). Calves born to heifers receiving mineral without selenium did not receive an injection of selenium. Second crop hay was fed for the first three weeks of the trial and first crop hay was fed for the remainder of the trial. In addition to the basal diet each heifer received 1.4 kg of rolled barley/d. Heifer BW and BCS was determined, and a blood sample was collected via coccygeal venipuncture at the initiation of the trial (11/22/02) and at calving (Average calving date 1/21/03). Blood samples were collected via jugular venipuncture from the calves within 24 h of birth. All blood samples were centrifuged at 1,500 x g for 20 min and serum was harvested and stored at -20° C until analyzed for selenium concentration (Brown and Watkinson, 1977). Calves that received the selenium injection were bled a minimum of six h after injection.

Intake of hay and mineral was determined approximately one week before the initiation of calving. Hay fed to each pen was weighed on a daily basis and orts were weighed back after five days to determine daily hay intake. The amount of mineral remaining at the end of the intake period was subtracted from the amount available at the beginning of the intake period to determine average daily mineral consumption. Daily selenium consumption was calculated by multiplying the selenium concentration of the hay and the mineral by daily consumption of each.

All data were analyzed as a completely random design using the general linear models procedure of SAS (SAS Institute, Cary, NC) using pen as the experimental unit.

## Results and Discussion

Selenium concentration of each crop of hay is presented in Table 1. First cutting hay that was fertilized with selenium had a 12 fold higher concentration ( $P < 0.05$ ) of selenium than first crop hay that was not fertilized with selenium (0.182 vs 0.015 ppm respectively). Second crop hay that was fertilized with selenium had a 4 fold greater ( $P < 0.05$ ) concentration of selenium than second crop hay that was not fertilized (0.084 vs 0.022 ppm respectively). Selenium fertilization increased forage selenium concentrations from deficient levels to recommended levels in the first cutting of hay and to just below recommended levels in the second cutting of hay (NRC, 1980). This agrees with previous research that shows fertilization with 10 g of selenium per hectare increases the selenium content of forages (Stephen

et al., 1989, Hathaway et al, 2001). In the selenium fertilized hay, selenium concentration decreased by 54% from the first crop to the second crop. Selenium was not detected in the drain water from the field prior to selenium fertilization. One month after selenium fertilization, the selenium concentration in the drain water was 0.55 ppb, and selenium concentration in the drain water dropped below detectable levels at months two to four after fertilization.

Heifer BW, BCS, and calf birth weight are presented in Table 2. Selenium fertilization treatment and selenium mineral treatment had no effect ( $P > 0.1$ ) on initial heifer BW and BCS, heifer BW and BCS change from initiation to calving, and calf birth weight. Intake of forage, mineral mix, and selenium is presented in Table 3. Treatments had no effect on hay intake. Mineral intake was greater ( $P < 0.05$ ) for heifers receiving the mineral without selenium than in heifers receiving the mineral with selenium (33.8 vs 24.2 g•heifer<sup>-1</sup>•d<sup>-1</sup> respectively). Hay selenium intake was greater ( $P < 0.05$ ) for heifers receiving selenium fertilized hay compared to heifers receiving non-selenium fertilized hay (2.13 vs 0.19 mg•heifer<sup>-1</sup>•d<sup>-1</sup> respectively). Intake of selenium from the mineral mix containing selenium was 4.35 mg•heifer<sup>-1</sup>•d<sup>-1</sup>. Total selenium intake was greatest ( $P < 0.01$ ) for the heifers consuming selenium fertilized hay and the mineral mix containing selenium (6.83 mg•heifer<sup>-1</sup>•d<sup>-1</sup>), with the heifers consuming the non-selenium fertilized hay and the mineral mix containing selenium being intermediate (4.28 mg•heifer<sup>-1</sup>•d<sup>-1</sup>), and heifers consuming selenium fertilized hay and mineral mix without selenium having the lowest intake of selenium (2.39 mg•heifer<sup>-1</sup>•d<sup>-1</sup>). The final selenium concentration of all diets was within the range recommended by the National Research Council (1996) with heifers receiving selenium fertilized hay and mineral containing selenium having a dietary selenium concentration of 0.55 ppm, heifers receiving non-selenium fertilized hay and mineral containing selenium having a dietary selenium concentration of 0.33 ppm, and heifers receiving selenium fertilized hay and mineral without selenium having a dietary concentration of 0.18 ppm selenium.

Serum selenium concentrations of heifers and calves are presented in Table 4. No differences ( $P > 0.10$ ) in serum selenium concentrations were detected at the initiation of the trial. At calving, heifers receiving selenium fertilized hay and mineral containing selenium had the highest ( $P < 0.05$ ) serum selenium concentrations, cows receiving non-selenium fertilized hay and mineral containing selenium were intermediate, and cows receiving selenium fertilized hay and mineral without selenium had the lowest serum selenium concentrations. Hathaway et al. (2001) saw similar increases in blood selenium concentrations when feeding selenium fertilized hay. Calves from heifers receiving selenium fertilized hay and mineral containing selenium had the highest ( $P < 0.05$ ) serum selenium concentrations with calves from heifers receiving selenium fertilized hay and mineral without selenium being intermediate and calves from heifers receiving non-selenium fertilized hay and mineral with selenium having the lowest serum concentrations of selenium. Serum

selenium concentrations in heifers and serum selenium concentrations in calves did not directly reflect selenium intake. Heifers consuming selenium fertilized hay and mineral without selenium had the lowest selenium intakes, however serum selenium concentration in this group were greater than in the group that consumed non-selenium fertilized hay. The discrepancy between selenium intake and serum selenium concentration may reflect potential differences in the form of selenium that was consumed. Throughout the study no animals displayed clinical symptoms of either selenium deficiency or toxicity.

### **Implications**

Selenium supplementation via fertilization of forage subsequently fed to beef cattle or provided in a salt mineral mix can improve selenium status. Selenium fertilized forage and selenium fortified salt mineral mixes can be used in conjunction to further enhance the selenium status of animals as long total dietary concentrations do not exceed current recommendations.

### **Literature Cited**

- Brown, M. W. and J. H. Watkinson. 1977. An automated fluorimetric method for determination of nanogram quantities of selenium. *Analytica Chimica Acta*. 89:29-35.
- Ekholm, P., M. Ylinen, M. Huttunen, P. Koivistoinen, and P. Varo. 1991. Selenium in Finnish food after beginning the use of selenium-supplemented fertilizer. *J. Sci. Food Agr.* 56:57-70.
- Gissel-Nielsen, G. 1986. Selenium fertilization and foliar application. Danish experiments. *Ann. Clin. Res.* 18:61-64.
- Hathaway, R. L., J. E. Oldfield, S. G. Paxton, and G. J. Pirelli. 2001. Selenium supplementation of beef cattle via fertilizer amendment. *Proc. West. Sec. Am. Soc. Anim. Sci.* 52:243-246.
- Morris, J. G., W. S. Cripe, H. L. Chapman, Jr., D. F. Walker, J. B. Armstrong, J. D. Alexander, Jr., R. Miranda, A. Sanchez, Jr., B. Sanchez, J. R. Blair-West, and D. A. Denton. 1984. Selenium deficiency in cattle associated with Heinz bodies and anemia. *Science*. 223:491-493
- Muth, O. H., J. E. Oldfield, L. F. Remmert, and J. R. Schubert. 1958. Effects of selenium and vitamin E on white muscle disease. *Science* 128:1090.
- NRC. 1996. Nutrient requirements of beef cattle (7<sup>th</sup> Ed.). National Academy Press. Washington, D.C.
- NRC. 1980. Nutrient requirements of beef cattle (6<sup>th</sup> Ed.) National Academy Press. Washington D. C.
- Schwarz, K. and C. M. Foltz. 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc.* 79:32292-3293
- Stabel, J. R. and J. W. Spears. 1993. Role of selenium in immune responsiveness and disease resistance. In *Human Nutrition a Comprehensive Treatise Vol. 8 Nutrition and Immunology* pp 333-356. D. M Klurfeld, ed. Plenum Press, New York, NY.
- Stephen, P. C., L. J. Seville, and J. H. Watkinson. 1989. The effects of sodium selenite applications on growth and selenium concentrations in wheat. *N.Z. J. Crop Hort. Sci.* 17:229-237.
- Underwood, E. J. 1981. The mineral nutrition of livestock (2<sup>nd</sup> Ed.) Slough, U.K. Commonwealth Agricultural Bureaux
- Wilkins, J. F. and B. A. Hamilton. 1980. Low release of selenium from recovered ruminal pellets. *Aug. Vet. J.* 56:87-89

Table 1: Selenium concentration of a grass/alfalfa hay fertilized with and without sodium selenite at 10g/ha.

Item	+ Se <sup>a</sup>	- Se <sup>a</sup>	SE <sup>b</sup>
First crop <sup>c</sup>			
Se mg/kg	0.182 <sup>d</sup>	0.015 <sup>c</sup>	0.016
Second crop <sup>c</sup>			
Se mg/kg	0.084 <sup>d</sup>	0.022 <sup>c</sup>	0.011

<sup>a</sup> Fertilization treatments + Se fertilized with 10g selenium/ha, - Se fertilized without selenium.

<sup>b</sup> Most conservative standard error of the mean (n = 2).

<sup>c</sup> First crop harvested 7/1/02, second crop harvested 8/25/02, fertilizer applied 4/20/02.

<sup>d,e</sup> Means within a row with different superscripts are different ( $P < 0.05$ )

Table 2: Effect of hay fertilized with sodium selenite and mineral with or without selenium fed to third trimester beef heifers, on BW, BW change, BCS, BCS change, and calf birth weight.

Item	Treatment <sup>a</sup>			SE <sup>b</sup>
	+ Se fertilizer/+ Se mineral	+Se fertilizer/- Se mineral	- Se fertilizer/+ Se mineral	
Initial BW, kg	502.1	520.1	514.3	8.3
Initial BCS	5.04	4.98	5.02	0.05
BW change, kg <sup>c</sup>	28.9	25.3	19.0	5.6
BCS change <sup>c</sup>	0.31	0.48	0.23	0.10
Calf birth weight, kg	36.1	35.6	35.7	1.2

<sup>a</sup> Treatments: + Se fertilizer/+ Se mineral = selenium fertilized hay (10g/ha) and mineral containing selenium (180 ppm), + Se fertilizer/- Se mineral = selenium fertilized hay and mineral containing no selenium, - Se fertilizer/+ Se mineral = non-selenium fertilized hay and mineral containing selenium.

<sup>b</sup> Standard error of the mean (n = 3).

<sup>c</sup> Change in BW and BCS is from initiation of the trial (11/22/02) to immediately post calving (average calving date 1/21/03).

Table 3: Intake of selenium from hay fertilized with sodium selenite and mineral with or without selenium fed to third trimester beef heifers.

Item	Treatment <sup>a</sup>			SE <sup>b</sup>
	+ Se fertilizer/+ Se mineral	+Se fertilizer/- Se mineral	- Se fertilizer/+ Se mineral	
Hay intake, kg	12.2	13.1	12.9	0.8
Mineral intake, g	25.7 <sup>c</sup>	33.8 <sup>d</sup>	22.7 <sup>c</sup>	2.1
Hay Se intake, mg	2.21 <sup>c</sup>	2.39 <sup>c</sup>	0.19 <sup>d</sup>	0.15
Mineral Se intake, mg	4.62 <sup>c</sup>	0.00 <sup>d</sup>	4.09 <sup>c</sup>	0.28
Total Se intake, mg	6.83 <sup>c</sup>	2.39 <sup>d</sup>	4.28 <sup>e</sup>	0.38

<sup>a</sup> Treatments: + Se/+ Se selenium fertilized hay and mineral containing selenium, + Se/- Se selenium fertilized hay and mineral containing no selenium, - Se/+ Se, non-selenium fertilized hay and mineral containing selenium.

<sup>b</sup> Standard error of the mean (n = 3).

<sup>c,d,e</sup> Means within a row within different superscripts are different ( $P < 0.05$ ).

Table 4: Serum Se concentrations in heifers and calves from heifers fed hay fertilized with selenium and mineral with or without selenium

Item	Treatment <sup>a</sup>			SE <sup>b</sup>
	+ Se fertilizer/+ Se mineral	+Se fertilizer/- Se mineral	- Se fertilizer/+ Se mineral	
Initial heifer Se conc <sup>c</sup>	28.7	32.3	31.8	0.9
Final heifer Se. conc <sup>c</sup>	56.1 <sup>d</sup>	50.1 <sup>d,e</sup>	45.7 <sup>c</sup>	0.3
Calf Se conc <sup>c</sup>	102.1 <sup>d</sup>	88.4 <sup>e</sup>	39.3 <sup>f</sup>	0.4

<sup>a</sup> Treatments: + Se/+ Se selenium fertilized hay and mineral containing selenium, + Se/- Se selenium fertilized hay and mineral containing no selenium, - Se/+ Se, non-selenium fertilized hay and mineral containing selenium.

<sup>b</sup> Standard error of the mean (n = 3).

<sup>c</sup> Serum selenium concentrations in ppb, initial = initiation of trial 11/22/02, final = at calving (average calving date 1/21/03) calf = within 24 h of birth.

<sup>d,e,f</sup> Means within a row within different superscripts are different ( $P < 0.05$ ).

## EFFECTS OF SULFATES IN WATER ON PERFORMANCE OF STEERS GRAZING RANGELAND

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**ABSTRACT:** Surface and subsurface water in South Dakota often contains high concentrations of total dissolved solids (TDS) and sulfates, which, in severe cases, can cause livestock deaths. Data from our laboratory have demonstrated that sulfate concentrations of 3,000 mg/L in water consumed by steers in dry-lot decreased ADG, feed intake, and water consumption. Little information is available on the effects of water sulfate concentrations on grazing livestock. This study evaluated the effects of water quality on the performance of steers grazing rangeland and the influence of vegetation community on responses. Eight native pastures at the SDSU Cottonwood Research Station were used. Four pastures were dominated by warm-season shortgrasses (SG) and four by cool-season midgrasses (MG). Yearling steers (105/year) were allotted to pastures in 2001 and in 2002 to attain a moderate stocking rate of 1.24 AUM/ha during a 4-month grazing season. In 2002, cattle were removed after two months due to drought, resulting in a stocking rate of 0.62 AUM/ha. Number of cattle per pasture varied from 7 to 30, depending on pasture size. Cattle in two of the SG and two of the MG pastures received high sulfate water (HS, 2001: 3,941 mg/L sulfates; 2002: 4,654 mg/L sulfates) with low sulfate water (LS, 2001: 404 mg/L sulfates; 2002: 441 mg/L sulfates) provided in the remaining pastures. ADG was greater for the LS steers than HS steers in 2001 ( $P=0.003$ , 0.84 and 0.76 kg/d, respectively) and in 2002 ( $P=0.001$ , 1.10 and 0.81 kg/d, respectively). An interaction between sulfate concentration in water and vegetation community in 2002 ( $P=0.078$ ) resulted from similar ADG for steers on SG (0.83 kg/d) and MG (0.79 kg/d) pastures for HS water, but greater ADG for steers on MG (1.15 kg/d) than SG (1.05 kg/d) pastures for LS water. During the two-year study, only one steer had health problems related to sulfur, with no deaths. Our study showed water with a sulfate concentration of 3,941 mg/L and greater reduced ADG of grazing steers, and that the response was influenced by vegetation.

Key Words: Steers, Sulfates, Water, Rangeland

### Introduction

Water is a critical resource on semi-arid rangelands of the western United States, including western South Dakota. Livestock production on these rangelands is absolutely dependent on adequate quantity and quality of water. Field observations from our laboratory since 1999 have shown both surface and subsurface water in South Dakota often exhibit high levels of total dissolved solids (TDS) and sulfates. We have shown that the majority of the salts (approximately 70%) in high TDS water sources in

the region are sulfate salts. Gould et al. (2002) concluded that 6% of 498 subsurface water samples taken in regions across the United States had sulfates greater than 1000 mg/L, with 50% of those coming from water in the North-Central Region (SD, ND, NE, and KS). The authors reported that in multiple locations in South Dakota, sulfur intake from water and forage exceeded the NRC (1996) maximum tolerable level of dietary sulfur (0.4% of DM). Drought conditions in 2002 further exacerbated water quality problems in South Dakota.

Data from our laboratory showed that growing steers in dry-lot receiving water with over 3000 mg/L sulfates had reduced average daily gain, DM intake, water intake, and gain/feed (Patterson et al., 2002). Steers receiving the high sulfate water had a high rate of polioencephalomalacia (**PEM**), a metabolic disorder induced by high sulfur ingestion. We also reported that water intake decreased linearly and average daily gain, dry matter intake and gain/feed decreased quadratically as TDS and sulfate levels increased for steers in dry-lot (Patterson et al., 2003). Little information is available on the effects of high sulfate water on the performance of cattle grazing rangeland. Such information is needed because of the prevalence of grazing livestock in the region that are affected by high TDS/sulfate water and the economic impact that potential reductions in animal performance may have. The type of forage available to grazing livestock affects animal weight gains (e.g. Lewis et al., 1956) and could interact with water quality in its influence on animal performance. Additionally, it is reasonable to expect that environmental and forage moisture differences between dry-lot and rangeland situations will affect water intake and consumption of sulfate salts.

The objective of this study was to determine the effects of water quality on the performance of steers grazing rangeland and the influence of vegetation community on those responses.

### Materials and Methods

The study was conducted at South Dakota State University's Cottonwood Range and Livestock Research Station, near Philip, SD in 2001 and 2002 on eight native pastures. Four pastures were dominated by warm-season shortgrasses (**SG**), including blue grama (*Bouteloua gracilis* (H.B.K.) Lag. Ex Griffiths) and buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.). The remaining four pastures are dominated by cool-season midgrasses (**MG**), including western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Love) and green needlegrass (*Stipa viridula* Trin.). One hundred and five crossbred yearling steers (288 and 294 kg

in 2001 and 2002, respectively) were stratified by weight and randomly assigned to one of eight pastures in each year to attain a moderate stocking rate of 1.24 AUM/ha during a 4-month grazing season. In 2002, cattle were removed after two months due to drought, resulting in a stocking rate of 0.62 AUM/ha in that year. Cattle grazed study pastures from 18 May to 6 September in 2001 and 22 May to 23 July in 2002. Number of cattle per pasture varied from 7 to 30, depending on pasture size. Cattle in two of the SG and two of the MG pastures received high sulfate water (HS, 2001: 3,947 mg/L sulfates; 2002: 4,654 mg/L sulfates) with low sulfate water (LS, 2001: 404 mg/L sulfates; 2002: 441 mg/L sulfates) provided in the remaining pastures. All water was obtained from natural sources, and sulfate levels were created by mixing water to form the desired salt level. The average analyses of water samples collected throughout the trial for each water treatment are shown in Table 1. Since we were working with natural water sources, exact target levels were not achieved. LS water was derived from our rural water system and sulfates varied little within or between years (375 – 420 mg/L in 2001 and 375 – 490 mg/L in 2002). HS water in 2001 was derived entirely from a stock dam in which sulfate concentrations increased from 3,170 – 4,600 mg/L over the grazing season. HS water in 2002 was a blend of water from two natural sources, with the blends ranging from 4,550 – 5,390 mg/L.

Water was supplied to each pasture in tanks. Water consumption was measured by the daily change in water depth adjusted for evaporation and precipitation. Measurements of evaporation and precipitation were taken from a weather station located at the research station headquarters, no more than 3 km from all pastures. Animal health was monitored daily. Cattle were diagnosed with PEM when showing clinical symptoms.

Steer weights were measured at the beginning and end of the experiment in each year. Access to feed and water was denied during a 12-h overnight period prior to weight measurements. Steer ADG was averaged within each experimental unit (pasture).

Analysis of variance (SYSTAT Linear Models II, SYSTAT, Richmond, CA) was used to evaluate the influence of sulfate level, pasture type, and their interaction on steer ADG and water intake.

## Results and Discussion

High sulfate water significantly reduced steer ADG in both years ( $P=0.003$  and  $0.001$  for 2001 and 2002, respectively) compared to LS water (Table 2). In 2001, the HS treatment resulted in a 10.7% reduction in ADG compared to LS, and in 2002 the reduction was 26.4%. Water intake was not different ( $P=0.456$ ) between water treatments in 2001, but in 2002 steers consumed more water in the LS treatments ( $P=0.061$ ). These results are similar to, though not as dramatic as, those from companion studies in the same years at the Cottonwood Research Station in which yearling steers in dry-lot experienced lower ADG and water intake on high sulfate compared to low sulfate water (Patterson et al., 2002; 2003).

The greater effect of high sulfate water on ADG and water intake in 2002 compared to 2001 may be

explained by environmental conditions. Spring precipitation in 2001 resulted in some pooling of water in low-lying areas of pastures, providing steers with intermittent alternate water sources during the first six weeks of the study. Extremely dry conditions in spring of 2002 precluded availability of alternate water sources in that year and forced HS animals to consume the HS water exclusively. The alternate water sources in 2001 likely reduced intake of water provided in pasture tanks and may explain the low water intake values measured in 2001 (Table 2). In that year, water intake by HS and LS steers was not different ( $P=0.456$ ). In 2002, when alternate water sources in pastures were unavailable, water intake was greater for LS steers compared to HS steers ( $P=0.061$ ). In 2002, steers were removed from pastures 48 d earlier than in 2001 due to drought. Although forage was in short supply in 2002, forage quality may have been elevated, especially for cool-season forages (Wilson 1982).

There was an interaction between pasture and water treatment for ADG (Figures 1 and 2) in both years of the study ( $P=0.027$  and  $0.078$  in 2001 and 2002 respectively). In both years, ADG was greater for steers grazing MG pastures compared to SG pastures when drinking water contained low sulfate concentrations. This is in keeping with the work by Lewis et al. (1956), who reported that MG pastures at the Cottonwood Research Station typically produced greater livestock gains compared to SG pastures. If, however, drinking water supplied to livestock contains high concentrations of sulfates, the data from this study indicate that there is no advantage in ADG for MG pastures, and there may, in fact, be an advantage for SG vegetation in such situations (Figures 1 and 2).

Throughout the two years of this study, only one steer (in the HS treatment in 2001) was identified as exhibiting symptoms of PEM. The steer was treated and recovered. That is in stark contrast to the incidence of PEM that occurred in dry-lot for steers drinking water with similar sulfate concentrations. In 2001, there was a 15% and 12.5% incidence of PEM for steers in dry-lot drinking water with sulfate concentrations of 3,087 and 3,947 mg/L, respectively (Patterson et al. 2002). In 2002, steers in dry-lot consuming water with a sulfate concentration of 4,654 mg/L had a 47.6% incidence of PEM (Patterson et al., 2003). We hypothesize that the differences in responses (ADG, water intake, PEM) to sulfate concentrations in drinking water between dry-lot and pasture steers are likely due to a number of factors. Moisture content of early-season forage on pasture is higher than in dry rations fed to dry-lot cattle, thus reducing the water requirement of grazing animals. Environmental conditions, including high temperatures, lack of shade, and reduced wind flow, likely result in greater heat stress in dry-lot situations and greater water requirements.

We conclude that water with 3,947 mg/L sulfates and higher reduced water consumption and animal performance for steers grazing native rangeland. We also determined that there was an interaction for ADG between pasture type and water sulfate concentration. Steers grazing midgrass-dominated pastures had greater weight gains than steers grazing shortgrass-dominated pastures when water sulfate concentrations were low. At high water

sulfate concentrations, steers grazing shortgrass-dominated pastures tended to have higher gains.

### Implications

These data, combined with field observations, suggest that water quality may be inadequate for optimal range livestock production in a significant portion of South Dakota. Reduction in animal weight gains can be expected for cattle on pasture drinking water with a sulfate concentration of 3,947 mg/L or greater. Midgrass-dominated pastures may be an advantage for steer weight gains when water sulfate levels are low, but that advantage is eliminated when water sulfate levels exceed 3,900 mg/L.

### Literature Cited

Gould, D H., D. A. Dargatz, F. B. Garry, D. W. Hamar, and P. F. Ross. 2002. Potentially hazardous sulfur conditions on beef cattle ranches in the United States. *J. Am. Vet. Med. Assoc.* 221:673-677.

Lewis, J.K., G.M. Van Dyne, L.R. Albee, and F.W. Whetzel. 1956. Intensity of grazing: Its effect on livestock and forage production. *SD AES Bulletin* 459, Brookings, SD.

NRC. 1996. *Nutrient Requirements of Beef Cattle*. 7<sup>th</sup> ed. National Academy Press, Washington, DC.

Patterson, H. H., P. S. Johnson, T. R. Patterson, D. B. Young, and R. Haigh. 2002. Effects of water quality on animal health and performance. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 53:217-220.

Patterson, H.H., P.S. Johnson, and W.B. Epperson. 2003. Effect of total dissolved solids and sulfates in drinking water for growing steers. *Proc. West. Sec. Amer. Soc. Anim. Sci.*: 54:

Wilson, J.R. 1982. Environmental and nutritional factors affecting herbage quality. Pages 111-131 in *Nutritional Limits to Animal Production from Pastures Proc.*, St. Lucia, Queensland, Australia. Commonwealth Agric. Bureaux, Farnham Royal, UK.

**Table 1.** Average TDS and sulfate analyses (mg/L) of water provided to growing steers on pasture in 2001 and 2002 across target sulfate levels.

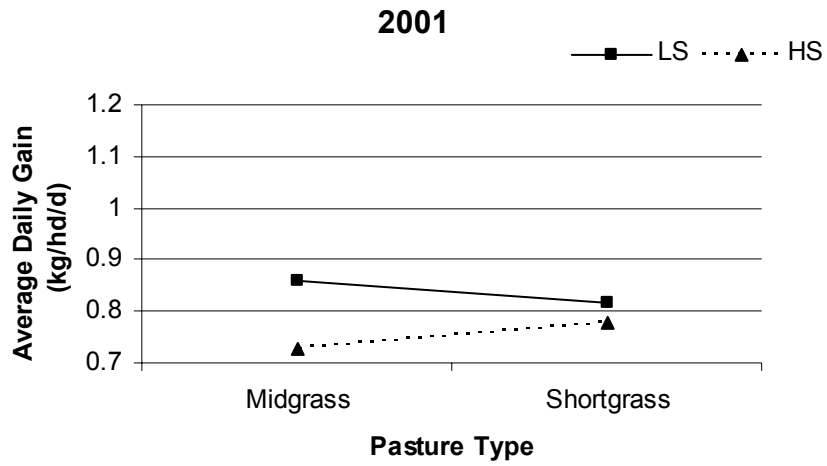
Item	Target Sulfate Level, mg/L			
	2001		2002	
	400	3,900	400	4,800
TDS	1,019	6,191	1,226	7,268
Sulfate	404	3,947	441	4,654

**Table 2.** Performance and water intake of growing steers on pasture supplied water with either low or high sulfate levels in western South Dakota in 2001 and 2002

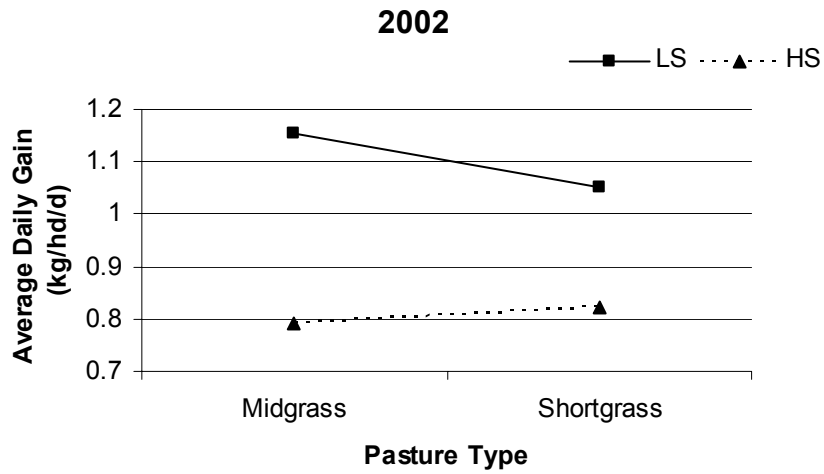
Item	Sulfate Level, mg/L			
	2001		2002	
	404	3,947	441	4,654
Initial Weight, kg	285.5	288.5	285.7	282.0
Final Weight, kg	379.3	372.9	360.7	341.9
ADG, kg/d <sup>a</sup>	0.84	0.75	1.10	0.81
Water Intake, L/d <sup>b</sup>	28.2	27.7	43.9	38.2

<sup>a</sup>Means within year significant (P=0.003 for 2001 and 0.001 for 2002).

<sup>b</sup>Means within year not significant (P=0.456) in 2001 and significant (P=0.061) in 2002.



**Figure 1.** Interaction between sulfate concentration (LS = 404 mg/L, HS = 3,947 mg/L) and pasture type (midgrass-dominated and shortgrass-dominated) for average daily gain (kg/d) for steers grazing native pastures in 2001 ( $P=0.027$ ).



**Figure 2.** Interaction between sulfate concentration (LS = 441 mg/L, HS = 4,654 mg/L) and pasture type (midgrass-dominated and shortgrass-dominated) for average daily gain (kg/d) for steers grazing native pastures in 2002 ( $P=0.078$ ).

## EFFECTS OF SULFATES IN WATER ON PERFORMANCE OF COW-CALF PAIRS

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**ABSTRACT:** Data from our laboratory showed water sulfate levels of 3000 mg/L reduced performance and health of growing steers during the summer. This experiment, conducted at the South Dakota State University Cottonwood Research Station, evaluated the effects of high sulfate water on cow and calf performance, milk production, and cow reproduction. Ninety-six crossbred, lactating cows (ages 2-13; average calving date of May 1) and their calves were assigned, after stratifying by age, weight, and previous winter management, to one of six pastures (16 cows/pasture) from June 3 to August 26, 2003. Pastures were randomly assigned to one of two water sulfate levels (three pastures/level). Treatments were low sulfate (LS) water (average 388 mg/L sulfates) or high sulfate (HS) water (average 2,608 mg/L sulfates). The HS water was created by adding sodium sulfate to the LS water. Weekly water sample composites ranged in sulfate concentration from 359-412 mg/L for LS and 1,631-3,055 mg/L for HS. Cow 12-h milk production was estimated by the weigh-suckle-weigh method at the initiation of the trial and again on July 3 and July 29. Initial milk production was used as a covariate in the analysis of subsequent milk production. Cows on LS gained 7 kg and cows on HS lost 17 kg during the experiment ( $P = 0.04$ ). Cows on HS lost more ( $P = 0.10$ ) body condition than LS (-0.27 and -0.48 for LS and HS, respectively). Twelve-hour milk production did not differ on July 3 ( $P = 0.33$ ; 4.8 and 4.3 kg for LS and HS, respectively) or July 29 ( $P = 0.48$ ; 5.4 and 5.0 kg for LS and HS, respectively). Calf ADG did not differ ( $P = 0.71$ ) between treatments. Pregnancy rates (52-d breeding season) were 98% and 94% for the LS and HS treatments, respectively ( $P = 0.36$ ). Sulfate levels averaging 2,608 mg/L in the drinking water of cow-calf pairs during the summer increased cow weight loss and condition loss but did not reduce calf performance or reproduction compared to sulfate levels averaging 388 mg/L.

Key Words: Cows, Sulfates, Water, Performance

### Introduction

Water available to livestock in South Dakota and the surrounding region can be high in sulfates (Gould et al., 2002). We previously reported that water with 3000 mg/L sulfates or greater reduced ADG, DMI, water intake, and gain/feed of growing steers in confinement compared to water with approximately 400 mg/L sulfates (Patterson et al., 2002). Additional work from our laboratory showed a quadratic decline in ADG, DMI, and gain/feed as sulfates in water for confined steers increased from approximately 400 to 4,700 mg/L (Patterson et al., 2003). In the work of Patterson et al. (2003), we documented a 48% incidence of

polioencephalomalacia (PEM) in confined steers receiving 4,700-mg/L sulfate water during the summer. Research also showed water provided to steers grazing native range during the summer with 3,900 mg/L sulfate or greater decreased ADG, but performance reductions were not as pronounced as with the confined cattle and few health problems were observed (Johnson and Patterson, 2004).

Grazing steers may not be as sensitive to sulfates in the water as are those in confinement (on a dry ration) due to: 1) less heat stress in pasture cattle, 2) ingestion of water in grazed forages, 3) the ability of pasture cattle to consume standing water following precipitation events, and 4) other digestive or behavioral differences. We hypothesized that the lactating cow and her calf would be highly sensitive to water sulfates due to the correlation between milk production and water intake (NRC, 1996). Since cow-calf production is the major livestock enterprise in South Dakota, the impacts of sulfates in water on both the cow and the calf are important to document. Therefore, the objective of this study was to evaluate the effects of sulfates in water for cow-calf pairs grazing native range during the summer on cow and calf performance, milk production, and cow reproduction.

### Materials and Methods

The study was conducted from June 3 to August 26, 2003 at South Dakota State University's Cottonwood Range and Livestock Research Station, near Philip, SD. Ninety-six crossbred, lactating cows (ages 2-13 yr; 632 kg) and their calves (average birth date May 1; ages 15-62 days; 79 kg) were assigned, after stratifying by age, weight, and previous winter management, to one of six pastures (16 cows/pasture). Pastures were randomly assigned to one of two water sulfate levels (three pastures/level). Treatments were low sulfate (LS) water or high sulfate (HS) water. Water was provided daily in aluminum stock tanks (round tanks; approximately 250 cm in diameter). The LS water was from a rural water system, and the HS water was created by adding sodium sulfate to LS water to a targeted 3000 mg/L sulfate level. The LS water was added to tanks daily and sodium sulfate was mixed directly into the stock tanks in the three HS pastures. Samples were taken daily from each HS pasture and from one LS pasture. Water samples were composited weekly and sent to the Water Resource Institute in Brookings, SD for sulfate analysis. Compiling all weekly composite sample results revealed the LS water averaged ( $\pm$  standard deviation)  $388 \pm 17$  mg/L sulfates, and the HS treatment averaged  $2,608 \pm 408$  mg/L sulfates. The volume (L) of water added to tanks (for calculation of sodium sulfate addition to HS) and the

volume of water consumed was calculated from the change in water depth and the tank surface area. Water consumption was adjusted for evaporation and precipitation (evaporation and precipitation measurements taken from a weather station located near the research pastures).

On June 3 (trial initiation) and August 26 (trial termination), both cows and calves were weighed and cows were assigned a body condition score (BCS; 1-9 scale; Richards et al., 1986) by two trained technicians (to the nearest 0.5 of a BCS). Cow-calf pairs were all on LS water and grazed native range prior to trial initiation. Cows were not allowed access to feed or water for approximately 12 h prior to initial weight measurements. At the end of the trial, all cows and calves were placed on LS water for three days prior to final weight measurements. Cows and calves were separated and housed in a drylot without access to feed or water for approximately 12 h prior to final weight measurements.

Once pasture assignments were made, cow-calf pairs were stratified by calving date within pasture group, and seven pairs/pasture (21/treatment) were selected to be used to estimate milk production (age of calves selected was between 18 and 43 days at trial initiation). Twelve-hour milk production was estimated by the weigh-suckle-weigh method (Boggs et al., 1980) on June 4 (initial), July 3, and July 29. Calves were separated from cows at approximately 0800 the day prior to measurements. Calves were returned to dams at 1800, allowed to suckle until content, and again removed. Calves were weighed the following morning at 0600, returned to dams and allowed to suckle until content, and then weighed again. The difference in calf weight prior to and post-suckling was used as an estimate of 12-h milk production. Data were not collected from seven calves on the LS treatment for both the July 3 and July 29 dates for reasons unrelated to treatment ( $n = 14$  for LS treatment for those dates).

One yearling bull was turned into each pasture on July 5. Bulls were rotated between pastures within treatment on July 29, and all bulls were removed on August 26. Pregnancy was determined by rectal ultrasonography in October of 2003.

Cow and calf weight and cow body condition score data were analyzed by ANOVA in PROC GLM of SAS (SAS Inst. Inc., Cary, NC) with pasture as the experimental unit. Twelve-hour milk production data from July 3 and July 29 were analyzed using initial measurements taken June 3 as a covariate (animal as experimental unit). Cow pregnancy rates were analyzed by Chi-Square in PROC GENMOD of SAS, with pasture as the observation and animal as the event within observation.

## Results and Discussion

Seven cows from one of the LS pasture groups escaped their treatment pasture during a hot period in July and suffered from water deprivation, therefore their data (cow and calf) were removed from the analysis. An additional cow from another LS pasture died of a suspected lightning strike, and one cow from the third LS pasture group was removed from the data set due to suspected hardware disease. One cow and one calf (each in a different pasture)

from the HS treatment died with no cause of death determined. No evidence existed to suggest the deaths in the HS treatment were or were not associated with treatment.

Water intake across all pastures averaged 78.16 L/cow-calf pair with no differences ( $P = 0.94$ ) between treatments (Table 1). Cows on LS gained 7 kg and cows on HS lost 17 kg during the 84-d experiment ( $P = 0.04$ ; Table 1). In addition, cows on HS lost 0.21 of a BCS more ( $P = 0.10$ ) during the experiment than LS cows, and HS cows had a lower BCS at trial end than LS cows ( $P = 0.04$ ). Calves, on average, weighed 79 kg at trial initiation and 169 kg at trial termination, with no differences ( $P > 0.5$ ) between treatment in calf weights or calf average daily gain (Table 1). Twelve-hour milk production, estimated by the weigh-suckle-weigh method, did not differ between treatments on July 3 or July 29 ( $P > 0.30$ ), and averaged (across treatments) 4.6 and 5.2 kg for July 3 and July 29, respectively (Table 2). The June 3 (trial initiation) estimate of milk production was a significant covariate for the July 3 estimate ( $P = 0.002$ ) but not the July 29 estimate ( $P > 0.9$ ). When the covariate was removed from the July 29 estimate (data not shown), there were still no differences between treatments ( $P > 0.50$ ). Pregnancy rates, measured by rectal ultrasonography in October, were 98% and 94% for the LS and HS treatments, respectively ( $P = 0.36$ ).

Previous research showed reductions in water intake of steers in the feedlot when the water contained elevated sulfate levels (Patterson et al., 2002; 2003). We did not observe differences in water intake in this study, despite a relatively low SE (Table 1). The lack of difference in water intake may help explain the absence of a treatment effect on milk production and calf gain but does not explain differences in cow performance. Johnson and Patterson (2004) showed an 11% reduction in ADG of foraging steers receiving water containing 3,947 versus 404 mg/L sulfates without differences in water intake. Nevertheless, foraging steers receiving water with 4,654 versus 441 mg/L sulfates had a 13% reduction in water intake and a 32% reduction in ADG (Johnson and Patterson, 2004). High levels of sulfur intake, independent of water intake, may have negative impacts on performance of cattle (Zinn et al., 1997). The impacts of the high sulfates in water on cow performance in this study were pronounced, but calf performance was not affected. Apparently, HS cows sacrificed body condition to support sustained milk production.

Unlike our work with growing steers in the feedlot (Patterson et al., 2002; 2003), in the current study with foraging cows, we did not document high rates of PEM in the high sulfate treatments. Patterson et al. (2002) reported a 15% incidence of PEM, with a 5% mortality rate, when confined steers were provided with water that averaged approximately 3,000 mg/L sulfates (slightly higher than this study). Working with feedlot cattle, Loneragan et al. (2001) reported reduced gain but no PEM with water sulfates levels up to 2,360 mg/L.

There are a few important points to consider when interpreting results of this study. The treatment period occurred during the summer months when high sulfate water issues are of greatest concern (Patterson and Johnson,

2004). This study does not, however, evaluate the effects of water high in sulfates supplied during late gestation or late lactation on calf gain and reproduction. This study was conducted, on average, from one to four months post-calving. At four to six months post-calving, calves would be expected to consume less milk (as a % of BW) and more water, which could make them more directly affected by water sulfates. We targeted a sulfate level of 3000 mg/L in the HS treatment, but we actually achieved an average of  $2608 \pm 408$  mg/L sulfates. Inference should not be drawn to sulfate levels greater than our reported average. We have documented that a threshold for cattle tolerance to water sulfates does indeed exist (Patterson et al., 2003). Finally, the bull to cow ratio used in this study was approximately 1:15. Lower bull to cow ratios could potentially impact reproduction in high sulfate situations.

We conclude that water provided to cow-calf pairs that averaged 2,608 mg/L in sulfates reduced cow weight and body condition change during an 84-d period during the summer compared to water averaging 388 mg/L sulfates. Milk production, calf average daily gain, and cow pregnancy rates were not affected by treatment.

### Implications

Calf gain and cow reproduction were not impacted when grazing cows received drinking water averaging approximately 2,600 mg/L sulfates from one to four months of lactation. Cows on high sulfate water lost more body weight and condition during the summer. The impacts of the body condition score loss on subsequent reproduction would be dependent on initial body condition score and cow management the following winter. More work is needed to determine critical sulfate levels in the drinking water for cow-calf pairs.

### Literature Cited

- Boggs, D. L., E. F. Smith, R. R. Schalles, B. E. Brent, L. R. Corah, and R. J. Pruitt. 1980. Effects of milk and forage intake on calf performance. *J. Anim. Sci.* 51:550-553.
- Gould, D. H., D. A. Dargatz, F. B. Garry, D. W. Hamar, and P. F. Ross. 2002. Potentially hazardous sulfur conditions on beef cattle ranches in the United States. *J. Am. Vet. Med. Assoc.* 221:673-677.
- Johnson, P. S. and H. H. Patterson. 2004. Effects of sulfates in water on performance of steers grazing rangeland. *J. Anim. Sci.* 82(Abstr.). *Accepted for publication, 2004 WSASAS Meetings.*
- Loneragan, G. H., J. J. Wagner, D. H. Gould, F. B. Garry, and M. A. Thoren. 2001. Effects of water sulfate concentration on performance, water intake, and carcass characteristics of feedlot steers. *J. Anim. Sci.* 79:2941-2948.
- NRC. 1996. *Nutrient Requirements of Beef Cattle.* 7<sup>th</sup> ed. National Academy Press, Washington, DC.
- Patterson, T. and P. Johnson. 2003. Effects of water quality on beef cattle. In: *Proc. The Range Beef Cow Symposium XVIII*, Mitchell, Nebraska. pp 121-128.
- Patterson, H. H., P. S. Johnson, and W. B. Epperson. 2003. Effect of total dissolved solids and sulfates in drinking water for growing steers. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 54: 378-380.
- Patterson, H. H., P. S. Johnson, T. R. Patterson, D. B. Young, and R. Haigh. 2002. Effects of water quality on animal health and performance. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 53:217-220.
- Richards, M. W., J. C. Spitzer, and M. B. Warner. 1986. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. *J. Anim. Sci.* 62:300-306.
- Zinn, R. A., E. Alvarez, M. Mendez, M. Montano, E. Ramirez, and Y. Shen. 1997. Influence of dietary sulfur level on growth performance and digestive function in feedlot cattle. *J. Anim. Sci.* 75:1723-1728.
- Boggs, D. L., E. F. Smith, R. R. Schalles, B. E. Brent, L. R. Corah, and R. J. Pruitt. 1980. Effects of milk and

Table 1. Performance and water intake of cow-calf pairs grazing native range and supplied water with low sulfates (average 388 mg/L) or high sulfates (average 2,608 mg/L) during the summer (Least Squares Means)<sup>a</sup>

Item	Treatment		SEM
	Low Sulfate (LS)	High Sulfate (HS)	
Cow initial weight, kg	633	631	8
Cow final weight, kg	640	615	12
Cow weight change, kg	7 <sup>b</sup>	-17 <sup>c</sup>	5
Cow initial body condition score	6.00	5.91	0.05
Cow final body condition score	5.73 <sup>b</sup>	5.43 <sup>c</sup>	0.06
Cow body condition score change	-0.27 <sup>d</sup>	-0.48 <sup>e</sup>	0.07
Calf initial weight, kg	77	81	1
Calf final weight, kg	166	172	4
Calf weight change, kg	89	91	4
Calf ADG, kg/d	1.06	1.08	0.04
Water Intake, L/d	78.26	78.07	1.52

<sup>a</sup>Trial lasted from June 3 to August 26, 2003 (84 days); Average calving date of May 1.

<sup>b,c</sup>Within a row, means with unlike superscripts differ (P = 0.04).

<sup>d,e</sup>Within a row, means with unlike superscripts differ (P = 0.10).

Table 2. Estimates of twelve-hour milk production for two dates using the weigh-suckle-weigh method for cow-calf pairs grazing native range and supplied water with low sulfates (average 388 mg/L) or high sulfates (average 2,608 mg/L) during the summer (Least Squares Means  $\pm$  SEM)<sup>a</sup>

Item	Treatment	
	Low Sulfate (LS) <sup>b</sup>	High Sulfate (HS) <sup>c</sup>
July 3, 2003, kg	4.8 $\pm$ 0.4	4.3 $\pm$ 0.3
July 29, 2003, kg	5.4 $\pm$ 0.5	5.0 $\pm$ 0.4

<sup>a</sup>Trial began June 3, and initial (June 3) estimate of milk production was used as a covariate. Covariate was significant for July 3 measurement (P = 0.002); Covariate was not significant for July 29 estimate (P > 0.9).

<sup>b</sup>n = 14 for each date.

<sup>c</sup>n = 21 for each date.

## COW-CALF PRODUCTION ON IRRIGATED PASTURES COMPOSED OF MONOCULTURES VERSUS A MIXTURE OF FORAGES

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**ABSTRACT:** Cow-calf production on irrigated pastures is an alternative for producers in public rangeland states. The objective of this study was to determine if cow-calf production on irrigated pastures could be enhanced using a blend of forages versus monocultures. A sprinkler irrigated plot (4.5 ha) was separated into 15 adjacent paddocks (0.3 ha) (14.6m x 201m). Paddocks were then randomly assigned to one of five forage treatments, three paddocks per treatment: 1) alfalfa (ALF) *Medicago sativa*, 2) meadow brome (MB) *Bromus biebersteinii*, 3) birdsfoot trefoil (BFT) *Lotus corniculatus*, 4) tall fescue (TF) *Festuca arundinacea*, and 5) an equal mixture of the above four forages (MIX). Thirty spring-calving cow-calf pairs were stratified by body weight into 15 groups of 2 cow-calf pairs each. Groups were then randomly assigned to each of the 15 paddocks. Management intensive grazing practices were used with cattle receiving a fresh pasture allotment each 24 h. Pasture forage harvested was estimated using a raised plate meter reading before and after grazing. The following are forages ranked by total DM harvested (kg DM/ha): TF, 17,614; ALF, 15,710 (P = 0.05); MIX, 14,050 (P = 0.10); MB, 11,489 (P = 0.0099); BFT, 8,720 (P = 0.006). Carrying capacity (pair/ha/y) followed a similar pattern: 5.51, 4.91 (P = 0.05), 4.38 (P = 0.09), 3.59 (P = 0.01), and 2.72 (P = 0.006), respectively. Calf gain (kg/ha) for the forage treatments were: TF, 1044; MIX, 883 (P = 0.10); MB, 773 (P = 0.41); ALF, 763 (P = 0.99); BFT, 480 (P = 0.001). Efficiency of calf gain (gain/DM) favored the MIX treatment: MIX, 0.0629; MB, 0.0608 (P = 0.415); TF, 0.0593 (P = 0.0018); BFT, 0.0549 (P = 0.0001); ALF, 0.0485 (P = 0.0007). Thus, ALF resulted in the least efficient calf gains. No differences (P = 0.3795) in cow body condition score change could be detected. The TF monoculture treatment was better than the other four forage treatments for cow-calf production on irrigated pastures.

Key Words: Forages, Production, Beef cattle

### Introduction

Many cow-calf producers in the intermountain west states utilize to varying degrees grazing permits on public lands. Many of these states consist of large amounts of public lands: Utah 75.2%, Idaho 70.4%, Wyoming 55.9%, Nevada 87.8%, Arizona 56.8% and New Mexico 47.4%. Livestock grazing on public lands is becoming more restrictive to producers. Cow-calf production on irrigated

pastures is an alternative for the producer because of the higher carrying capacity versus that of range conditions.

Recent studies at Utah State University (USU) have shown that irrigated pastures using tall fescue out performs other cool season grass species (Wiedmeier, et al, unpublished data). Cool-season grasses tend to decrease in yield during summer months. Many legumes continue to grow well and produce high yields during the summer months. This would be complementary forage to cool season grasses. Studies have shown that cattle tend to perform better on pastures that contain a blend of forages compared to those pastures that have monoculture forage. Mouriño et al (2003) stated, "that average daily gain and gain per hectare are usually greater on legume-grass systems." A mixture of cool-season grasses and legumes seems to be advantageous because of the possibility of increased yield and increased nutritive value of the diet selection.

In order for cow-calf production to be advantageous for the producer it must be economically feasible. The objectives of this study are to measure animal performance on mixed versus monoculture pastures and measure the inputs to estimate a profit/loss calculation. It will compare each forage type and its economic feasibility.

### Materials and Methods

**Pasture Establishment.** A sprinkler irrigated plot (4.5 ha) was separated into 15 adjacent paddocks (0.3 ha)(14.6m x 201m) at the USU Ag Experiment Station in North Logan, Utah. The paddocks were randomly assigned to one of the five forage treatments, three paddocks/treatment: 1) alfalfa, 2) meadow brome, 3) birdsfoot trefoil, 4) fuego tall fescue, and 5) an equal mixture of the above four forages. All paddocks were seeded on 4/12/02 at approximately 22.45 kg/ha. The choice paddocks were seeded into four adjacent strips (3.65m x 201m), one strip per forage. One crop of hay was taken from each paddock treatment. Ammonium nitrate and superphosphate was applied to paddocks in the fall of the establishment year. In early summer of the second year (2003), one crop of hay was taken off of each paddock except BFT before grazing.

**Pasture Management.** Grazing began in July of the second year (2003). Prior to grazing, each paddock was

fenced with electric wire fences and gates. Two polywire cross fences were moved one in front of cattle and one behind cattle each d. An intensive watering system was developed allowing water to be moved each d with cattle. By using the electronic raised plate meter system (RPM), taking clip plots of 0.1 m<sup>2</sup> and drying clip plot samples in 60°C drying oven, an equation estimating total DM/m<sup>2</sup> was developed for each forage treatment.

Cow-calf pairs received a new paddock each 24 h at 0800 h. Taking the electronic RPM values and putting them into a spreadsheet determined the amount of DM and surface area to be given each d. An after grazing RPM was taken to determine the amount of forage refused. Clip plots of 0.1 m<sup>2</sup> were also taken and dried at 60°C in a drying oven for 72 h to determine DM/m<sup>2</sup>. DMI was adjusted to allow for an after grazing stubble height of approximately 10 cm. The DMI was recorded each d by taking pre and post grazing RPM. All clip plot samples were dried, weighed, ground to pass through a 1mm screen and composited into one sample for each of the different forage types. The dried samples were analyzed in the laboratory for DM (8 h at 105°C), ADF and NDF (Komareck and Sorois, 1993) and CP (rapid combustion @ 850°C and conversion of all N to N<sub>2</sub> for subsequent measurement by thermoconductivity, LECO CHN – 1000 Combustion Analyzer)(Yeomans and Bremmer, 1991).

Grazing management was designed to allow cow-calf pairs to move in a linear path across plots. After grazing each plot, the cow-calf pairs were removed from plots. A small harrow was dragged over each plot to breakup manure. All forages except alfalfa were fertilized with approximately 33.68 kg/ha of Nitrogen from Ammonium nitrate. A sprinkler irrigation system was moved behind cattle after grazing and a few days ahead of cattle. All irrigation sets received 0.5197 cm/h for 12 h. Cattle were placed back on plots when all plots had a stubble height of approximately 30 cm.

*Cattle.* Selection of 30 spring calving cow-calf pairs of closely the same breeding and size were randomly selected and stratified to the 15 different groups by calf weight. All cows (627.27 kg) and calves (204.09 kg) were weighed and cows received a BCS (5.1) prior to stratification. Animals were weighed at both pre and post grazing periods to calculate change in cow weight, BCS and calf ADG.

*Economics.* Records were kept throughout the study on water, fertilizer and other input costs. This information was given to an agricultural economist to analyze and determine cost of production and profit/loss estimates on each treatment production system.

*Statistics.* Data was analyzed using the Proc Mixed procedure in SAS (SAS Inst. Inc., 1996, Cary, NC). Carrying capacity, kg/ha harvested, calf gain/ha, calf ADG, efficiency, change in cow BCS and change in cow BW were analyzed under random block model with

forage species as the treatment effect and grazing period as the block effect.

## Results and Discussion

Cattle were placed on paddocks July 7, with cows having a mean BW of 627 kg and BCS of 5.1, and calves having a mean BW of 204 kg. The grazing study ended September 30, with cows having a mean BW of 618 kg and BCS of 4.7. Calves were weaned on October 1, with an average WW of 291.5 kg. The ADG over all the treatments was 1.15 kg/d. Calf ADG differed significantly between treatments. The ADG for calves on the MIX (1.26 kg) treatment was statistically significant compared to calves receiving ALF (0.97 kg,  $P < 0.0001$ ) or BFT (1.10 kg,  $P = 0.0046$ ) treatments but not the TF or MB treatments. However, the ALF treatment was significantly different from all other treatments (0.92 kg/d,  $P < 0.0001$ )(Table 1).

Calf gain (kg/ha) was statistically significantly different among treatments. The TF had a mean gain of 1,044 kg/ha, which is significantly different than ALF (763 kg/ha,  $P = 0.0012$ ), MB (773 kg/ha,  $P = 0.0018$ ) and BFT (480 kg/ha,  $P < 0.0001$ ). The TF and MIX treatments were not different statistically (Table 1). The efficiency in which DM utilized (kg gain/DM) was significantly different among treatments. Calves on MIX were significantly more efficient (0.06287 kg/DM) than calves on treatments ALF (0.04850 kg/DM,  $P < 0.0001$ ) and BFT (0.05492 kg/DM,  $P = 0.0051$ ). The ALF treatment was significantly lower in efficiency than all other treatments (Table 1).

Calf performance was enhanced on the MIX treatment with increased ADG and increased efficiency of gain. This is similar to what other research has found. A mixture of alfalfa and smooth brome grass resulted in higher ADG when compared to a monoculture of grass (Hermann et al, 2000). Though not measured in our study, an increase in milk production by cows contributes to higher gain and efficiency in the calves. A recent study showed an increase in milk production of 28% when a legume-grass seeding was adjacent to each other, allowing a choice for consumption (Marotti et al., 2001).

Cow performance seemed to favor the MIX treatment over Monoculture treatments. There was no difference in body condition change among treatments. Cows on the ALF treatment had a decrease in BCS of -0.4167 (Table 1), although this was not significantly different than any other treatment ( $P = 0.3795$ ). Cow BW change was significantly different among treatments. The change in BW for the ALF treatment (-31.44 kg) is significantly lower compared to MB (6.44 kg,  $P = 0.0217$ ) and MIX (2.50 kg,  $P = 0.0471$ ) treatments (Table 1).

Productivity of forage species throughout the study tended to favor the monoculture TF treatment. Carrying capacity of TF (5.51) was significantly different to MIX (4.38,  $P = 0.0008$ ), MB (3.59,  $P < 0.0001$ ) and BFT (2.72,

P < 0.0001) treatments. The TF treatment tended to be different from the ALF treatment (4.91, P = 0.0525)(Table 2). Total kg/ha DM harvested follows the same trend as carrying capacity. Where TF is significantly different than MIX (P = 0.0009), MB (P < 0.0001) and BFT (P < 0.0001. The MIX (14,050) treatment and ALF (15,710) treatment are not significantly different (P = 0.1039). The TF (17,614) and ALF (15,710) treatments tend to be different (P = 0.0550)(Table 2). We found that BFT produces in spring for a first graze but does not continue to produce for continuous grazing periods. The lower carrying capacity and lower amount of DM kg/ha harvested are due to this situation. Another study shows that pure stands of BFT have lower forage production and fewer total steers d/ha (Wen et al, 2002).

One of the main objectives of this study was to estimate the economics of a cow-calf production system on irrigated pastures. The profit/loss calculations show that TF, ALF and MIX treatments showed a profit of 38.08, 37.73 and 20.33 \$/hd, respectively. The MB and BFT showed a loss of 59.63 and 69.80 \$/hd, respectively (Table 3). In order to have a profit on irrigated pasture, a producer must maintain a high carrying capacity throughout the year. To offset the input costs, forages need to produce high enough yields to maintain a high carrying capacity.

### Implications

Due to the lower carrying capacity and lower production of MB and BFT and the percentage of those species in the

mix causes a lower profit for the MIX pastures. Based on this study, when using irrigated pastures with intensive management grazing, TF pastures seem to have an advantage in maintaining high production yield with higher carrying capacity with increased profit possibility.

### Literature Cited

- Hermann M.L., J.R. Russell and S.K Barnhart. 2002. Evaluation of hay-type and grazing-tolerant alfalfa cultivars in season-long or complementary rotational stocking systems for beef cows. *J. Anim. Sci.* 80:768-779
- Marotti D.M., G.P. Cosgrove, D.F. Chapman, A.J. Parsons, A.R. Egan and C.B. Anderson. 2001. Novel methods of forage presentation to boost nutrition and performance of grazing dairy cows. *Aust. J. Dairy Technol.* 56(2):159
- Mouriño F., K.A. Albrecht, D.M. Schefer and P. Berzaghi. 2003. INTEGRATED SYSTEMS! Steer performance on Kura Clover-Grass and Red Clover-Grass mixed pastures. *Agron. J.* 95:652-659
- Wen L., R.L. Kallenbach, J.E. Williams, C.A. Roberts, P.R. Beuselinck, R.L. McGraw and H.R. Benedict. 2002. Performance of steers grazing rhizomatous and nonrhizomatous birdsfoot trefoil in pure stands and in tall fescue mixtures. *J. Anim. Sci.* 80:1970-197

Table 1. Performance of cow-calf pairs and production of irrigated pastures as effected by forage species treatment

	Forage Species					SEM <sup>f</sup>
	MIX <sup>a</sup>	TF <sup>b</sup>	ALF <sup>c</sup>	MB <sup>d</sup>	BFT <sup>e</sup>	
Calf ADG (kg)	1.26 <sup>g</sup>	1.18 <sup>gh</sup>	0.97	1.22 <sup>gh</sup>	1.10 <sup>h</sup>	0.04
Calf gain (kg/ha)	883 <sup>g</sup>	1044 <sup>g</sup>	763 <sup>gh</sup>	773 <sup>gh</sup>	480	62.55
Efficiency of gain (kg/DM)	.0629 <sup>g</sup>	.0593 <sup>gh</sup>	0.0485	.0608 <sup>ghi</sup>	.0549 <sup>hi</sup>	0.0014
Change in cow BCS	.1667 <sup>g</sup>	.1667 <sup>g</sup>	-.4167 <sup>g</sup>	-.1667 <sup>g</sup>	-.1667 <sup>g</sup>	0.2386
Change in cow BW (kg)	2.50 <sup>g</sup>	-1.89 <sup>gh</sup>	-31.44 <sup>hi</sup>	6.44 <sup>ghj</sup>	-18.94 <sup>ghij</sup>	11.45
Carrying capacity (pairs/ha)	4.38 <sup>g</sup>	5.51 <sup>h</sup>	4.91 <sup>gh</sup>	3.59	2.72	0.18
Total kg/ha harvested	14050 <sup>g</sup>	17614 <sup>h</sup>	15710 <sup>gh</sup>	11489	8720	589

<sup>a</sup> Mixture of equal parts of each forage

<sup>b</sup> Monoculture Tall Fescue, Fuego

<sup>c</sup> Monoculture Alfalfa, Alfagraz

<sup>d</sup> Monoculture Meadow Brome

<sup>e</sup> Monoculture Birdsfoot Trefoil, Empire

<sup>f</sup> Standard error of mean

<sup>ghij</sup> Means in the same row with different superscripts differ, P < 0.05

Table 2. Productivity of irrigated pasture as effected by forage species treatment

	Forage Species					
	MIX <sup>a</sup>	TF <sup>b</sup>	ALF <sup>c</sup>	MB <sup>d</sup>	BFT <sup>e</sup>	SEM <sup>f</sup>
Carrying capacity (pairs/ha)	4.38 <sup>g</sup>	5.51 <sup>h</sup>	4.91 <sup>gh</sup>	3.59	2.72	0.18
Total kg/ha harvested	14050 <sup>g</sup>	17614 <sup>h</sup>	15710 <sup>gh</sup>	11489	8720	589

<sup>a</sup> Mixture of equal parts of each forage

<sup>b</sup> Monoculture Tall Fescue, Fuego

<sup>c</sup> Monoculture Alfalfa, Alfagraze

<sup>d</sup> Monoculture Meadow Brome

<sup>e</sup> Monoculture Birdsfoot Trefoil, Empire

<sup>f</sup> Standard error of mean

<sup>gh</sup> Means in the same row with different superscripts differ,  $P < 0.05$

Table 3. Profit/Loss for cow-calf production system on summer grazing irrigated pastures and total cost / year on five forage treatments

	Forage Species				
	MIX <sup>a</sup>	TF <sup>b</sup>	ALF <sup>c</sup>	MB <sup>d</sup>	BFT <sup>e</sup>
Pasture Feed Cost (\$)	148.97	129.54	123.06	198.35	222.64
Other Feed Cost (\$)	127.42	127.41	145.37	127.42	127.42
Total Feed Cost (\$)	276.39	256.95	268.43	325.77	350.06
Non Feed Cost (\$)	271.88	271.88	271.88	271.88	271.88
Total Annual Cow Cost (\$)	548.27	528.83	540.31	597.65	621.94
Profit/Loss <sup>f</sup>	20.33	38.08	37.73	(-59.63)	(-69.80)

<sup>a</sup> Mixture of equal parts of each forage

<sup>b</sup> Monoculture Tall Fescue, Fuego

<sup>c</sup> Monoculture Alfalfa, Alfagraze

<sup>d</sup> Monoculture Meadow Brome

<sup>e</sup> Monoculture Birdsfoot Trefoil, Empire

<sup>f</sup> Calculated based on (market value of calf - ranch value of calf )

# INTAKE AND DIGESTIBILITY RESPONSE TO FORAGE KOCHIA (*KOCHIA PROSTRATA*) IN A LOW QUALITY FORAGE DIET<sup>1</sup>

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**ABSTRACT:** Forage kochia (*Kochia prostrata*), a half-shrub native to arid regions of central Eurasia, has potential to be a source of forage for the beef industry in the western U.S., but little is known about its nutritional value. Our objective was to evaluate intake and digestibility using different dietary ratios of forage kochia and tall wheatgrass (*Elytrigia elongata*). Four ruminally fistulated beef steers (mean initial BW = 348 kg) were allocated to four treatments in a 4 × 4 Latin square design. Treatments were: 100% wheatgrass (0K); 75% wheatgrass:25% kochia (25K); 50% wheatgrass:50% kochia (50K); and 25% wheatgrass:75% kochia diet (75K). Steers were fed twice daily at 110% of mean intake over the previous 5 d. Steers were allowed an 11 to 13 d adaptation period. Feed intake and fecal output were measured and sampled during the following 7 d. Immediately following that period, duplicate in-situ bags of wheatgrass or kochia were incubated in the rumen for 0, 2, 6, 12, 18, 24, 48, and 96 h. Data were analyzed in a Latin square-design in the MIXED procedure of SAS. Intake increased linearly ( $P = 0.019$ ) as the amount of kochia increased in the diet. In vivo DM digestibility differed among treatments ( $P = 0.014$ ), and tended ( $P = 0.107$ ) to display a quadratic response, with maximum DM digestibility at 25K. In vivo NDF digestibility decreased linearly ( $P = 0.012$ ) as the amount of kochia increased in the diet. In situ rate of DM and NDF digestion of wheatgrass samples increased ( $P < 0.02$ ) as the amount of kochia increased in the diet. The in situ rate of DM digestion of kochia samples also increased ( $P = 0.005$ ) as the amount of kochia increased in the diet. The in situ rate of NDF digestion for kochia samples tended to increase ( $P = 0.056$ ) as the amount of kochia increased in the diet. Although fiber digestibility decreased as kochia was added to the diet, the steers were able to increase feed intake because of an increase in the rate of digestion of DM and NDF. Incorporating forage kochia into a low-quality grass diet improved nutrient utilization.

Key words: Beef cattle, forage utilization, forage kochia

## Introduction

Forage kochia (*Kochia prostrata*) is a semi-evergreen half shrub that grows to a height of 0.3 to 0.9 m. It is native to the arid and semiarid regions of Central Eurasia (Keller and Bleak, 1974) and has adapted well to a variety of environmental conditions in the western United States. It is drought tolerant and capable of growing in areas receiving 12 to 50 cm of annual precipitation (ZoBell et al., 2003).

Potential uses of forage kochia include forage for livestock and wildlife (Gade and Provenza, 1986), food and cover for upland game birds (Stevens et al. 1985), ground cover on disturbed sites, greenstrips that reduce wildfire size and/or spread (Clements et al. 1997), and competition against the invasive annual weeds cheatgrass (*Bromus tectorum*), Russian thistle (*Salsola tragus*), medusahead (*Taeniatherum caput-medusae*), and halogeton (*Halogeton glomeratus*).

Fall and winter grazing studies have been conducted on forage kochia (ZoBell et al., 2003; Koch and Asay, 2002) but nutritional value and the optimum amount of kochia in the diet that will be most beneficial to an animal has not been evaluated.

The objective of this study was to evaluate intake and digestibility responses by beef cattle to different dietary ratios of forage kochia and tall wheatgrass (*Elytrigia elongata*) straw.

## Materials and Methods

Four ruminally fistulated beef steers (mean initial BW = 348 kg) were allocated to one of four treatments in a 4 × 4 Latin square design. The 4 treatments consisted of varying mixtures of tall wheatgrass straw and kochia mixed to provide diets of: 100% wheatgrass (0K); 75% wheatgrass:25% kochia (25K); 50% wheatgrass:50% kochia (50K); and 25% wheatgrass:75% kochia diet (75K) on an as-fed basis. The forage kochia was from an irrigated, pure stand with the intent of the seed being harvested but was instead harvested as hay at full maturity with the seed-heads attached for the purpose of this study. The tall wheatgrass straw was harvested in late fall from an irrigated, pure stand after seed had been harvested. Both forages were intended to mimic stockpiled forage used for winter grazing. The tall wheatgrass and kochia were chopped to an average length of 3 cm. Treatment diets were fed as mixed rations.

Steers were housed in individual metabolism crates (2.4 x 1.1 m) located inside a shed that was enclosed on three sides and was open facing the south. Steers were allowed free access to water and a trace-mineralized salt block (Table 1). Steers were fed twice daily at 0700 and 1900. Orts were collected and weighed daily before the morning feeding. Steers were then offered 110% of mean intake over the previous 5 d. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Utah State University.

<sup>1</sup> Research supported by Utah Agricultural Experiment Station.

Experimental periods were 24 to 26 d, with 11 to 13 d of adaptation. Period 1 consisted of a 13-d period to allow steers to adjust to their respective treatments. Period 2 adaptation was reduced to 12 d and periods 3 and 4 were reduced to 11 d because of a limited supply of forage kochia. The following 7-d period was used to measure feed intake and fecal output. Individual feed, mixed diet, and orts samples were collected during this period. Total feces voided were collected in tubs and weighed twice daily at 0700 and 1900. A representative 2% subsample (wet weight) was obtained each time feces were weighed. The subsample was immediately weighed and dried at 60°C in a forced-air oven until no more weight loss.

Feed, diet and ort samples were dried at 60°C to obtain partial DM. Feed, diet, orts and fecal samples were then ground in a Wiley mill to pass a 1-mm screen. Feed, diet, orts and fecal samples were composited within steer and period.

Samples of wheatgrass and kochia that were representative of the feed used during each period were ground in a Wiley mill to pass a 2-mm screen for in situ procedures. Duplicate 5 g samples of each were weighed into 10 × 20 cm nylon bags (Ankom, Fairport, NY) with a 50 ± 15µm pore size, and heat-sealed using an impulse sealer (model MP-8; Midwest Pacific from Ankom, Fairport, NY) for each time point and each animal. Samples for each time point were placed in 36 × 42 cm polyester mesh bags to ensure similar location within the rumen and to assist in retrieval. Bags were placed in reverse order to allow 0 (never placed in the rumen), 2, 6, 12, 18, 24, 48, and 96 h of fermentation. Bags for 96 h were placed into the rumen of each animal on the day of the last fecal collections. All bags were removed simultaneously at 0 h and placed immediately into an ice-water bath to stop microbial activity. Bags were then immediately rinsed in a Kenmore heavy-duty, top-loading washing machine (Sears, Roebuck, and Co., Chicago, IL) for 10 cold-water rinse cycles. Each rinse cycle consisted of a one-minute agitation and a two-minute spin per rinse. The 0-h time bags were treated identically to the rest of the samples except for not being placed in the rumen. After rinsing, the bags were dried at 105° C for 48 h and then weighed to determine rate of DM digestion.

**Laboratory Analysis.** All samples were dried overnight at 105°C to determine DM (AOAC, 1996). Feed and diet samples were analyzed for ADF content and feed, diet, and fecal samples were analyzed for NDF content using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). In situ residues were also analyzed for NDF content. Feed and diet samples were analyzed for N content with the combustion method (AOAC, 1996) using a N analyzer (Leco, St. Joseph, MI) and nitrogen was multiplied by 6.25 to determine CP.

**Statistical analyses.** The nonlinear regression procedure of SAS (PROC NLIN, SAS Institute, Cary, NC) was used with the in situ residue data to calculate ruminal rate of disappearance of DM and NDF. DM intake and extent and rate of digestion of DM and NDF were analyzed using the MIXED procedure of SAS in a Latin square-repeated measure design. The model included period,

treatment, and steer. Steer was designated a random effect and treatment was designated a repeated measure. Linear and quadratic polynomial contrasts were constructed to evaluate the influence of increasing levels of forage kochia in the diet.

## Results and Discussion

Chemical composition of wheatgrass and kochia are reported in Table 2. The low CP and high fiber of the wheatgrass typified a low-quality, dormant forage available for winter grazing. The CP level of kochia reported in this study was similar to levels that have been reported for fall and winter grazing by ZoBell et al. (2003) and Koch and Asay (2002), but slightly higher than that reported for the same period by Schauer et al. (2004). However, fiber values were lower than that reported by ZoBell et al. (2003) and Koch and Asay (2002). Differences could be due to different maturities at time of harvest or growing conditions that contributed to differences in plant stature and structural components.

Dry matter intake expressed on a percentage of BW basis increased linearly as the amount of kochia increased in the diet (Table 3).

In vivo DM digestibility tended to display a quadratic response (Table 3). Maximum DM digestibility occurred at 25K. In vivo NDF digestibility decreased linearly as the amount of kochia increased in the diet.

The in situ rate of DM and NDF digestion of wheatgrass increased linearly as the amount of kochia increased in the diet (Table 4). The rate of DM digestion of kochia increased linearly and the rate of NDF digestion for kochia samples tended to increase as the amount of kochia increased in the diet. The incremental increases in kochia caused increased rate and extent of digestion of the wheatgrass, indicating that kochia caused a positive associative effect to occur.

Forage intake is often positively related to rate and extent of digestion in the rumen (Forbes, 1996). The rate of digestion increased as kochia was added to the diets with a concomitant rise in intake. The rise in intake is further evidence of a positive associative effect resulting from addition of kochia to the diet.

Forage kochia has been considered a potential source of forage that has the ability to increase CP supplied to livestock in the winter grazing period (Welch and Davis, 1984; ZoBell et al., 2003). Besides that, forage kochia also increased digestibility of low quality forage in this study. Incorporation of forage kochia in grass stands intended for winter grazing has the potential to improve livestock performance and decrease supplementation costs. ZoBell et al. (2003) showed that cattle grazing a mixed stand of forage kochia and crested wheatgrass (*Agropyron desertorum*) during winter were able to select a diet that was higher in nutritional value than the average of available forage. In a grazing situation in which forage kochia is available, livestock will have the opportunity to choose the best ratio of forage kochia to other available forage. Alternatively, livestock producers with pure grass stands can use these results to determine the amount of forage kochia that should be interseeded for an optimum combination.

## Implications

Although extent of fiber digestion decreased as kochia was added to the diet, the steers were able to increase feed intake because of an increase in the rate of digestion of DM and NDF. Incorporating forage kochia into a low-quality grass diet improved nutrient utilization. Based on the results reported herein, forage kochia should be interseeded into existing grass stands to achieve a 25 to 50% proportion of the available DM as kochia to optimize the combination of rate and extent of diet digestion and intake.

## Literature Cited

- AOAC. 1996. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Arlington, VA.
- Clements, C.D., K.J. Gray, and J.A. Young. 1997. Forage kochia: To seed or not to seed. *Rangelands* 19:29-31.
- Forbes, J.M. 1996. Integration of regulatory signals controlling forage intake in ruminants. *J. Anim. Sci.* 74:3029-3035.

- Gade, A.E., and F.D. Provenza. 1986. Nutrition of sheep grazing crested wheatgrass versus crested wheatgrass-shrub pastures during winter. *J. Range Manage.* 39:527-530.
- Keller, W., and A. T. Bleak. 1974. *Kochia prostrata*: A shrub for western ranges? *Utah Sci.* 35:24-25.
- Koch, D.W., and W. Asay. 2002. Forage kochia-a forage with fall and winter grazing potential. *Univ. Wyoming Ext. Bull.* B-1122.5.
- Schauer, C.S., D.W. Bohnert, M.F. Carpinelli, and S.J. Falck. 2004. Nutritional and seed responses of forage kochia to ruminal incubation. *Rangelands* 26:8-11.
- Stevens, R., K.R. Jorgensen, E.D. McArthur, and J.N. Davis. 1985. "Immigrant" forage kochia. *Rangelands* 7:22-23.
- Welch, B.L. and J.N. Davis. 1984. In vitro digestibility of *Kochia prostrata* (L.) Schrad. *Great Basin Nat.* 44:296-298.
- ZoBell, D.R., B.L. Waldron, K.C. Olson, R.D. Harrison, and H. Jensen. 2003. Forage kochia for fall/winter grazing. *Utah State Univ. Ext. Pub.* AG-2003-07.

Table 1. Composition of trace-mineralized salt block <sup>a</sup>

Item	Concentration
Salt (NaCl) minimum	95.5 %
Salt (NaCl) maximum	98.5 %
Zinc (Zn) minimum	3,500 ppm
Iron (Fe) minimum	2,000 ppm
Manganese (Mn) minimum	1,800 ppm
Copper (Cu) minimum	280 ppm
Copper (Cu) maximum	420 ppm
Iodine (I) minimum	100 ppm
Cobalt (Co) minimum	60 ppm

<sup>a</sup> Ingredients: salt, zinc oxide, ferrous carbonate, manganous oxide, magnesium oxide, copper oxide, calcium iodate, cobalt carbonate, red iron oxide for color.

Table 2. Chemical composition of tall wheatgrass and forage kochia used in diets

Item	Wheatgrass	Kochia
DM, %	94.1	93.6
	----- % of DM -----	
CP	3.6	9.6
NDF	77.7	53.8
ADF	50.6	32.2

Table 3. Least squares mean responses for intake and extent of digestion to dietary level of forage kochia

Item	Kochia, % as fed				SE	Contrasts	
	0	25	50	75		Linear	Quadratic
DM intake, %BW	0.975	1.666	1.881	2.324	0.098	0.02	0.28
DM Digestibility, %	51.29	53.67	50.73	52.17	0.355	0.78	0.11
NDF Digestibility, %	55.0	55.7	49.6	46.8	1.25	0.01	0.24

Table 4. Least squares mean responses for in situ rate of digestion of DM and NDF of tall wheatgrass and forage kochia

Item	Kochia, % as fed				SE	Contrasts	
	0	25	50	75		Linear	Quadratic
Tall wheatgrass							
DM rate, % h <sup>-1</sup>	1.11	2.55	1.87	2.52	0.225	0.02	0.15
NDF rate, % h <sup>-1</sup>	1.07	2.56	2.11	2.92	0.208	0.002	0.18
Forage kochia							
DM rate, % h <sup>-1</sup>	8.1	10.3	14.1	17.5	2.37	0.005	0.73
NDF rate, % h <sup>-1</sup>	4.5	7.4	11.9	19.3	1.77	0.06	0.27

## A COMPARISON OF LOW-QUALITY FORAGE UTILIZATION BY CATTLE VERSUS HORSES

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**ABSTRACT:** Both cattle and horses have extensive gastrointestinal fermentation capacity. Cattle, however, have two major fermentation chambers in the rumen and cecum/colon while horses ferment in the cecum/colon only. The objective of this study was to compare cattle and horses regarding DM intake (DMI) and digestibility (DMD) when fed a low-quality forage diet. Two mature mares (477 kg) and two mature cows (511 kg) were fed a diet composed of ammoniated wheat straw (AWS) supplemented with alfalfa hay pellets (ALF). Horses and cattle received 0.022 kg ALF/kg of metabolic body weight/d plus ad libitum access to AWS. Animals were adapted to the diets for 21 d period followed by a 5 d collection period. During the collection period samples of feces and feed were taken twice d for subsequent proximate analysis and analysis for acid insoluble ash (AIA). AIA was used as an internal marker for apparent digestibility. Intake of AWS was also measured during the collection period. After the initial collection period the horses and cattle continued to receive the diet for an additional 10 d adaptation period followed by another 5 d collection period. Cattle consumed 11.3 kg DM/d while horses consumed only 8.25 kg/d ( $P < 0.05$ ). However, DMD did not differ ( $P > 0.05$ ) between the two species: 52.14% and 48.39% for cattle and horses, respectively. Cattle consumed about 47% more DDMI than horses, 5.90 kg/d versus 4.01 kg/d ( $P < 0.05$ ). Cattle also exhibited higher digestibility of cellulose (69.24% versus 52.46%) and hemicellulose (87.29% versus 66.38%) for cattle versus horses, respectively ( $P < 0.05$ ). The DDMI of the two species indicates that horses can use AWS diets for maintenance purposes only while utilization by cattle indicates maintenance plus some production.

Key Words: Low-quality forage, Horses & cattle, Utilization

### Introduction

Cattle have extensive fermentation capacity in the rumen and secondarily in the cecum/colon. Although horses have an extremely small stomach for an animal their size, they do have more extensive fermentation capacity in the cecum/colon than do cattle. All feed consumed by cattle first enters the rumen making supplementation programs that stimulate ruminal fibrolytic microorganism a relatively simple process. In horses, such supplements are digested and absorbed in the small intestine and thus will have limited affect on fibrous polysaccharide hydrolysis in the cecum/colon. This agrees with research findings from Mésoschina et al., that the ruminant digestive system

is more efficient than the hind-gut fermentation of horses. Thus we assume that the utilization of low-quality forages by horses will be lower than that of cattle, but be adequate for the maintenance of mature horses during the wintering period. The objective of this study was to measure the difference in the utilization of low-quality forages between mature cattle and mature horses.

### Materials and Methods

Two mature mares (477 kg) and two mature cows (511 kg) were used for the study. Both the cows and the mares were seven years old at the time of the study. The mares were sired by a single Tennessee Walker stallion while the cows were sired by a single Hereford sire.

Mares and cows were placed in adjacent individual pens (4m x 9m). Each pen had a covered loafing area at one end and a covered feeder at the other end. The loafing area was regularly bedded with wood shavings during the study. The feeding area was paved with concrete and had a covered feeder and waterer. Fresh, clean water was available at all times.

The diet was composed of alfalfa pellets (ALF) (19.7% CP, 43% NDF) as a supplement, a vitamin-mineral supplement (VM), and ad libitum access to ammoniated wheat straw (AWS). The VM was designed to rectify deficiencies associated with an AWS diet and emphasized phosphorus and fat-soluble vitamins. It was formulated to be highly palatable and readily consumed with the addition of dried molasses. Wheat straw was ammoniated using the bale-stack method. A stack of small square bales was covered with a 6-mil polyethylene film that was anchored at the base of the stack with road-base gravel. Anhydrous ammonia was slowly injected into the stack through a perforated steel pipe embedded near the bottom of the stack at a rate of 3% of DM. The stack remained covered for about 120 d after ammoniation (August through November). One end of the stack was then opened to allow dissipation of excess ammonia. The study began the first week of December so the stack was adequately cleared of excess ammonia.

Mares and cows were fed the diet for a 21 d adaptation period followed by a 5 d sample collection period. During the adaptation period animals were fed twice per day at 0800 and 1500 h. The ALF supplement was offered at a rate of 0.022 kg/kg of metabolic BW to adjust for the weight difference between the mares and the cows. The VM was fed only at the 0800 h feeding since only

200g were fed per d. The AWS was fed such that there were limited refusals at each feeding (2-3%). Mares were much more adept at sorting the chaff and leaves from the AWS than the cows. Feeding for limited refusals helped insure consumption of all portions of the AWS. The intake of AWS was recorded each d. The amount offered at each feeding was adjusted based on the refusals remaining from the previous feeding. The AWS was not ground or processed prior to feeding. Rather, it was offered directly from the bales to mimic practical feeding conditions.

During the 5 d collection period samples of diet and feces were collected at each of the two feedings. Core samples were taken from the bale flake or AWS offered to each animal. A 200g sample of ALF was also taken at each feeding along with a 20g sample of VM. Fecal samples were taken from fresh droppings in the pens at each feeding rather than rectal grab samples, which stresses that animal under such a sampling regimen. Fecal samples were immediately dried in a forced-air oven at 60°C for 72 h. All samples were then ground to pass a 1mm screen in a Wiley Mill. All samples were then proportionately composited by d and then analyzed for laboratory DM (105°C, 8 h), NDF, ADF, ADL, hemicellulose, and cellulose using the filter bag system (Komareck and Sorois, 1993), nitrogen (CP) using an elemental analyzer (rapid combustion @ 850°C and conversion of all N to N<sub>2</sub> for subsequent measurement by thermoconductivity, LECO CHN – 1000 Combustion Analyzer)(Yeomans and Bremmer, 1991), and acid insoluble ash (AIA) using the Van Keulen and Young (1977) method. Acid insoluble ash was used as an internal marker to estimate apparent nutrient digestibility.

## Results and Discussion

A comparison of intake and apparent nutrient digestibility between mature mares and cows is presented in Table 1. Mature cows consumed 37% more DM when offered an AWS diet than mature mares. The mares (477 kg) consumed only 1.73% of BW while cows (511 kg) consumed 2.21% of BW. Surprisingly, the digestibility of DM did not differ between the two species, both apparently digesting about half of the dietary DM. However, DMD was numerically higher in the cows. The lower DMI exhibited by the mares may account for this observation. Lower intake is usually associated with slower rates of digesta passage allowing more time for microbial fibrolysis. It is often observed that horses are more variable regarding nutrient utilization than cattle. In this study the SEM for horses regarding DMD was 3.38 while that for the cows was 0.64. Most importantly the cows consumed 47% more DDM than the mares.

Most of the improvement in DDMI can be attributed to increased fibrous carbohydrate digestibility exhibited by the cows. Cellulose digestibility was about 16.8 percentage points higher in cows. Similarly hemicellulose digestibility was 20.9 percentage points higher in cows than mares. The digestibility of

hemicellulose is quite high in both species. The ammoniation of low-quality forages such as cereal straw is noted for increasing the solubility and therefore digestibility of hemicellulose.

Crude protein digestibility did not differ between the two species and was relatively low in both species. The ammoniation of low-quality forages such as wheat straw usually increases the CP content by 100 to 200%. The CP content of the wheat straw used in this study was 3.25% before ammoniation and 9.49% after. The utilization of this added CP is usually considered to be limited.

The digestibility of GE was about 21% higher in cows than mares. The GE content of the diet consumed by the mares was 3.882 Mcal/kg DM and that of the cows was 3.834 Mcal/kg DM. This infers that this diet contained 1.74 Mcal DE/kg DM in mares and 2.08 Mcal DE/kg DM in cows. The mares consumed 8.25 kg DM of this diet and thus consumed 14.36 Mcal of DE. Cows consumed 11.31 kg DM and thus consumed 23.52 Mcal of DE. On a metabolic BW basis mares consumed 0.141 Mcal DE/kg metabolic BW and cows consumed 0.219 Mcal DE/kg metabolic BW. The maintenance energy requirement of the mares (477 kg) is estimated to be  $(1.4 + 0.03 \times \text{BW})$  15.71 Mcal DE/d. The mares on this study were about 91.4% of maintenance energy requirement on this AWS-based diet (NRC, 1989). The estimated maintenance TDN requirement of the cows (511 kg) is estimated to be 4.67 kg/d. Converted to a DE basis this would be 20.55 Mcal DE/d. The cows were receiving 23.52 Mcal DE/d from this diet and were thus receiving 114% of maintenance energy requirement (NRC, 1996).

## Implications

Physiological differences between horses and cattle result in utilization differences in feeds. Especially when fed low-quality forage, cattle have rumen microorganisms that increase the quality of the low-quality diet. When fed a supplement, the microorganisms are stimulated, thus increasing fibrolytic activity. Horses do not contain microorganisms and therefore do not have the fibrolytic capability of cattle. This study validated these facts and showed that utilization is much higher in cattle than horses when fed a low-quality diet.

## Literature Cited

- Komared, A.R., and P.K. Sorois. 1993. Improved efficiency of ADF analysis using a filter bag procedure. *J. Anim. Sci.* 71 (Suppl. 1):284.
- Mésochina, P., W. Martin-Rosset, J.-L. Peyraud, P. Duncan, D. Micol and S. Boulot. 1998. Prediction of the digestibility of the diet of horses: evaluation of faecal indices. *Grass and Forage Science.* 53:189-196

NRC. 1989. Nutrient Requirements of Horses, Fifth Revised Edition, 1989, National Research Council, Nutrient Requirement of Domestic Animals, National Academy Press, Washington, D.C.

NRC. 1996. Nutrient Requirements of Beef Cattle, Seventh Revised Edition, 1996, National Research Council, Nutrient Requirement of Domestic Animals, National Academy Press, Washington, D.C.

Van Keulen, J., and B.A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci. 44:282.

Yeomans, J.C., and J.M. Bremmer. 1991. Carbon and nitrogen analysis of soils by automated combustion techniques. Comm. in Soil Sci. Plant Anal. 22:843-850.

Table 1. Comparison of mature mares and cows regarding digestibility and intake of an ammoniated wheat straw diet supplemented with alfalfa

Item	Species		
	Mares	Cows	SEM <sup>c</sup>
Dry matter intake, kg/d	8.25 <sup>b</sup>	11.31 <sup>a</sup>	0.315
Dry matter digestibility, %	48.39	52.14	2.01
Digestible dry matter intake, kg	4.01 <sup>b</sup>	5.9 <sup>a</sup>	0.303
Cellulose digestibility, %	52.46 <sup>b</sup>	69.24 <sup>a</sup>	2.29
Hemicellulose digestibility, %	66.38 <sup>b</sup>	87.29 <sup>a</sup>	2.47
Crude protein digestibility, %	60.52	57.37	5.03
Gross energy digestibility, %	44.86 <sup>b</sup>	54.17 <sup>a</sup>	2.14

<sup>a,b</sup>Means in the same row with different superscripts differ,  $P < 0.05$

<sup>c</sup>Standard error of mean

## COMPARATIVE PRODUCTIVITY OF FIVE COOL-SEASON PASTURE GRASSES UNDER INTERMITTENT FLOOD IRRIGATION AND GRAZED BY BEEF COW-CALF PAIRS USING MANAGEMENT INTENSIVE GRAZING PRACTICES

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**ABSTRACT:** Irrigated pastures are an alternative resource for cow-calf producers in public rangeland areas. The objective of this study was to compare the productivity of five cool-season grasses under intermittent flood irrigation common to the mountain valleys of the Intermountain West: meadow brome, *Bromus biebersteinii* "Regar" (MB); orchard grass, *Dactylis glomerata* "Ambassador" (OG); perennial ryegrass, *Lolium perenne* "BG3" (PRG); tall fescue, *Festuca arundinacea* "Fawn" (FWTF); tall fescue, *Festuca arundinacea* "Fuego" (FGTF). A 5.1 ha field was subdivided into 10, 0.51 ha plots using flood irrigation burms and electric fencing. Each plot was then sown to one of the five species above with two plots per species. Cow-calf pairs grazed plots after one year of establishment. Management intensive grazing practices were used with cattle receiving a new pasture allotment each 24 h. Pastures were grazed for three consecutive years. Intakes were established by taking clip plot samples before and after grazing. Flood irrigation of the plots was intermittent due to drought conditions. Plots were irrigated three times during a 160 d grazing season with flows of water usually inadequate for complete coverage and saturation. Plots received 33.7 kg N/ha prior to each irrigation. Average DM harvested (kg DM/ha) was as follows: MB, 9,704; OG, 13,074; PRG, 9,345; FWTF, 14,916; FGTF, 14,781. Productivity of MB and PRG were similar ( $P > 0.05$ ) as was the productivity of FWTF and FGTF. The TF species produced 56% more DM than MB or PRG ( $P < 0.05$ ) and 13% more DM than OG ( $P < 0.05$ ). Based on this study we recommend improved TF species for cow-calf production on irrigated pastures in the Intermountain West.

Key Words: Irrigated pastures, Cow-calf, Cool-season grasses

### Introduction

In public-land states such as Utah 70 - 80% of the cow-calf producers are currently heavily dependent on leasing of public lands for grazing. Use of public lands for livestock grazing can be precarious from year to year due to changes in policies and political situations. One alternative that can be considered by cow-calf producers faced with curtailments in public land use would be intensive management and production on privately owned irrigated pastures. When developing productive irrigated

pastures, one of the first decisions is the species of grass best matched to the environment of the Intermountain West. Since most research in this regard has been conducted in other regions (Blaser, et al., 1956; Gesshe and Walton, 1981; Heinemann and Hanks, 1982; Welty, 1979), localized information will be crucial to success. It was the objective of this study to determine the productivity of five species of cool-season grasses commonly used for irrigated pastures as measured by the grazing of cow-calf pairs in the Great Basin.

### Materials and Methods

A 5.1 ha field used for silage corn production the previous three years was prepared for a late fall, dormant seeding of cool-season grass species commonly used for irrigated pastures. Four equally spaced burms were raised separating the field into five strips (39m x 268m). Electric fence was placed on each burn. The burn aided in control of flood irrigation. Each of the five strips was then subdivided into two equal plots (39m x 134m). Each of the resulting ten .5 ha plots was then randomly assigned one of five grass species, two plots per species:

1. Regar Meadow Brome, *Bromus biebersteinii*
2. Ambassador Orchard Grass, *Dactylis glomerata*
3. BG3 Perennial Ryegrass, *Lolium perenne*
4. Fawn Tall Fescue, *Festuca arundinacea*
5. Fuego Tall Fescue, *Festuca arundinacea*

Seeding rate for all of the species was 23 kg/ha. Seeding was accomplished using a roller packing type drill.

Plots were not grazed the first year, allowing proper establishment and weed control procedures including use of herbicides and hay crop removal:

1. Regar Meadow Brome, 1889 kg DM/ha
2. Ambassador Orchard Grass, 3954 kg DM/ha
3. BG3 Perennial Ryegrass, 4807 kg DM/ha
4. Fawn Tall Fescue, 7143 kg DM/ha
5. Fuego Tall Fescue, 7323 kg DM/ha

Intervals between flood irrigation were longer than would be considered ideal for irrigated pasture due to drought conditions during the study.

Year	Dates of flood irrigation		
Establishment	May 29	July 8	Sept. 4
Grazing 1	June 4	July 12	Aug. 29
Grazing 2	May 21	July 17	Sept. 3
Grazing 3	June 5	July 12	Aug. 7, 25

Flows of irrigation water were often inadequate for thorough coverage and saturation. Before each irrigation, nitrogen in the form of ammonium nitrate was applied to each plot at a rate of 34 kg nitrogen/ha.

Plots were grazed by 32 beef cow-calf pairs. Average body weights of the cows were 645 kg and that of the calves were 182 kg though the grazing period (May through October). It was originally planned that all plots would be grazed simultaneously by small groups of cattle requiring 14 to 21 d to graze each plot. However, due to lack of irrigation water, the cattle were stratified into two groups of 16 pairs each. Thus, two plots were grazed at a time. This allowed the fairly rapid removal of forage on the plots (4 – 7 d). The grazed plots were then fertilized and irrigated while the two groups of cattle grazed two other plots. At the beginning of each grazing season the different species of grasses reached grazing readiness at different times in the following order:

1. Meadow Brome
2. Orchard Grass
3. Fuego Tall Fescue
4. Fawn Tall Fescue
5. Perennial Ryegrass

The two plots of each of these grass species were grazed in this order. After the first grazing period, plots were grazed as they reached readiness, yielding at least 1685 kg DM/ha.

Management intensive grazing was used with cattle receiving a new allotment of pasture each 24 h. Daily pasture allotments were controlled using polywire electric fencing in front of and behind the cattle. Forage harvested was estimated by the difference in clip plot analysis of dry matter before and after grazing each daily allotment.

Data were analyzed using Proc Mixed procedure of SAS (SAS Inst. Inc., 1996, Cary, NC). Model included kg DM/ha as the dependant variable and species of forage, year and species by year interaction as independent variables. Least square means were computed and differences between means were determined using the adjusted Tukeys Method with difference considered significant if  $P < 0.05$ .

## Results and Discussion

Table 1 is a summary of the grazed forage yields from the pasture grass species for three consecutive years, keeping in mind that irrigation water was limited. The year within species effect was not significant. Under the conditions

described, the tall fescue species produced 13.4% more grazed forage than orchard grass. The tall fescue species produced 57.1% more grazed forage than either the meadow brome or perennial ryegrass, which were similar. Meadow brome and perennial ryegrass appear to be declining in productivity while orchard grass and tall fescue species were increasing in productivity. There was little difference regarding the productivity of the two tall fescue species.

Comparing the productivity of irrigated orchard grass and perennial ryegrass pastures, Heinemann and Hanks (1982) measured 22% higher carrying capacity for orchard grass. In an early study from Virginia, Baser, et al., (1956) compared the productivity of orchard grass and tall fescue as well as grass-legume mixtures and reported a 33% higher carrying capacity for tall fescue than orchard grass. Regarding carrying capacity, tall fescue also appears to be the preferred species for irrigated pasture in the Great Basin.

Estimating the average DM requirement of the cow-calf pairs used in this study to be approximately 20.9 kg DM/pair/d, Table 2 would be an estimate of the carrying capacity of the grass species under the conditions described.

## Implications

Due to higher carrying capacity and grazed forage harvested (kg DM/ha), the tall fescue species in this study out performed the other three cool-season grasses on irrigated pastures with intermittent flood irrigation.

## Literature Cited

- Blaser, R.E., R.C. Hammes, Jr., H.T. Bryant, C.M. Kincaid, W.H. Skrdla, T.H. Taylor and W.L. Griffeth. 1956. The value of forage species and mixtures for fattening steers. *Agron. J.* 48:508-513
- Gesshe, R.H. and P.D. Walton. 1981. Grazing animal preferences for cultivated forages in Canada. *J. Range Mangmnt.* 34:42-45
- Heinemann, W.W. and E.M. Hanks. 1982. Irrigated orchard grass and perennial ryegrass pastures grazed by lightweight steers. Washington State Agricultural Research Center. Bulletin No. XB 0926. Washington State University, Pullman.
- Welty, L.E. 1979. Evaluation of four irrigated pastures for beef production. Montana Agricultural Experiment Station. Bulletin No. 714. Montana State University, Bozeman.

Table 1. Forage harvested through grazing on five flood irrigated cool-season grasses over three years

Species	Harvested Forage, kg DM/ha			
	Year 1	Year 2	Year 3	Average
Regar Meadow Brome	9165	11120	8828	9704 <sup>b</sup>
Ambassador Orchard Grass	11816	13343	14085	13074 <sup>c</sup>
Fawn Tall Fescue	13298	15410	16039	14916 <sup>a</sup>
Fuego Tall Fescue	13591	14981	15859	14781 <sup>a</sup>
BG3 Perennial Ryegrass	9390	10086	8581	9345 <sup>b</sup>
Average	11456 <sup>b</sup>	12962 <sup>a</sup>	12669 <sup>a</sup>	

<sup>abc</sup>Means in same row and column with different superscripts differ,  $P < 0.05$

Table 2. Carrying capacity on five cool-season grasses

Species	kg DM/ha/yr	lbs DM/pair/d	Grazing days	Carrying Capacity, pairs/ha
Regar Meadow Brome	9704	20.9	180	2.58
Ambassador Orchard Grass	13074	20.9	180	3.48
Fawn Tall Fescue	14916	20.9	180	3.96
Fuego Tall Fescue	14781	20.9	180	3.93
BG3 Perennial Ryegrass	9345	20.9	180	2.48

## UTILIZATION OF FORAGE KOCHIA FOR FALL/WINTER GRAZING

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**ABSTRACT:** The objective of this study was to evaluate forage kochia as a resource for fall/winter grazing beef cows compared to a traditional stock-piled roughage feeding program. In mid-November, 42 beef cattle were randomly assigned to one of two treatments: Control - received stock-piled alfalfa hay free-choice, or Treated - placed on pastures containing a mix of forage kochia and crested wheatgrass. All groups were replicated three times. Initial and final data were obtained for body condition score and backfat. Pastures and alfalfa were analyzed for nutritive properties throughout the trial. A preference study was also conducted utilizing cannulated beef cows on the pastures. Pasture results from this 84 d study showed that clipped forage samples of forage kochia had higher crude protein than crested wheatgrass and lower NDF but higher ADF than the grass samples. Forage quality of both forage kochia and crested wheatgrass decreased as the winter progressed. Crude protein for the forage kochia was 10.7% in November and gradually decreased to 5.3% by the end of January. Crude protein for crested wheatgrass was 6.7% in November and dropped to 5.1% by late January. Forage yield for all three pastures averaged 971.2 kg/ha (DM basis). The average yield for forage kochia was estimated to be 660.2 kg/ha and crested wheatgrass was 311.0 kg/ha. Pasture yield decreased from 1302.4 kg/ha in November to 462.6 kg/ha by the end of January. Cow performance data indicated that BCS and BF changed over time for the alfalfa and kochia fed cows ( $P<0.05$ ). Cows in the drylot pens averaged 13.6 kg/day of alfalfa hay which was more than adequate to meet requirements. The quality of cow diets based on the preference study was always higher than quality of the forage available to them. The grazing system would have been more profitable due to lower costs. It was concluded that forage kochia has tremendous potential advantages for beef producers using it as a roughage source for grazing beef cows during late fall and early winter as an alternative to feeding harvested forage.

Key words: Beef Cows, Forage Kochia, Feed Costs

### Introduction

Winter feeding costs in the Intermountain West can represent 50 to 70 percent of the input costs per cow per year (Hathaway, 2003). Research and rancher experience suggests that using forage kochia for fall/winter grazing may help reduce these costs (Koch, 2002; ZoBell et al, 2003). Forage or prostrate kochia (*Kochia prostrata*) is native to the heavily grazed rangeland regions of Central

Eurasia and is an important fall and winter forage for various domestic and wildlife species (Waldron, 2001). It is a long lived, semi-evergreen half-shrub that averages .30 to 1.0 meter high. It is drought, saline, and alkaline tolerant, and grows on a wide range of soils in areas receiving 13 to 50 cm of yearly precipitation (McArthur and Sanderson, 1996). It is well adapted to marginal rangelands, out-competing cheatgrass (*Bromus tectorum*) and halogeton (*Halogeton glomeratus*) and stabilizing disturbed soils. Forage kochia is different than the weed annual kochia (*Kochia scoparia*), in that forage kochia is a perennial semi-shrub, will not spread into perennial plant stands, and does not have nitrate or oxalate toxicity (Harrison, 2000). Forage kochia can also be used as greenstrips to reduce the spread of wildfires (Harrison et al., 2002).

The objective of this study was to evaluate forage kochia as a resource for fall/winter grazing beef cows compared to a traditional stock-piled roughage feeding program.

### Materials and Methods

An 84-day study was conducted in Box Elder County in cooperation with the USDA Farm Service Agency and the Salt Wells Cattle Company. In mid-November, 2002) 42 late-gestation Black Angus beef cattle (average age 7 years) were divided into six groups to provide three replicate groups of each feed treatment. Control cows were fed alfalfa hay in drylot pens and treated cows grazed pastures planted to a mixture of kochia and crested wheatgrass. The treatment pastures were 16.2 hectares in size. Pastured cows received no supplement for the duration of the experiment but had free access to salt and water.

Cow body condition score (BCS - scoring system from 1-9 wherein 1 was emaciated and 9 was obese) and ultrasound backfat (BF) thickness were collected initially and at termination on each cow.

Forage clip samples were taken every 28 days on all three pastures to estimate forage yield and quality. Clipped forage samples were taken in representative areas of the pastures using a 1 m<sup>2</sup> plot and clipped to stubble that assumed 70% utilization. Grass and kochia were clipped separately and forage and alfalfa quality were analyzed by determining crude protein, acid detergent fiber (ADF) and neutral detergent fiber (NDF). In vitro true digestibility (IVTD) was also determined on forage samples from the pastures.

Samples of the diet selected by cows on pasture were collected in November and January using ruminally cannulated cows which measured CP, ADF and NDF. Quality of the diet selected by cows was compared to the forage quality of the available grass and forage kochia from the clip plots, as well as the alfalfa hay.

Cow BCS and backfat responses and diet CP and NDF were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC) in a completely randomized design. Body condition scores and backfat thickness were evaluated in a model that included treatment, period (i.e. initial and final) and their interaction. Period was designated a repeated measure. When interactions occurred, means were separated within each level of each main effect using LSD. Diet variables were evaluated in a model that included month, pasture, and cow within pasture. The test of interest was the month effect. Pasture and cow were designated as random effects.

## **Results and Discussion**

### ***Forage Quality***

Clipped forage samples of forage kochia had higher crude protein than crested wheatgrass (Table 1). This was expected because shrubs retain higher levels of crude protein than grasses during the winter. The kochia had lower NDF but higher ADF than the grass samples. The reason that ADF was higher in kochia than grass is because shrubs have higher lignin levels than grasses, and lignin is a component of ADF. Higher NDF in grasses is reflective of higher levels of fiber in the cell wall of grasses. The grass samples had higher digestibility because fiber is potentially digestible while lignin is totally indigestible. This relationship of higher crude protein from shrubs and higher digestibility from grass is typical. Thus, allowing ruminants to consume a combination of kochia and dormant grass is most desirable for obtaining a balance of nutrients and energy in the diet.

Forage quality based on the clipped samples of both forage kochia and crested wheatgrass decreased as the winter progressed (data not shown). Crude protein for the forage kochia was 10.7% in November and gradually decreased to 5.3% by the end of January (study termination). Additionally, crude protein for crested wheatgrass was 6.7% in November and dropped to 5.1% by late January. Reduction of forage quality as the grazing season progresses is to be expected for two reasons. First, cattle graze selectively and remove the best material first, leaving poorer quality material. Second, the forage weathers throughout the winter, losing nutritional value in the process.

The quality of cow diets based on the preference study was always higher than quality of the forage available to them (Table 2). As stated previously, this is to be expected because grazing livestock always select a diet that is higher in nutritional value than the average of all the forage available. Diet quality declined from November to January. This is also to be expected because the value of the forage that remains late in the grazing season is less than what was available in November. Despite the rather

dramatic decline from November to January, January diets still had adequate crude protein to support ruminal digestion of forage (7% is considered the minimum crude protein that will support rumen fermentation). Additionally, diets that are 60% digestible should be adequate to meet requirements of nonlactating cows in mid- to-late gestation. This is supported by their ability to maintain body condition, even in January when diet quality was lowest.

### ***Forage Yield***

Mean forage yield for the pastures throughout the grazing period was 971.2 kg/ha (DMB). The average yield for forage kochia and crested wheatgrass was 660.2 kg/ha and 311.0 kg/ha, respectively. Pasture yield decreased substantially throughout the duration of the study. Total yield decreased from 1302.4 kg/ha in November to 462.6 kg/ha by the end of January. Over time there was an estimated 208.2 kg decrease in forage kochia and a 131.8 kg decrease in crested wheatgrass. The forage kochia yielded significantly more than did the crested wheatgrass on average (348.0 kg/ha more) ( $P < 0.05$ ). Despite the drop in forage availability, the cattle had access to adequate forage to select a diet that met or exceeded their nutrient requirements. However, it appears that cows should be removed from pastures at about the level of residual forage that we observed so that diet quality does not fall below maintenance requirements.

### ***Animal Performance***

Treatment and period interacted ( $P = 0.04$ ) for BCS and tended to interact ( $P = 0.08$ ) for backfat (Table 3). Both BCS and backfat increased for cows in both treatments, but the interactions occurred because cows receiving alfalfa hay had a statistically greater increase in BCS and backfat than cows grazing kochia. Cows in drylot were offered 13.6 kg/d of alfalfa hay, with very little being wasted. This exceeded nutrient requirements for cows in late gestation based on NRC (1996). The experiment ended within days of the onset of parturition. The final BCS of 6 observed with alfalfa feeding was greater than necessary for cows to quickly return to estrus and be fertile by initiation of breeding (Perry et al., 1991). The final BCS of 5.3 observed with kochia grazing would optimize reproductive performance and winter feed costs.

From an economic sense, the grazing system would have been more profitable due to lower costs. This is based on approximately \$45-\$50 / AUM to feed stored feeds and approximately \$16-\$20 / AUM to pay for pasture rent and fees. Grazing systems are also much less labor intensive.

## **Conclusions**

Forage kochia is a nutritious perennial that is well adapted to the Intermountain West region of the U.S. There are tremendous potential advantages for beef producers using it as a roughage source for grazing beef cows during late fall and early winter as an alternative to feeding harvested forage. Viability and sustainability of beef production in the western U.S. can be increased if feed

costs are decreased. Forage kochia could be an important management option to reduce winter feed costs and improve livestock ranching profitability.

### Literature Cited

- Harrison, R.D., N.J. Chatterton, B.L. Waldron, B.W. Davenport, A.J. Palazzo, W.H. Horton, and K.H. Asay. 2000. Forage Kochia – Its Compatibility and potential aggressiveness on Intermountain rangelands. Utah Ag. Exp. Sta. Res. Rpt. 162. (Available on-line at <http://www.agx.usu.edu/agx/ResearchReports/KOCHIA/kochia.html>).
- Harrison, R.D., B.L. Waldron, K.B. Jensen, R. Page, T.A. Monaco, W.H. Horton, and A.J. Palazzo. 2002. Forage kochia helps fight range fires. Rangelands. 24(5):3-7.
- Hathaway, Ronald. 2003. Alternative winter nutritional management strategies. Western Beef Resource Committee, Cow-Calf Management Guide CL314.
- Koch, D. 2002. Kochia – a forage with winter grazing potential. University of Wyoming Extension (Available on-line at <http://wwwuwyo.edu/ces/psas/SMRR/kochia.html>).
- McArthur, E.D., and S.C. Sanderson. 1996. Adaptation of forage kochia accessions across an environmental gradient in Rush Valley, Utah. Arid Soil Research and Rehabilitation 10:125-138.
- NRC. 1996. Nutrient Requirements of Beef Cattle. 7<sup>th</sup> ed. National Academy Press, Washington, DC.
- Perry, R. C., L. R. Corah, R. C. Cochran, W. E. Beal, J. S. Stevenson, J. E. Minton, D. D. Simms, and J. R. Brethour. 1991. Influence of dietary energy on follicular development, serum gonadotropins, and first postpartum ovulation in suckled beef cows. J. Anim. Sci. 69: 3762-3773.
- Waldron, B.L., R.D. Harrison, N.I. Dzyubenko, A. Khusainov, S. Shuvalov, and S. Alexanian. 2001. *Kochia prostrate* germplasm collection expedition to Kazakhstan. P. 113-117. In D.E. McArthur and D.J. Fairbanks (comps) Shrubland Ecosystem Genetics and Biodiversity Symp., Provo, UT. June 13-15, 2000. Proceedings RMRS-P-21. USDA, Forest Service, Rocky Mountain Res. Station. Ogden, UT.
- ZoBell, D.R., B.L. Waldron, K.C. Olson, R.D. Harrison, and H. Jensen. 2003. Forage Kochia for Fall/winter Grazing. Utah State University Extension Publ. AG-2003-07. (Available on-line at <http://extension.usu.edu/files/publications/zobell7.pdf>).

Table 1. Nutritional quality of forage samples clipped from the pastures (% of DM)

Item	forage kochia	crested wheatgrass
Crude protein	7.2	5.9
NDF	59.5	63.6
ADF	47.3	42.5
IVTD	52.1	55.8

Table 2. Backfat and Body Condition Score for alfalfa and kochia

Item	Backfat, cm		BCS	
	Initial	Final	Initial	Final
Alfalfa	0.41 <sup>a,x</sup>	1.24 <sup>b,y</sup>	4.95 <sup>a,x</sup>	6.02 <sup>b,y</sup>
Kochia	0.38 <sup>a,x</sup>	0.66 <sup>a,z</sup>	4.86 <sup>a,x</sup>	5.31 <sup>b,z</sup>

<sup>a,b</sup> Numbers with different superscripts differ (P<0.05) across rows for BF and BCS.

<sup>x,y,z</sup> Numbers with different superscripts differ (P<0.05) down columns for BF and BCS.

Table 3. Nutritional quality of diets selected by cows from the pastures (% of DM)

Item	November	January	P
Crude protein	12.6	7.3	0.02
NDF	53.8	64.6	0.01
IVTD	62.2	60.1	0.60

## POSTPARTUM INTERVAL IN FIRST-CALF SUCKLED BEEF COWS EXPOSED TO FAMILIAR OR UNFAMILIAR BULLS

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**ABSTRACT:** The objective of this experiment was to determine if the proportion of first-calf suckled beef cows that resumed ovulatory cycles differ after exposure to either "unfamiliar" bulls or cows on d 35 postpartum after exposure to either "familiar" bulls or cows for the first 35 d after calving. Fifty AxH cows were stratified by calving date, calf BW, and calf sex by d 3 postpartum, and assigned to be exposed to familiar epididectomized (EX) bulls (BEF; n = 25) or familiar mature ovariectomized (OVX) cows (CEF; n = 25). Thirty d later, 12 BEF cows were assigned to be exposed to unfamiliar EX bulls (BEU); likewise, 12 CEF cows were assigned to be exposed to unfamiliar OVX cows (CEU). Cows were in their treatments for either 95 d (BEF and CEF) or 60 d (BEU and CEU) before the breeding season. A distance of approximately 0.35 km separated bull pen areas from cow pen areas. Cows were suckled ad libitum. Blood samples were collected every third d from the beginning to the end of the experiment. A rise in progesterone concentration of  $> 0.05$  ng/mL in consecutive samples was used as the criterion for resumption of ovulatory cycles. Data were analyzed by chi square analyses. Exposing cows to bulls on d 5 after calving and then switching these cows to be exposed to unfamiliar bulls did not ( $P > 0.10$ ) increase the proportion of cows that resumed cycling activity compared to BEF cows by the end of the experiment. However, more ( $P < 0.05$ ) cows that were exposed to bulls (BEF and BEU) resumed cycling activity before cows that were exposed continuously to OVX cows (CEF and CEU). We conclude that the familiarity of first-calf cows to either bulls or ovariectomized cows, did not affect the interval from calving to resumption of ovulatory cycles. However bull exposure, whether familiar or unfamiliar, stimulates first-calf cows to resume ovulatory cycles before the beginning of the breeding season

Key Words: Biostimulation, Postpartum, Bulls, Bovine

### Introduction

Presence of bulls accelerates resumption of postpartum ovarian cycling activity in multiparous (Zalesky et al., 1984; Rekwot et al., 2001) and primiparous (Custer et al., 1990; Fernandez et al., 1993; Berardinelli et al., 2001) suckled beef cows. The physiological mechanism(s) whereby the presence of bulls affects the reproductive neuroendocrine-endocrine system of the postpartum anestrus cows is not known.

The postpartum anestrus condition is prolonged by social (cow-calf bond; Griffith and Williams, 1996) and lactational (Lamb et al., 1998) factors. The cow-calf bond is determined by specific exteroceptive cues (olfactory and visual) and appears to be an essential component in regulating the anestrus interval to resumption of ovulatory cycles in suckled cows (Silveira et al., 1993; Stagg et al., 1998).

If a "bull-cow" bond forms during the early postpartum anestrus period that is similar in nature, but opposite in effect, to the cow-calf bond, then substituting an "unfamiliar" bull for a "familiar" (a bonded bull) should have the opposite effect on this system. Therefore, the objective of this experiment was to determine if the proportions of first-calf suckled beef cows that resumed ovulatory cycles differ after exposure to either "unfamiliar" bulls or cows on d 35 postpartum after exposure to either "familiar" bulls or cows for the first 35 d after calving.

### Materials and Methods

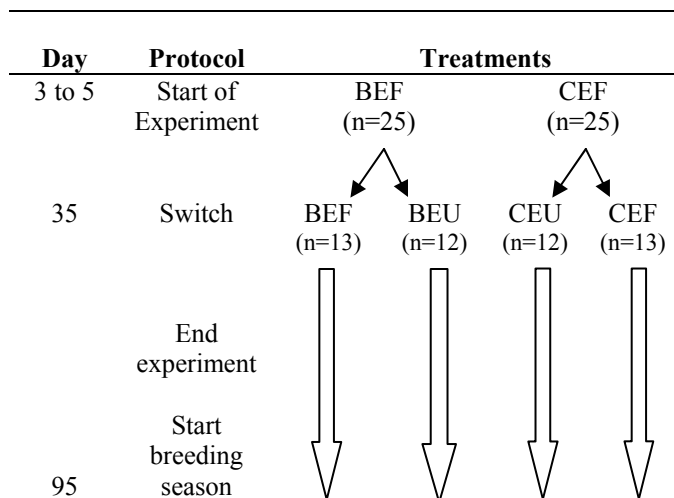
#### *Animals and Treatments*

Fifty spring-calving two-yr-old Angus X Hereford first-calf suckled beef cows, four epididectomized (EX) Angus X Hereford bulls, and four mature, ovariectomized (OVX) Angus X Hereford cows were used in this experiment conducted at the Montana State University Livestock Teaching and Research Center. Animal care, handling, and protocols were approved by the Montana State University Institutional Animal Care and Use Committee.

Cows and calves were maintained in a single pasture for calving. Average calving date was February 12. Cows and calves had no contact with bulls or mature cows for approximately 10 mo before the start of the experiment. Calves were weighed within 24 h after birth.

<sup>1</sup>Supported by USDA, CREES, NRI, CGP Grant 99-35203-7932 and the Montana Agricultural Experiment Station. Contributing project to Multisate Research Project, W-112, Reproductive Performance in Domestic Ruminants.

Body weights and BCS were obtained from each cow within three d after calving and at the end of the experimental period. Cows were stratified by calving date, calf birth weight, calf sex ratio, and cow BCS. Once stratified, each cow was assigned randomly to one of two treatments; exposure to mature EX bulls (BEF;  $n = 25$ ) or exposure to mature OVX cows (CEF;  $n = 25$ ). We reasoned that establishment of “familiarity” might be accomplished by exposing postpartum cows to bulls or cows for the first 30 d after calving; the period during which the cow-calf bond is thought to be established. After 30 d of familiarization, 12 BEF cows were assigned randomly to be exposed to unfamiliar EX bulls (BEU); likewise, 12 CEF cows were assigned to be exposed to unfamiliar OVX cows (CEU). Cows were in their treatments for either 95 d (BEF and CEF) or 60 d (BEU and CEU) before the breeding season. The cow to bull and cow to OVX cow ratio was approximately 1:12, respectively during the first 35 d and then 1:6, respectively for the remainder of the experiment. Figure 1 depicts the experimental design, number of cows per treatment, and protocols.



**Figure 1.** Experimental design, number of cows per treatment, and protocols. BEF represents exposure to mature bulls from d 3 to 5 postpartum; CEF represents exposure to cows from d 3 to 5 postpartum; BEU and CEU represent cows exposed to familiar bulls or cows for 30 d and switched to unfamiliar bull or cow exposure, respectively. Day represents d postpartum.

#### Pen Areas

Cows were maintained in pen areas at the Bozeman Livestock Teaching and Research Center. Each pen within an area is 48 m long by 18 m wide and is identical in configuration and aspect with access to shelter. The north pen area is 0.35 km away from the south pen area. Cows assigned to the BEF and BEU treatments were placed into pens in the north pen area, while those assigned to the CEF and CEU treatments were placed into separate pens. Treatments, within pen areas, were separated by a pen width and double paneled

fences on each side that were covered with tarpaulins. The prevailing winds blow from the southeast to the northwest. Cows housed in the southern pen area could not see or smell bulls or cows housed in the northern pen area. However, there is the possibility that they could hear the bulls. This arrangement has proven to be effective in previous experiments (Berardinelli et al., 2001; Joshi et al., 2002).

#### Nutrition

Cows had free access to good quality, chopped mixed-grass alfalfa hay, and any pasture grasses that were available. Once cows and calves were moved into pens they were given free access to the same hay,  $0.5 \text{ kg} \cdot \text{hd}^{-1} \cdot \text{d}^{-1}$  cracked barley, water, and a trace mineral-salt supplement. The TDN of the diet exceeded the NRC requirement for lactating beef cows with a mature weight of 545 kg by approximately 18% (NRC, 1996).

#### Blood Sampling for Progesterone

Blood samples (7 mL/sample) were obtained from each cow in each treatment by jugular venepuncture starting on d 5 postpartum, and every third d until the end of the experiment. Samples were placed on ice, allowed to clot overnight, and then centrifuged at  $1850 \times g$  for 30 min at  $4^{\circ}\text{C}$ . Serum was harvested and stored at  $-20^{\circ}\text{C}$  until assayed for progesterone. Serum was assayed for progesterone concentration using a solid-phase RIA kit (Diagnostic Systems Laboratories Inc., Webster TX) validated for bovine serum in our laboratory. Progesterone concentrations were used to assess the resumption of ovarian cycling activity. A rise in progesterone concentration of  $> 0.5 \text{ ng/ml}$  in three consecutive samples provided evidence that cows resumed ovarian cycling activity.

#### Statistical Analyses

Calving date, calf BW, calf sex ratio, and BCS were analyzed by analysis of variance for a completely random design using PROC GLM of SAS (SAS, Cary, NC). The model included treatment. Means were separated by PDIF procedure of SAS (SAS, Cary, NC). Proportions of cows that resumed ovarian cycling activity within the experimental period were analyzed by contingency chi-square analyses (SAS, Cary, NC).

#### Results

Calving date, calf BW, calf sex ratio, and cow BCS did not differ ( $P > 0.10$ ) among treatments.

There was no interaction ( $P > 0.10$ ) among treatments for the proportions of cows that resumed ovarian cycling activity by the end of the experiment (Table 1).

Table 1. Percentages of first-calf suckled beef cows exposed to familiar bulls (BEF) or cows (CEF) and switched to exposure to unfamiliar bulls (BEU) or cows (CEU) that resumed cycling activity by the end of the experiment<sup>a</sup>

Item	Treatment <sup>b</sup>			
	BEF	BEU	CEU	CEF
	13	12	12	13
% Cycling	75.0	83.3	41.7	58.3

<sup>a</sup>BEF and CEF cows continuously exposed to same bulls or cows from d 5 postpartum. BEU and CEU cows exposed to same bulls or cows until 35 d postpartum then introduced to unfamiliar bulls or cows for the remainder of the experiment.

<sup>b</sup>Interaction  $X^2 = 5.4$ , d.f. = 3,  $P < 0.10$

The data were then pooled to evaluate the effect of the “primary” treatment (bulls vs cows). By the end of the experiment, the proportion of cows that had resumed ovarian cycling activity was 31.8% greater ( $P < 0.05$ ; Table 2) for cows exposed to bulls (BEF and BEU) than for cows exposed to OVX cows (CEF and CEU).

Table 2. Percentages of first-calf suckled beef cows exposed to familiar and unfamiliar bulls (BEF and BEU) or familiar and unfamiliar cows (CEF and CEU) that resumed cycling activity by the end of the experiment

Item	Treatment		$X^2$	$P$ value
	BEF and BEU	CEF and CEU		
n	25	25		
% Cycling	79.2 <sup>a</sup>	48.0 <sup>b</sup>	4.5	< 0.05

<sup>a,b</sup>Percentages that lack a common superscript differ.

## Discussion

The objective of this experiment was to determine if the proportions of first-calf suckled beef cows that resumed ovulatory cycles differ after exposure to either “unfamiliar” bulls or cows on d 35 postpartum after exposure to either “familiar” bulls or cows for the first 35 d after calving. This idea was formulated based upon the following notions: 1) cows interact not only with their calves to form bonds but that they might also interact with bulls to form a social bond; and, 2) the cow-calf bond can be “broken” by substituting a cow's own calf for an “alien” calf; doing so reduces the interval to resumption of ovarian cycling activity (Silveira et al., 1993). We reasoned that if a “bull-cow” bond forms during the early postpartum anestrus period, that is similar in nature, but opposite in effect, to the cow-calf bond, then substituting an “unfamiliar” bull for a “familiar” (a bonded bull) bull should reduce the number of cows that resume cycling activity. To the contrary, we found that exposing cows to bulls soon after calving and

then substituting unfamiliar bulls does not decrease the proportion of cows that resumed cycling activity by the end of the experiment compared to cows exposed continuously to familiar bulls. Thus, it appears that cows probably do not form a bond analogous, but opposite in effect, to the inhibitory effect of “cow-calf” bond.

We used OVX mature cows as a control for the effect of substituting bulls because it seemed more reasonable than simply including a negative control in which cows would have no exposure to the substitution process. By pooling the data for the primary affect of bulls, we found that more cows exposed to bulls (BEF and BEU) resumed cycling activity before by the end of the experiment than cows exposed OVX cows (CEF and CEU). To our knowledge, there is no reported in the literature regarding the effect of OVX cows on postpartum anestrus in cows. The proportions CEU and CEF cows that resumed cycling activity were not different, and are quite similar to those reported by our laboratory for cows not exposed to bulls (Custer et al., 1990; Fernandez et al., 1993; Berardinelli et al., 2001). This might have been predicted because the biostimulatory effect of bulls appears to be testosterone-dependent (Burns and Spitzer, 1992).

We conclude that the familiarity of first-calf cows to either bulls or ovariectomized cows, did not affect the interval from calving to resumption of ovulatory cycles. However bull exposure, whether familiar or unfamiliar, stimulates first-calf cows to resume ovulatory cycles before the beginning of the breeding season

## Implications

The biostimulatory effect of bulls does not appear to involve a unique social bonding mechanism analogous to the “cow-calf” bond. The mechanism may operate through some other sensory pathway linked to the reproductive neuroendocrine-endocrine system of the postpartum cow. This sensory pathway may either directly attenuate the negative effect of the “cow-calf” bond or directly stimulate the neural centers responsible for altering GnRH release from the hypothalamus.

## Literature Cited

- Berardinelli, J.G., B. Robinson, K. Anderson, and R. Adair. 2001. Effect of presence of bulls and restricted suckling on breeding performance of first-calf suckled beef cows using the OV-synch protocol. Proc. West. Sec. Amer. Soc. Anim. Sci. 52:377-380.
- Burns, P. D., and J. C. Spitzer. 1992. Influence of biostimulation on reproduction in postpartum beef cows. J. Anim. Sci. 70:358-362.
- Custer, E.E., J.G. Berardinelli, R.E. Short, M. Wehrman, and R. Adair. 1990. Postpartum interval to estrus and patterns of LH and progesterone in first-calf suckled beef cows exposed to mature bulls. J. Anim. Sci. 68:1370-1377.

- Fernandez, D., J.G. Berardinelli, R. E. Short, and R. Adair. 1993. Temporal requirement for the presence of mature bulls to alter the postpartum period to resumption of ovarian cycling activity and reproductive performance in first-calf suckled beef cows. *Theriogenology* 39:411-419.
- Griffith, M. K., and G. L. Williams. 1996. Roles of maternal vision and olfaction in suckling-mediated inhibition of luteinizing hormone secretion, expression of maternal selectivity, and lactational performance of beef cows. *Biol. Reprod.* 54:761-768.
- Joshi, P., J. Berardinelli, A. Jacobs, K. Anderson, and R. Adair. 2002. Resumption of postpartum ovarian cycling activity in first-calf restricted suckled beef cows exposed to pheromonal products of bulls. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 53:69-72.
- Lamb, G. C., J. M. Lynch, D. M. Grieger, J. E. Minton, and J. S. Stevenson. 1997. Ad libitum suckling by and unrelated calf in the presence or absence of a cow's own calf prolongs postpartum anovulation. *J. Anim. Sci.* 75:2762-2769.
- NRC. 1996. Pages 227 in *Nutrient Requirements of Beef Cattle*. 7th revised ed. Natl. Acad. Press, Washington, DC.
- Rekwot, P. I., D. Ogwu, E. O. Oyedipe, and V. O. Sekoni. 2001. The role of pheromones and biostimulation in animal reproduction. *Anim. Reprod. Sci.* 65:157-170.
- Silveira, P. A., R. A. Spoon, D. P. Ryan, and G. L. Williams. 1993. Evidence for maternal behavior as a requisite link in suckling-mediated anovulation in cows. *Biol. Reprod.* 49:1338-1346.
- Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. *J. Anim. Sci.* 68:799-816.
- Stagg, K., L. J. Spicer, J. M. Sreenan, J. F. Roche, and M. G. Diskin. 1998. Effect of calf isolation on follicular wave dynamics, gonadotropin and metabolic hormone changes, and interval to first ovulation in beef cows fed either of two energy levels postpartum. *Biol. Reprod.* 59:777-783.
- Zalesky, D. D., M. L. Day, M. Garcia-Winder, K. Imakawa, R. J. Kittok, M. J. D'Occhio, and J. E. Kinder. 1984. Influence of exposure to bulls on resumption of estrous cycles following parturition in beef cows. *J. Anim. Sci.* 59:1135-1139.

**BREEDING PERFORMANCE OF FIRST-CALF SUCKLED BEEF COWS EXPOSED TO FAMILIAR OR UNFAMILIAR BULLS USING A MODIFIED CO-SYNCH PROTOCOL**

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**ABSTRACT:** The objective was to evaluate breeding performance of first-calf suckled beef cows exposed to “familiar” bulls for 35 d after calving then switched to exposure to “unfamiliar” bulls 30 d before using a modified CO-Synch protocol and timed AI. Fifty AxH cows were assigned on d 5 postpartum to be exposed to familiar epididectomized bulls (BEF;  $n = 25$ ) or familiar mature OVX cows (CEF;  $n = 25$ ). Thirty d later, 50% of BEF cows were assigned to be exposed to unfamiliar bulls (BEU;  $n = 12$ ), and 50% of CEF cows were assigned to be exposed to unfamiliar OVX cows (CEU;  $n = 12$ ). Cows were in their treatments for either 95 d (BEF and CEF) or 60 d (BEU and CEU) before the breeding season and for 10 d after timed AI. Each cow received GnRH (100  $\mu$ g) on d -10 of the CO-Synch protocol and PGF<sub>2 $\alpha$</sub>  (25 mg) 7 d later. Cows detected in estrus by 60 h after PGF<sub>2 $\alpha$</sub>  were bred by AI 12 h later; cows not detected in estrus by 60 h after PGF<sub>2 $\alpha$</sub>  received GnRH (100  $\mu$ g/hd) and timed AI (TAI) at 70 h after PGF<sub>2 $\alpha$</sub> . Pregnancy rates were determined ultrasonically 35 d after timed AI. Data were analyzed by chi square analyses. A greater ( $P < 0.05$ ) proportion of bull-exposed cows were cycling on d -10 than cows exposed to OVX cows. Proportions of cows that showed estrus after PGF<sub>2 $\alpha$</sub> , or were timed AI, did not differ ( $P > 0.10$ ) among treatments. Pregnancy rate for cows bred by AI within 60 h after PGF<sub>2 $\alpha$</sub>  did not differ ( $P > 0.10$ ) among treatments; however, timed AI pregnancy rate tended to be greater ( $P = 0.09$ ) for cows exposed to bulls than for cows exposed to OVX cows. We conclude that bull exposure did not alter AI pregnancy rate of cows that exhibited estrus within 60h after PGF<sub>2 $\alpha$</sub>  in the CO-Synch protocol, however, it did appear to increase timed AI pregnancy rate of first-calf beef cows whether the exposure was to familiar bulls or to unfamiliar bulls before the breeding season.

**Key Words:** Biostimulation, Postpartum, Estrous Synchronization, Bovine

**Introduction**

Extended postpartum anestrus is the major cause of cows failing to rebreed or breeding late in the breeding season. This is especially true for first-calf suckled cows that require 15 to 25 d longer to return to estrus than multiparous cows (Short et al., 1994). This problem can create a challenge to successfully synchronizing estrus or ovulation in first-calf suckled beef cows. Gonadotropin releasing hormone (GnRH)-based estrous synchronization (ES) protocols are more successful if postpartum cows have resumed cycling activity (Jordan et al., 2002). Work reported in our laboratory shows that the presence of bulls accelerates resumption of postpartum ovarian activity in first-calf suckled beef cows (Custer et al., 1990; Fernandez et al., 1993; Berardinelli et al., 2001).

Anderson et al. (2002) reported that timed AI pregnancy rate was higher in bull-exposed cows if cows remained with bulls for five d after breeding. This indicates the possibility that the physical presence of a bull before, during, and after estrous synchronization (ES) and AI is necessary to improve pregnancy rates. We reported that unfamiliar bulls are as effective as familiar bulls in increasing the number of cows that resume cycling. Exposure to ovariectomized cows appeared to have the same effect as no bull exposure (Berardinelli et al., 2004). This allows an opportunity to test whether bull exposure per se before, during, and after a GnRH-based ES that included timed AI (TAI) enhances breeding performance of first-calf suckle cows.

The objective was to evaluate AI pregnancy rates of first-calf suckled beef cows exposed to “familiar” bulls or cows for 35 d after calving then switched to exposure to “unfamiliar” bulls or cows 65 d before using a modified CO-Synch protocol and timed AI.

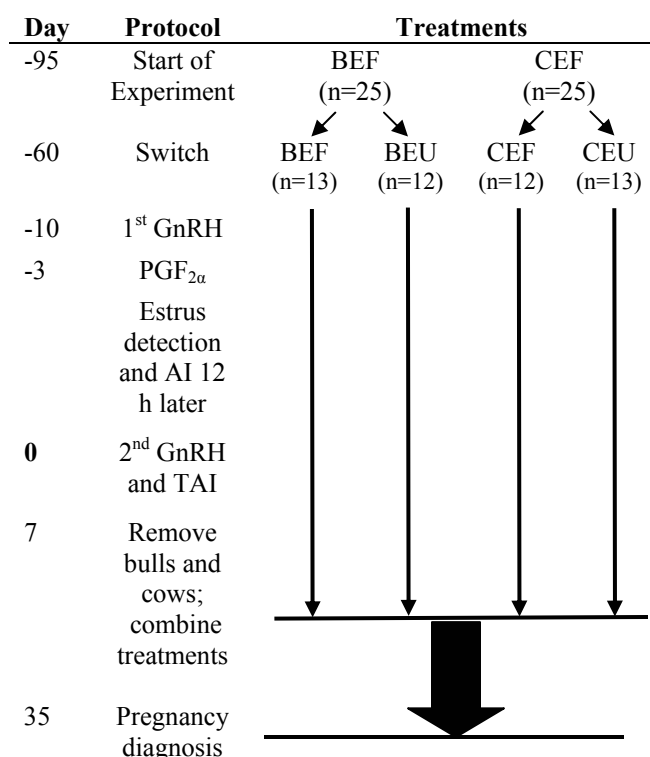
**Materials and Methods**

*Animals and Treatments*

Fifty spring-calving two-yr-old Angus X Hereford first-calf suckled beef cows, four epididectomized (EX) Angus X Hereford bulls, and four mature, ovariectomized (OVX) Angus X Hereford cows were used in this experiment conducted at the Montana State University Livestock Teaching and Research Center. Animal care, handling, and protocols were approved by the Montana State University Institutional Animal Care and Use Committee.

<sup>1</sup>Supported by USDA, CREES, NRI, CGP Grant 99-35203-7932 and the Montana Agricultural Experiment Station. Contributing project to Multisate Research Project, W-112, Reproductive Performance in Domestic Ruminants.

Cows and calves were maintained in a single pasture for calving. Average calving date was February 12. Cows and calves had no contact with bulls or mature cows for approximately 10 mo before the start of the experiment. Calves were weighed within 24 h after birth. Body weights and BCS were obtained from each cow within three d after calving and at the end of the experimental period. Cows were stratified by calving date, calf birth weight, sex of calf, and body condition score. Once stratified, each cow was assigned randomly to one of two treatments; exposure to mature EX bulls (BEF;  $n = 25$ ) or exposure to mature OVX cows (CEF;  $n = 25$ ). These two treatments represent the “familiar” relationship because cows were exposed to these bulls or cows from 5 to 35 d after calving; the period during which the cow-calf bond is established. After 30 d of familiarization, 12 BEF cows were assigned randomly to be exposed to unfamiliar EX bulls (BEU); likewise, 12 CEF cows were assigned to be exposed to unfamiliar OVX cows (CEU). Cows were in their treatments for either 95 d (BEF and CEF) or 60 d (BEU and CEU) before the start of the estrous synchronization protocol. The cow to bull and cow to OVX cow ratio was approximately 1:12, respectively during the first 35 d and then 1:6, respectively for the remainder of the experiment. Figure 1 depicts the experimental design, number of cows per treatment, and protocols.



**Figure 1.** Experimental design, number of cows per treatment, and protocols. BEF = exposure to mature bulls from d 3 to 5 postpartum; CEF = exposure to cows from d 3 to 5 postpartum; BEU and CEU represent cows exposed to familiar bulls or cows for 30 d and switched to unfamiliar bull or cow exposure, respectively.

## Pen Areas

Cows were maintained in pens at the Bozeman Livestock Teaching and Research Center. Each pen is 48 m long by 18 m wide and is identical in configuration and aspect with access to shelter. The north pen area is 0.35 km away from the south pen area. Prevailing winds blow from the southwest to northeast so NE cows were housed in the south pen area and BE cows were housed in the north pen area. This reduced the chances of incidental overlap exposure between treatments (Fernandez et al., 1996).

## Nutrition

Cows had free access to good quality, chopped mixed-grass alfalfa hay, and any pasture grasses that were available. Once cows and calves were moved into pens they were given free access to the same hay, 0.5 kg•hd<sup>-1</sup>•d<sup>-1</sup> cracked barley, water, and a trace mineral-salt supplement. The TDN of the diet exceeded the NRC requirement for lactating beef cows with a mature weight of 545 kg by approximately 18% (NRC, 1996).

## Estrous Synchronization and AI

Each cow was injected i.m. with GnRH (100 µg/hd) on d -10 (Figure 1), followed by an injection of PGF<sub>2α</sub> (25mg/hd) seven d later (d -3). Cows were then observed for estrus twice daily (0700 and 1900 h). Cows exhibiting estrus on d -2 and -1 were inseminated artificially by one of two experienced AI technicians 12 h later. Cows that failed to show estrus by 70 h after PGF<sub>2α</sub> were given a second injection of GnRH and inseminated by appointment at this time (TAI). All cows were inseminated using semen from a single sire. Cows remained in their treatments for seven d following TAI.

## Blood Sampling for Progesterone and Pregnancy Determination

Blood samples were collected from each cow by jugular venepuncture at three-d intervals from the start of the experiment. Serum was assayed for progesterone concentration using a solid-phase RIA kit (Diagnostic Systems Laboratories Inc., Webster TX) validated for bovine serum in our laboratory. The criterion for resumption of cycling activity was a rise in progesterone of greater than 0.5 ng/mL in three consecutive samples. The uterus of each cow was examined ultrasonically 35 d after breeding for the presence of an embryo.

## Statistical Analyses

Proportions of cows among treatments that exhibited resumption of ovarian cycling activity at the start (d -10) of the CO-Synch protocol, estrus response after PGF<sub>2α</sub>, and pregnancy rates at 35 d after TAI were analyzed by contingency chi-square analyses. The model included treatment.

## Results

There was no interaction ( $P < 0.10$ ) among treatments for proportions of cows cycling at the start of the CO-Synch protocol, proportions of cows showing estrus before the TAI, or AI pregnancy rates. Therefore, these data were pooled and re-analyzed for the primary effect (bull exposed vs cow exposed). A greater ( $P < 0.05$ ) proportion of bull-exposed cows (BEF and BEU) was cycling on d -10, at the start of the CO-Synch protocol, than cows exposed to OVX cows (CEF and CEU; Table 1).

Proportions of cows that showed estrus after PGF<sub>2α</sub>, or were timed AI, did not differ ( $P > 0.10$ ) between bull-exposed (BEF and BEU) and cow-exposed (CEF and CEU) treatments (Table 1). Pregnancy rate, at 35 d after TAI, for cows bred by AI within 60 h after PGF<sub>2α</sub> did not differ ( $P > 0.10$ ) between bull-exposed (BEF and BEU) and cow-exposed (CEF and CEU) treatments (Table 1). However, TAI pregnancy rate tended to be greater ( $P = 0.09$ ) for cows exposed to bulls (BEF and BEU) than for cows exposed to cows (CEF and CEU; Table 1).

Table 1. Percentages of first-calf suckled beef cows exposed to familiar and unfamiliar bulls (BEF and BEU) or familiar and unfamiliar cows (CEF and CEU) by the start of the CO-Synch protocol, and that were bred by AI 12 h after estrus or bred at the second GnRH injection (timed AI), and were pregnant 35 d after AI<sup>1</sup>

Item	Treatment		$X^2$	$P$ value
	BEF	CEF		
	and BEU	and CEU		
n	25	25		
% Cycling	79.2 <sup>a</sup>	48.0 <sup>b</sup>	4.5	< 0.05
Bred AI 12 h after estrus	42.9 <sup>a</sup>	39.1 <sup>a</sup>	0.06	> 0.10
Timed AI	57.1 <sup>a</sup>	60.9 <sup>a</sup>	0.06	> 0.10
Pregnancy rate (35 d) for AI 12 h after estrus	72.7 <sup>a</sup>	60.7 <sup>a</sup>	0.9	> 0.10
Pregnancy rate (35 d) for AI 12 h after estrus	64.2 <sup>a</sup>	31.6 <sup>b</sup>	2.9	< 0.09

<sup>1</sup>BEF and CEF cows continuously exposed to same bulls from d 5 postpartum. BEU and CEU cows exposed to same cows until 35 d postpartum then introduced to unfamiliar bulls or cows for the remainder of the experiment.

<sup>a,b</sup>Percentages within rows that lack a common superscript differ.

## Discussion

The objective of this experiment was to evaluate AI pregnancy rates of first-calf suckled beef cows exposed to “familiar” bulls or cows for 35 d after calving then switched to exposure to “unfamiliar” bulls or cows 65 d before using a modified CO-Synch protocol and timed AI. We found that the proportion of cows cycling at the start of the ES protocol did not differ between cows exposed to “familiar” or “unfamiliar” bulls, but was greater than that for cows exposed to “familiar” or “unfamiliar” cows. This indicates that the effect of bulls does not depend upon familiarization to have a biostimulatory action on postpartum anestrus cows.

Modifying the CO-Synch protocol by breeding cows that show estrus before the second injection of GnRH and increasing the interval from of PGF<sub>2α</sub> injection to the second GnRH injection was shown by Anderson et al. (2002) to be an effective protocol for using this GnRH-based ES method. Using this protocol they reported that TAI pregnancy rate was greater for cows exposed to the physical presence of bulls, before, during, and for five d after TAI than for cows not exposed to bulls. In the present study, we found that the presence of bulls did not alter the proportion of cows that show estrus and were bred before the second GnRH injection, proportion of cows that were TAI, or AI pregnancy rates of cows bred before the second GnRH injection. Again this is similar to the results reported by Anderson et al. (2002). However, we found a tendency for TAI pregnancy rates to be greater for cows exposed to bulls before, during, and for seven d after TAI. This result is consistent with that of Anderson et al. (2002) in that the physical presence of bulls enhances TAI pregnancy rate using this modified CO-Synch ES protocol.

Berardinelli, et al. (2001) reported that the presence of bulls did not affect the breeding performance of first-calf suckled cows. In that experiment bulls were removed at time of PGF<sub>2α</sub>, whereas in a subsequent study TAI by Anderson et al. (2002) the present study cows remained with the bull for five d after breeding. It may be that the continued physical presence of a bull is necessary to enhance the usefulness of synchronization programs that employ timed AI.

We conclude that bull exposure did not alter AI pregnancy rate of cows that exhibited estrus within 60h after PGF<sub>2α</sub> in the CO-Synch protocol, however, it did appear to increase timed AI pregnancy rate of first-calf beef cows whether the exposure was to familiar bulls or to unfamiliar bulls before the breeding season.

## Implications

The biostimulatory action of bulls on postpartum cows reduces postpartum anestrus (Zalesky et al., 1984; Custer et al., 1990). Results of the present study and that reported by Anderson et al. (2002) indicate the possibility that there is another component to the biostimulatory effect of bulls, namely, an enhancement of breeding

performance when used in combination estrous synchronization protocols that include TAI.

### **Literature Cited**

- Anderson, K. A., J.G. Berardinelli, P.S. Joshi, and B. Robinson. 2002. Effects of exposure to bull or excretory products of bulls on the breeding performance of first-calf restricted suckled beef cows using a modified CO-synch protocol. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 2002; 53:457-460.
- Fernandez, D. L., J. G. Berardinelli, R. E. Short, and R. Adair. 1996. Acute and chronic changes in LH secretion, and postpartum interval to estrus in first-calf suckled beef cows exposed continuously or intermittently to mature bulls. *J. Anim. Sci.* 74:1098-1100.
- Berardinelli, J.G., B. Robinson, K. Anderson, and R. Adair. 2001. Effect of presence of bulls and restricted suckling on breeding performance of first-calf suckled beef cows using the OV-synch protocol. *Proc. West. Sec. Amer. Soc. Anim. Sci.*; 52:377-380.
- Custer, E.E., J.G. Berardinelli, R.E. Short, M. Wehrman, and R. Adair. 1990. Postpartum interval to estrus and patterns of LH and progesterone in first-calf suckled beef cows exposed to mature bulls. *J. Anim. Sci.* 68:1370-1377.
- Fernandez, D., J.G. Berardinelli, R.E. Short, and R. Adair. 1993. Temporal requirement for the presence of mature bulls to alter the postpartum period to resumption of ovarian cycling activity and reproductive performance in first-calf suckled beef cows. *Theriogenology* 39:411-419.
- Fernandez, D.L., J.G. Berardinelli, R.E. Short, and R. Adair. 1996. Acute and chronic changes in LH secretion, and postpartum interval to estrus in first-calf suckled beef cows exposed continuously or intermittently to mature bulls. *J. Anim. Sci.* 74:1098-1100.
- Jordan, E. R., M. J. Shouten, J. W. Quast, A. P. Belschner, and M. A. Tomaszewski. 2002. Comparison of two time artificial insemination (TAI) protocols for management of first insemination postpartum. *J. Dairy Sci.* 85:1002-1008
- NRC. 1996. Pages 227 in *Nutrient Requirements of Beef Cattle*. 7th revised ed. Natl. Acad. Press, Washington, DC.
- Short R.E., R.A. Bellows, R.B. Staigmiller, D.C. Adams, and J.G. Berardinelli. 1994. Effects of suckling on postpartum reproduction. In: M.J. Fields, and R.S. Sand RS (eds.), *Factors Affecting Calf Crop* p. 179-187. CRC Press, Boca Rotan, FL.
- Zalesky, D. D, M. L. Day, M. Garcia-Winder, K. Imakawa, R. J. Kittok, M. J. D'Occhio, and J. E. Kinder. 1984. Influence of exposure to bulls on resumption of estrous cycles following parturition in beef cows. *J. Anim. Sci.* 59:1135-1139

**EFFECT OF EXOGENOUS GONADOTROPIN RELEASING HORMONE FIVE DAYS AFTER ARTIFICIAL INSEMINATION ON OVARIAN STRUCTURES, SERUM PROGESTERONE, AND CONCEPTION RATE IN DAIRY COWS**

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**ABSTRACT:** The objectives of this study were to investigate the effect of exogenous GnRH after artificial insemination (AI) on ovarian structures, serum progesterone, and conception rate in dairy cows. In Exp 1, twenty-three Holstein cows (~60 DIM) were synchronized using the Ovsynch protocol. Cows received 100 µg of GnRH (i.m.) on d -10 followed by 25 mg PGF<sub>2α</sub> (i.m.) on d -3. On d -1 a second dose of GnRH (100 µg) was given and the presence of a preovulatory follicle was determined by ultrasound. Twelve to sixteen hours later (d 0) cows were inseminated. On d 5 after AI, cows were assigned randomly to receive saline (n = 11) or 100 µg of GnRH (n = 12). The presence of corpora lutea (CL) was determined by ultrasound on the day of treatment (d 5) and every other day until d 14. Blood samples were obtained on d 5 and 14 to measure serum progesterone concentration. GnRH treatment five days after AI affected ( $P < 0.05$ ) ovarian structures, as all cows in the GnRH-treated group developed an accessory CL, whereas cows in the saline group had no accessory CL. Mean serum progesterone concentrations were not different between GnRH- and saline-treated groups on d 5 ( $1.64 \pm 0.46$  ng/ml vs.  $2.04 \pm 0.48$  ng/ml, respectively). On d14 serum progesterone concentrations were higher ( $P < 0.05$ ) in the GnRH-treated group compared to control ( $5.22 \pm 0.46$  ng/ml vs.  $3.36 \pm 0.48$  ng/ml, respectively). In Exp 2, 230 lactating cows were used to test the effect of exogenous GnRH 5 d after AI on conception rate. Cows detected in estrus according to chalk removal (roughened tailhead hair) received AI immediately and 5 d later were assigned randomly to receive either GnRH (n = 106) or saline (n = 124). Pregnancy status was confirmed by trans rectal palpation of uterine contents approximately 40 days after AI. Pregnancy data were analyzed by logistic regression. Conception rates were not different between GnRH- and saline-treated groups (27.3% vs. 25.8%, respectively). Exogenous GnRH 5 d after AI increased serum progesterone by developing accessory CL but did not improve conception rate in lactating dairy cattle.

Key words: GnRH, progesterone, conception rate.

**Introduction**

Reproductive efficiency in today's dairy industry is considerably lower than desired and has tremendously reduced the advantage of AI. During the

last few decades reproduction has decreased drastically due to many factors including inefficiency and inaccuracy of estrus detection, inbreeding, improper timing of insemination, delayed ovulation, and anovulation (Hermas et al., 1987; Nebel and Jobst, 1998; Lucy, 2001). Another factor contributing to low pregnancy rates is embryonic loss. (Lamming et al., 1989). Although fertilization rates in cattle are reported to be greater than 90% (Diskin and Sreenan, 1980), the majority of embryonic mortality (70 to 80% of the total loss) is sustained between days 8 and 16 after insemination (Sreenan et al., 2000; Dunn et al., 2001). Maintenance of pregnancy, in part, is dependent on secretion of progesterone during early pregnancy. Luteal deficiency during the very early stages of pregnancy has been hypothesized as a cause of pregnancy failure (Henricks et al., 1970; Butler et al., 1996; Mann and Lamming, 2000). Many factors including nutrition (Gombe and Hansel, 1973), heat stress (Wilson et al., 1998; Wolfenson et al., 2002), genetics (Lucy et al., 2001), and rate of steroid metabolism in high producing cows (Wiltbank et al., 2000) affect progesterone levels during early pregnancy. Regardless of the cause of low progesterone during early embryonic development, it appears that a subnormal progesterone level may contribute to low fertility in dairy cows.

Understanding how early embryonic development may be compromised by suboptimal progesterone may allow researchers to develop methods to increase progesterone and perhaps improve pregnancy rate. Johnson et al. (1958) demonstrated that the administration of 500 mg of progesterone (i.m.) to cows after first service improved the conception rate of the treated group by 28% compared the control group. Schmitt et al. (1996) have shown that injecting GnRH 5 d after estrus increased progesterone concentrations in Holstein heifers, which became evident between d 11 and 16. Arnett et al. (2002) demonstrated that administration of GnRH 1 or 2 d before embryo transfer in recipient beef heifers altered follicular dynamics, induced luteal tissue development, and increased systemic progesterone; pregnancy rate also increased by more than 45% in heifers that received GnRH compared to the control group. Willard et al. (2003) indicated that administration of GnRH post-insemination increased blood progesterone concentration, and may improve conception rate under heat stress conditions.

Others (Rusbridge et al., 1992; Schmitt et al., 1996) have shown that GnRH administration, during

certain stages of estrous cycle, induces ovulation and results in increased progesterone concentration. Therefore, it is possible that exogenous GnRH administered 5 d after AI causes ovulation and (or) luteinization of the tertiary follicle, increases blood progesterone, and improves fertility. Several investigations have been conducted to test such a putative effect of GnRH, but the results have not been consistent. The objective of this study was to examine the effect of exogenous GnRH (100 µg) given 5 d after AI on CL development, progesterone concentration, and conception rate in lactating dairy cows.

## Materials and Methods

**Experiment 1, Animals and treatments:** The University of Idaho Institutional Animal Care and Use Committee approved all the procedures used in this experiment. Twenty-three lactating, non-pregnant Holstein cows at the University of Idaho dairy center were used in this study. Animals were housed in a freestall facility and fed a total mixed ration to meet or exceed NRC requirements for lactating dairy cows (NRC, 2001). The voluntary waiting period for breeding was 60 days. Cows were synchronized (~60 d postpartum) using the Ovsynch program (Pursley et al., 1997). The first injection of GnRH (100 µg) (Cystorelin<sup>®</sup>; Merial, Iselin, NJ) was given i.m. (d -10). Seven days later (d -3) 25 mg of PGF<sub>2α</sub> (Lutalyse; Pharmacia Animal Health; Kalamazoo, MI) was given i.m. Forty-eight h later (d -1) a second dose of GnRH (100 µg i.m.) was administered. All animals were inseminated 12-16 h after GnRH (d 0). On d 5 after AI, cows were assigned randomly to receive 2 ml of 0.9% saline (n = 11) or 100 µg of GnRH (n = 12).

**Ultrasonography:** Ovarian status was monitored by ultrasonography on the day (d -1) of the second GnRH injection to determine the location of any follicle greater than 10 mm in diameter (presumptive ovulatory follicle). Additionally, on the day of treatment (d 5), ovaries were monitored by ultrasound for the presence of a CL from the previously ovulated follicle. The location of the CL, and the number and size of follicles were also recorded. After the treatment, ovarian activities were monitored every other day until d 14 (Figure 1).

**Blood Collection procedures and progesterone quantification:** Blood samples were collected from the coccygeal vein on d 5 and 14 and analyzed for progesterone concentration. Serum progesterone was quantified using a solid-phase <sup>125</sup>I RIA kit (Diagnostic Product Corp., Los Angeles, CA). Standards and samples were done in duplicate. The standard curve ranged from 0.1 to 40.0 ng/ml. The assay was conducted in one run with an intrassay CV of 5.18%.

**Experiment 2, Animals and treatment:** Two hundred thirty cows from a commercial herd were used

for this experiment. The estrus synchronization protocol, as set by the dairy, was the Heat synch protocol. A luteolytic dose of PGF<sub>2α</sub> was administered (i.m.) to all animals. Fourteen days later, 100 µg GnRH was administered (i.m.; d 0) followed by 25 mg PGF<sub>2α</sub> on d 7. On d 8 all cows received 1 mg (i.m.) estradiol cypionate (ECP<sup>®</sup>, Pharmacia Animal Health, Kalamazoo, MI). Cows detected in estrus according to chalk removal (roughened tailhead hair) received AI immediately by a single technician. Five days after AI cows were assigned randomly to receive either GnRH (n = 106) or saline (n = 124) as described in experiment 1. Pregnancy status was confirmed via trans rectal palpation of uterine contents approximately 40 days after AI. Animals that were sold or died before pregnancy diagnosis were excluded from the data.

**Statistical analysis:** Serum progesterone concentrations were analyzed by least-squares analysis of variance using the GLM procedure of SAS (V.8.12). The statistical model included treatment, cow within treatment, day, day × cows (treatment) and residual error. If treatment or day × cow (treatment) was significant (P < 0.05), non-orthogonal contrasts were used to compare least squares means using the improved Bonferroni F-test. Pregnancy data were analyzed by logistic regression using SAS<sup>®</sup>.

## Results

**Experiment 1.** At the initiation of the experiment both groups were similar in days in milk, milk yield, and body condition score (BCS). Mean values for DIM, milk yield, and BCS were similar and were 66.45 ± 3.07 vs 68.45 ± 3.07, 41.75 ± 3.31 kg vs 41.24 ± 3.46 kg, and 2.81 ± 0.07 vs 2.90 ± 0.08 for GnRH-and saline-treated groups, respectively.

Mean serum progesterone concentrations and presence of accessory CL for both treatment groups are shown in Table 1. On the day of treatment (d 5) all cows had a CL. Nine days after treatment (d 14), all cows

Table 1. Mean<sup>1</sup> serum progesterone concentration on d 5 and d 14 after AI and the percentage of cows that exhibited accessory CL as viewed by ultrasound on d 14 in Holstein dairy cows treated with GnRH or saline.

Item	Treatment	
	Saline (n = 11)	GnRH (n = 12)
Day 5 post-AI (ng/ml)	2.04 ± 0.48	1.64 ± 0.46
Day 14 post-AI (ng/ml)	3.36 ± 0.48 <sup>a</sup>	5.22 ± 0.46 <sup>b</sup>
Accessory CL presence	0/11 (0%)	12/12 (100%)

<sup>1</sup>Least squares means ± standard error.

<sup>a,b</sup> Means with different superscripts within a row differ (P < 0.05).

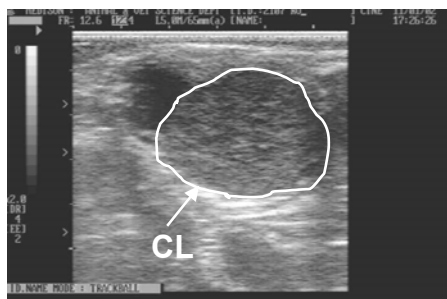


Figure 1a: Sonogram image of a CL on ovary in a saline-treated cow on d 14.

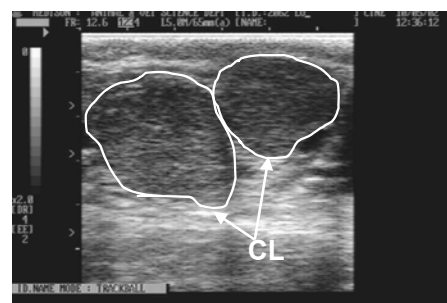


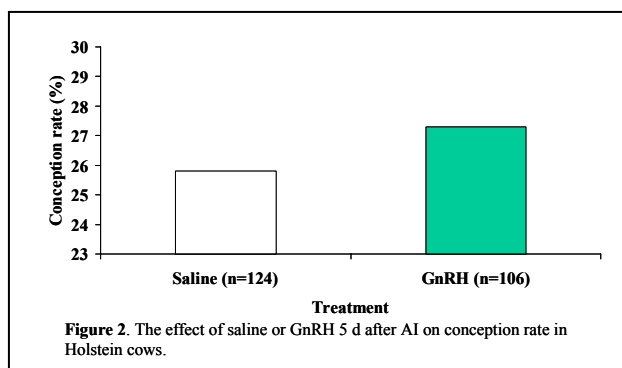
Figure 1b: Sonogram image of two CL side by side on the ovary of a GnRH-treated cow on d 14.

(100%) in the GnRH-treated group developed an accessory CL (Figure 1b). In contrast, none of the cows in the saline-treated group had developed an accessory CL by d 14. Blood samples were collected on d 5 and 14 to assess serum progesterone concentrations. On d 5 mean serum progesterone concentrations for the GnRH- and saline-treated groups did not differ (Table 1). Mean serum progesterone concentrations increased to  $5.22 \pm 0.46$  ng/ml in the GnRH-treated group and  $3.36 \pm 0.48$  ng/ml in the saline-treated group by d 14, and were different ( $P < 0.05$ ) between groups (Table 1).

**Experiment 2.** The results of experiment 1 indicated that GnRH administered 5 d after AI increased blood progesterone. Therefore, experiment 2 was conducted to determine whether or not administration of GnRH 5 d after AI improved conception rate in dairy cattle. Conception rate was defined as the number of cows diagnosed pregnant in a treatment group divided by the number of cows in each treatment group. There was no effect of GnRH treatment on conception rate. Conception rate for the saline-treated group was 25.8% and was 27.3% for the GnRH-treated group (Figure 2).

### Discussion

All cows treated with GnRH 5 d after AI developed an additional CL and exhibited increased progesterone concentrations by d 14 after AI. The cows given saline did not develop an accessory CL or luteal tissue and thus serum progesterone concentration was lower by d 14. Schmitt et al. (1996) have shown that injecting GnRH 5 d after estrus increased progesterone concentrations in Holstein heifers, which became evident between d 11 and 16. Willard et al. (2003)



indicated that administration of GnRH 5 d after insemination increased serum progesterone concentrations in heat-stressed dairy cows, which was evident between d 9 to 19 after AI compared to untreated cows. The mechanism by which progesterone concentrations increase involves many events. Foster and co-workers (1980) reported that day of administration of GnRH during the luteal phase had no effect on LH response. Consequently, exogenous GnRH administered on d 5 or 6 of the estrous cycle overrides the progesterone negative feedback causing the release of both LH and FSH from the anterior pituitary. In addition to ultrasonography results, the increase in mean serum progesterone observed on d 14 in the GnRH-treated group is consistent with the hypothesis that GnRH given on day 5 of the estrus cycle induced ovulation of the first wave dominant follicle resulting in formation of accessory CL.

The injection of GnRH during the luteal phase induces both short- and long-term progesterone secretions (Rettmer et al., 1992). The short-term increase of progesterone starts 15 min after the injection of GnRH and remains elevated until 6 h after injection. The long-term increase of progesterone starts 4 d after the treatment and remains elevated until luteolysis. Peters et al. (2000) have also observed an increase in progesterone when GnRH is given between days 11 and 14 after first insemination.

It was previously suggested that low progesterone during early embryonic development might cause pregnancy failure and subsequently reduce the pregnancy rate (Lucy et al., 2001). Embryonic death due to low progesterone in cattle occurs at two different stages (King et al., 1982; Van Cleeff et al., 1991; Lucy, 2001). The first stage occurs shortly after conception (d 5) while the next stage appears around d 15 to 17 after conception. This later stage is considered critical, because the embryo must be developed sufficiently to suppress uterine secretion of  $\text{PGF}_{2\alpha}$ . Peters et al. (1992) hypothesized that high progesterone post-insemination might enhance embryo development by suppressing the luteolytic mechanism. Garrett and co-workers (1988) found that bovine embryos recovered from progesterone-treated cows on d 14 after inseminations were more advanced compared to those from controls. Increasing peripheral progesterone concentrations at an early stage (d 5) and late stage (d 15) might help, at least partially, overcome the problem of embryonic death in cattle. In fact, researchers (Johnson et al., 1958; Macmillan et al., 1991) showed that increasing

progesterone in diestrus shortly after AI improved embryonic development. In the present study, exogenous GnRH 5 d after AI did not affect conception rate, despite stimulation of luteal function. The present results are similar to others (Leslie et al, 1986; Peters et al., 1992) who showed that GnRH given 4 days or 8 to 10 days after AI did not improve conception rates in dairy cows. In contrast, Willard et al. (2003) indicated that GnRH given 5 d post-insemination tended to improve pregnancy rates in heat-stressed dairy cattle. Moreover, Peters et al. (2000) showed that exogenous GnRH given 11 to 14 days after AI increased the overall pregnancy rate in dairy cattle.

Although accessory CL and increased progesterone concentration were accomplished following GnRH administration 5 d after AI (experiment 1), the results of experiment 2 do not provide sufficient evidence that GnRH given 5 d after AI improves conception rate.

### Implications

Data on the effect of exogenous GnRH after AI on conception rate are inconsistent. Definitive studies on a larger scale have yet to be conducted to address whether supplemental GnRH after AI improves fertility. Supplemental GnRH after AI may be beneficial in improving pregnancy rate only in certain situation. Until then the beneficial effects of routine GnRH administration 5 d after AI remains questionable.

### Literature Cited

- Arnett, M., J. D. Rhinehart, J. D. Bailey, R. B. Hightstone, and L. H. Anderson. 2002. Administration of gonadotropin-releasing hormone (hormone) on d 5 or 6 of the estrous cycle alters follicle dynamics and increases pregnancy rates in beef cattle. *J. Anim. Sci.* 80(suppl. 1):133-134 (Abstr.).
- Butler, W. R., J. J. Calaman, and S. W. Beam. 1996. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. *J. Anim. Sci.* 74:858-65.
- Dsikin, M. G., and J. M. Sreenan. 1980. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. *J. Reprod. Fertil.* 59:463-68.
- Dunne, L. D., M. G. Diskin, J. M. Sreenan. 2000. Embryo and fetal loss in beef heifers between day 14 of gestation and full term. *Anim Reprod Sci.* 58:39-44.
- Foster J. P., G. E. Lamming, and A. R. Peters. 1980. Short-term relationships between plasma LH, FSH and progesterone concentrations in post-partum dairy cows and the effect of Gn-RH injection. *J. Reprod. Fertil.* 59:321-7.
- Garrett, J. E., R. D. Geisert, M. T. Zavy, and G. L. Morgan. 1988. Evidence for maternal regulation of early conceptus growth and development in beef cattle. *J. Reprod. Fertil.* 84:437-446.
- Gombe, S., and W. Hansel. 1973. Plasma luteinizing hormone (LH) and progesterone levels in heifers on restricted energy intakes. *J. Anim. Sci.* 37:728-33.
- Henricks, D. M., J. F. Dickey, and G. D. Niswender. 1970. Serum luteinizing hormone and plasma progesterone levels during the estrous cycle and early pregnancy in cows. *Biol. Reprod.* 2:346-51.
- Hermas, S. A., C. W. Young, and J. W. Rust. 1987. Effects of mild inbreeding on productive and reproductive performance of Guernsey cattle. *J. Dairy Sci.* 70:712-5.
- Johnson, K. R., R. H. Ross, and D. L. Fourt. 1958. Effect of progesterone administration on reproductive efficiency. *J. Anim. Sci.* 17:386-390.
- King, G. J., B. A. Atkinson, and H. A. Robertson. 1982. Implantation and early placentation in domestic ungulates. *J. Reprod. Fertil.* 31 (suppl.): 17.
- Lamming, G. E., A. O. Darwash, and H. L. Back. 1989. Corpus luteum function in dairy cows and embryo mortality. *J. Reprod. Fertil. Suppl.* 37:245-52.
- Leslie, K. E., W.T. Bosu, K. Lissemore, and D. Kelton. 1986. The effects of gonadotrophin releasing hormone administration four days after insemination on first-service conception rates and corpus luteum function in dairy cows. *Can. J. Vet. Res.* 50:184-7.
- Lucy, M. C. 2001. Reproductive loss in high-producing dairy cattle: where will it end? *J. Dairy Sci.* 84:1277-93.
- Lucy, M.C., and J. S. Stevenson. 1986. Gonadotropin-releasing hormone at estrus: Luteinizing hormone, estradiol, and progesterone during the periestrual and post insemination periods in dairy cattle. *Biol. Reprod.* 35:300-11.
- Macmillan, K L., V. K. Taufa, A. M. Day, and A. J. Peterson. 1991b. Effect of supplemental progesterone on pregnancy rates in cattle. *J. Reprod. Fertil.* 43(suppl.):304. (Abstr.).
- Mann, G. E., and G. E. Lamming. 2001. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction.* 121:175-80.
- National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7<sup>th</sup> rev. ed. Natl. Acad. Sci., Washington, DC.

- Nebel, R. L., and S. M. Jobst. 1998. Evaluation of systematic breeding programs for lactating dairy cows: a review. *J. Dairy Sci.* 81:1169-74.
- Peters A. R., S. B. Drew, G. E. Mann, G. E. Lamming, and N. F. Beck. 1992. Experimental and practical approaches to the establishment and maintenance of pregnancy. *J. Physiol. Pharmacol.* 43 (Suppl. 1):143-52.
- Peters, A. R., T. A. Martinez, and A. J. Cook. 2000. A meta-analysis of studies of the effect of GnRH 11-14 days after insemination on pregnancy rates in cattle. *Theriogenology*. 54:1317-26.
- Pursley, J. R., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H. A. Garverick, and L. L. Anderson. 1997. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *J. Dairy Sci.* 80:295-300.
- Rensis, F. D., and R. J. Scaramuzzi. 2003. Heat stress and seasonal effects on reproduction in the dairy cow-a review. *Theriogenology*. 60:1139-51.
- Rettmer, I., J. S. Stevenson, and L. R. Corah. 1992a. Endocrine responses and ovarian changes in inseminated dairy heifers after an injection of a GnRH agonist 11 to 13 days after estrus. *J. Anim. Sci.* 70:508-17.
- Rusbridge, S. M., T. A. Bramley, and R. Webb. 1992. A comparison of GnRH-induced corpora lutea and spontaneously formed CL in heifers. *J. Reprod. Fertil. Abstr. Series* 9:33 (Abstr.).
- SAS Inst., Inc. 2000. SAS Online Doc, Version 8. SAS Inst., Inc. Cary, NC.
- Schmitt, E. J., T. Diaz, C. M. Barros, R. L. de la Sota, M. Drost, E. W. Fredriksson, C. R. Staples, R. Thorne, and W. W. Thatcher. 1996. Differential response of the luteal phase and fertility in cattle following ovulation of the first-wave follicle with human chorionic gonadotropin or an agonist of gonadotropin-releasing hormone. *J. Anim. Sci.* 74:1074-83.
- Sreenan, J.M., M.G. Diskin, and D.G. Morris. 2000. Embryo survival rate in cattle: a major limitation to the achievement of high fertility. *Occ. Publ. Br. Soc. Anim. Sci.* 26:93-104.
- Takekida, S., J. Deguchi, T. Samoto, H. Matsuo, and T. Maruo. 2000. Comparative analysis of the effects gonadotropin-releasing hormone agonist on the proliferative activity, apoptosis, and steroidogenesis in culture porcine granulosa cells at varying stages of follicular growth. *Endocrine* 12:61-67.
- Uemura, T., T. Namiki, A. Kimura, T. Yanagisawa, and H. Minaguchi. 1994. Direct effects of gonadotropin-releasing hormone on the ovary in rats and humans. *Horm. Res.* 41(suppl. 1):7-13.
- Van Cleeff, J., M. Drost, and W. W. Thatcher. 1991. Effects of postinsemination progesterone supplementation on fertility and subsequent estrous responses of dairy heifers. *Theriogenology* 36: 795.
- Willard, S., S. Gandy, S. Bowers, K. Graves, A. Elias, and C. Whisnant. 2003. The effects of GnRH administration postinsemination on serum concentrations of progesterone and pregnancy rates in dairy cattle exposed to mild summer heat stress. *Theriogenology*. 59:1799-810.
- Wilson, S. J., R. S. Marion, J. N. Spain, D. E. Spiers, D. H. Keisler, and M. C. Lucy. 1998. Effects of controlled heat stress on ovarian function of dairy cattle. 1. Lactating cows. *J. Dairy Sci.* 81:2124-31.
- Wiltbank, M. C., P. M. Fricke, S. Sangsritavong, R. Sartori, and O. J. Ginther. 2000. Mechanisms that prevent and produce double ovulations in dairy cattle. *J. Dairy Sci.* 83:2998-3007.
- Wolfenson, D., H. Sonego, A. Bloch, A. Shaham-Albalancy, M. Kaim, Y. Folman, and R. Meidan. 2002. Seasonal differences in progesterone production by luteinized bovine thecal and granulosa cells. *Domest. Anim. Endocrinol.* 22:81-90.
- Wolfenson, D., W. W. Thatcher, L. Badinga, J. D. Savio, R. Meidan, B. J. Lew, R. Braw-Tal, and A. Berman. 1995. Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. *Biol. Reprod.* 52:1106-13.

## THE EFFECTS OF MELATONIN SUPPLEMENTATION ON FECAL SHEDDING OF *E. coli* O157:H7 IN WETHERS

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**ABSTRACT:** To determine if exogenous melatonin (MEL) influences *E. coli* O157:H7 shedding patterns and immune function, 16 mature wethers of mixed breeding (avg. wt. 53±7 kg) were randomly assigned to one of two treatment groups: 1) control (CONT) or 2) 25 mg MEL daily for 21 d (MEL). Both groups were exposed to 16 h light and 8 hr dark (16L/8D) to simulate a summer photoperiod. Treated wethers were dosed with a gel capsule containing 25 mg MEL and 500 mg of ground alfalfa meal. Control animals received a vehicle capsule containing 500 mg ground alfalfa. All animals received *ad libitum* access to water, alfalfa pellets and hay. Seven days (d 7) post initial dosing of MEL, all animals were experimentally infected with *E. coli* O157:H7 via oral gavage followed by daily (14 d total) fecal sampling to examine shedding patterns. Blood was collected on d 6, 14, and 21 to determine total WBC counts and differentials. On day 21 all animals were sacrificed to determine ruminal, ileal, cecal and rectal populations of the experimental strain. Daily fecal shedding patterns were analyzed as repeated measures using the MIXED procedure of SAS. Total WBC counts, differentials and gut *E. coli* O157:H7 were analyzed using GLM procedure of SAS, with all data reported as least square means. Daily shedding patterns of *E. coli* O157:H7 were non-significant between treatments with fecal populations of *E. coli* O157:H7 decreasing daily ( $P < 0.001$ ). No differences were observed in the populations of *E. coli* O157:H7 in the luminal contents of the rumen, ileum, cecum or rectum of CONT or MEL treated animals. Total white blood cells did not differ between treatment groups ( $10.6 \times 10^3/\text{mm}^3$  vs.  $9.6 \times 10^3/\text{mm}^3$  for CONT and MEL, respectively). Differential leukocyte counts were similar between treatments with lymphocytes accounting for 59.1 and 56.3% of the white blood cells in CONT and MEL, respectively. Monocytes and polymorphonuclear leukocytes accounted for 3.9 and 5.4% and 36.3 and 39.8% of white blood cells in CONT and MEL treated animals, respectively. Administration of exogenous MEL did not alter bacterial shedding patterns of experimentally infected wethers exposed to a long photoperiod.

**Key Words:** *Escherichia coli* O157:H7, melatonin, wethers

### Introduction

*Escherichia coli* O157:H7 is responsible for a number of foodborne illnesses in the U.S. with an estimated 62,500 human cases reported per year (0.45% of all foodborne illnesses) (APHIS, 2001). A greater incidence of illness occurs during the warmer months (CDC, 2002) which may be correlated with seasonal changes in shedding patterns observed in various livestock species. Seasonal occurrences of *E. coli* O157:H7 in fecal samples of dairy and beef cattle have been well documented with a greater prevalence of outbreaks exhibited during the summer and spring months (Hancock et al., 1997; Barkocy-Gallagher, 2003; Miller et al., 2003). In the southern plains region of the U.S., environmental factors typically associated with such time periods include: increased ambient temperatures, relative humidity, and day length (photoperiod). Research examining the effects of heat stress on shedding has been minimal, and of that completed, little correlation between the two was shown (Edrington et al., 2004). However, the idea that photoperiod, or the hormones associated with photoperiod, may play a role in bacterial shedding is not out of the ordinary. Quorum sensing is a system in which bacteria produce and release chemicals (autoinducers) that increase in concentration to regulate gene expression of virulence factors and cellular differentiation (Miller and Bassler, 2001). In addition, bacteria are also capable of using the host's environmental factors to further regulate the production of these autoinducers. The catecholamine, norepinephrine, has been shown to stimulate autoinducer production and bacterial growth of *E. coli* O157:H7 (Lyte et al., 1996; Freestone et al., 1999; Freestone et al., 2002). However, little to no work with hormones other than the catecholamines has been done. The duration and peak serum concentrations of pineal melatonin are lower during the photophase (Zinn et al., 1988) and summer and spring seasons (Eloranta et al., 1992; Alila-Johansson et al., 2001). This is also the time when *E. coli* O157:H7 populations are greatest in dairy and beef cattle. Melatonin also possesses antioxidant-like properties (Tan et al., 2000) and can alter humoral responses, T cell blastogenesis, and immune reactions (Bubenik et al., 1998). Therefore, the objective of this study was to determine if supplemental melatonin would alter daily fecal shedding patterns and GIT populations of *E. coli* O157:H7 and overall immune function.

## Materials and Methods

Mature wethers ( $n = 16$ ) of mixed breeding were equally divided into two treatment groups: control (CONT) or melatonin (MEL). All animals were housed in isolation rooms with 4 animals per room and were acclimated for 7 d to a photoperiod consisting of 16 h light and 8 h dark (16L:8D). Following the acclimation period, wethers were orally dosed for 21 d with gel capsules containing 500 mg ground alfalfa or 25 mg MEL (Sigma-Aldrich Inc., St. Louis, MO) plus 500 mg ground alfalfa for CONT- and MEL-treated animals, respectively. Wethers received ad libitum access to water, commercial sheep pellets and bermudagrass hay. Seven d (d 7) post initial dosing, all animals were experimentally infected with *E. coli* O157:H7 (strain 2336;  $10 \text{ mL}$  of  $6.4 \times 10^8/\text{mL}$ ) via oral gavage. The *E. coli* O157:H7 was made resistant to rifampicin in our laboratory via successive cultivation in tryptic soy broth (TSB) containing  $20 \text{ }\mu\text{g/mL}$  rifampicin. Fecal samples were collected daily and plated on MacConkey agar containing  $20 \text{ }\mu\text{g}$  rifampicin/mL to monitor shedding patterns of the experimental strain. To evaluate gut populations of the experimental strain, contents from the rumen, ileum, cecum and rectum were collected. Sheep were euthanized (Euthasol®, euthanasia solution, Del Marva Laboratories, Inc., Midlothian, VA) on d 14 post inoculation. Luminal contents from each segment were serially diluted and plated similarly to that of the fecal samples.

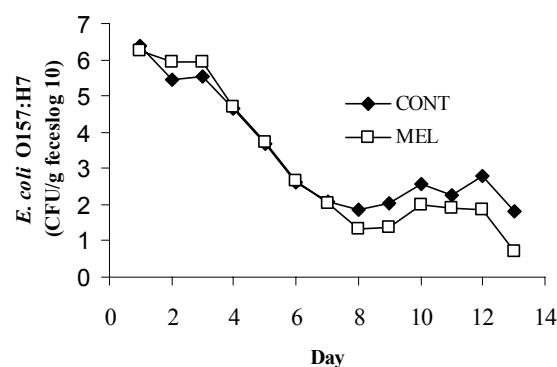
Blood was collected via jugular venipuncture on d 6, 14, and 21 to determine total WBC and differential leukocyte counts. Counts were done immediately following blood collections. Blood was collected in  $5 \text{ mL}$  evacuated tubes containing  $0.05 \text{ mL}$  heparin. To determine total WBC counts, blood cells were lysed with ultra-pure water, washed with Hanks' balanced salt solution (HBSS), centrifuged at  $1200 \times g$  for  $15 \text{ min}$  and then counted using a hemacytometer. For leukocyte differential counts, approximately  $10 \text{ }\mu\text{L}$  blood was smeared on pre-cleaned microscope slides, stained and then counted within 5 d of the collection time.

Daily fecal shedding patterns were analyzed as repeated measures using the MIXED procedure of SAS while WBC, differential leukocyte counts, and luminal *E. coli* populations were analyzed using the GLM procedure of SAS.

## Results and Discussion

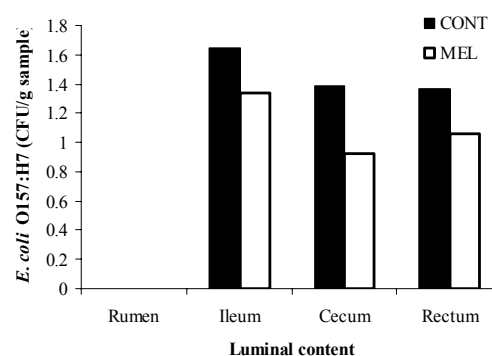
Fecal swabs taken prior to inoculation demonstrated animals were not shedding *E. coli* O157:H7 capable of growth on rifampicin supplemented MacConkey agar (data not shown). Daily fecal shedding patterns (Figure 1) of the experimental strain were similar between treatment groups with shedding decreasing daily ( $P < 0.001$ ) over the 14 d period. Patterns in fecal shedding of the experimental strain were comparable to that observed by Edrington et al. (2003). However, *E. coli* numbers were slightly lower than that observed by Laven et al. (2003) when naturally infected cattle were observed for *E. coli* O157:H7 at slaughter.

Experimentally infecting mature animals, who have an established gut microflora or who are not stressed, may be less effective due to competitive exclusion of the experimental strain by the mature bacterial populations of the gut.



**Figure 1.** Effect of treatment on daily shedding patterns of *E. coli* O157:H7 from d 0 through 14 post inoculation of the experimental strain 2336 (fecal counts and fecal counts by day,  $P > 0.10$ ).

Similar to daily fecal shedding patterns, no differences were observed in the populations of *E. coli* O157:H7 in the luminal contents of the rumen, ileum, cecum, or rectum of CONT- and MEL-treated animals (Figure 2). *Escherichia coli* populations found in the content of the GIT were consistent with that observed in the feces. Laven et al. (2003) also found *E. coli* O157 in sections of the GIT, including the rumen. Rumen samples taken by Laven et al. (2003) were perhaps more representative than that collected in the present study. Samples from multiple compartments were collected vs. a

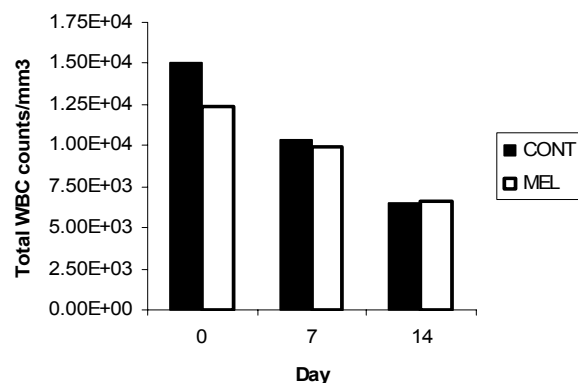


**Figure 2.** Effect of treatment on the prevalence of *E. coli* O157:H7 in the luminal contents of the rumen, ileum, cecum, and rectum on d 14 post inoculation with the experimental strain ( $P > 0.10$ ).

single random sample taken in this study and may explain why no bacteria were detected in our rumen samples. Conversely, previous research indicates the hind gut is the

primary site of colonization and the ratio of *E. coli* to the already present microflora increases in the lower tract of the gastrointestinal system (Grauke, 2002). As observed in this experiment, populations of *E. coli* O157:H7 seen in the GIT of slaughter cattle investigated by Levan et al. (2003) were consistent with populations found in the feces of those same cattle.

To evaluate the overall health status of our animals, total white blood (Figure 3) and differential



**Figure 3.** Effects of treatment on total WBC counts on d 6, 14, and 21 for CONT and MEL treated wethers (Treatment effect,  $P > 0.10$ ; day effect,  $P < 0.01$ ; treatment by day effect,  $P > 0.10$ ).

Table 1. Effects of treatment on differential leukocyte counts<sup>1</sup> in wethers.

	Treatment, % <sup>2</sup>		SE <sup>3</sup>
	CONT	MEL	
Lymphocytes	59.1	56.3	4.9
Monocytes	3.9	5.4	2.0
Polymorphonuclear leukocytes	36.3	39.8	3.5

<sup>1</sup> Values reported as a percentage of total WBC counts.

<sup>2</sup> Melatonin (25 mg) orally dosed for 21 d ( $P > 0.10$ ).

<sup>3</sup> Standard error of the mean.

leukocyte (Table 1) counts were analyzed. Although neither a treatment, or treatment by day effect was observed, total WBC counts decreased over the course of the study ( $P < 0.01$ ) in all animals across treatments. Differential leukocyte counts were also similar between treatment groups with no day effect observed. Despite the decrease in total WBC, leukocyte numbers were in accordance with that reported by Plumb (1995) for healthy sheep ( $4\text{--}12 \times 10^3$ ).

### Implications

Supplementing melatonin had no effect on fecal shedding patterns or gut populations of *E. coli* O157:H7 in experimentally infected sheep. Animals in this study were considered healthy with stress kept to a minimum. A pathogenic invasion may be greater in animals under

stress due to lower immuno-competence, therefore resulting in increased GIT populations and fecal shedding of *E. coli*. In that case, MEL may have played a greater role in altering pathogenicity either via a direct effect on gut populations or by altering host immune responses. Further research is needed to understand host and microorganism interactions.

### Literature Cited

- Alila-Johansson, A., L. Eriksson, T. Soveri, and M. L. Laakso. 2001. Seasonal variation in endogenous serum melatonin profiles in goats: a difference between spring and fall? *J. Biol. Rhythms*. 16(3): 254-263.
- Animal and Plant Health Inspection Service. October 2001. *Escherichia coli* O157 in United States Feedlots. NAHMS Feedlot '99. Available at: [http://www.aphis.usda.gov:80/vs/ceah/cahm/Beef\\_Feedlot/bffeed.htm](http://www.aphis.usda.gov:80/vs/ceah/cahm/Beef_Feedlot/bffeed.htm).
- Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants.
- Bubenik, G. A., D. E. Blask, G. M. Brown, G. J. M. Maestroni, S. F. Pang, R. J. Reiter, M. Viswanathan, and N. Zisapel. 1998. Prospects of the clinical utilization of melatonin. *Biol. Signals Recept.* 7: 195-219.
- Centers for Disease Control. 2002. Foodnet 2000 annual Report. Foodnet Active Surveillance Project. available at: [http://www.cdc.gov/foodnet/annual/2000/2000\\_summary.htm](http://www.cdc.gov/foodnet/annual/2000/2000_summary.htm).
- Edrington, T. S., C. L. Schultz, K.J. Genovese, T.R. Callaway, M.L. Looer, K.M. Bischoff, J.L. McReynolds, R.C. Anderson, and D.J. Nisbet. 2004. Examination of heat stress and stage of lactation (early vs. late) on fecal shedding of *E. coli* O157:H7 and *Salmonella* in dairy cattle. *Foodborne Pathogens and Disease*. (Accepted)
- Eloranta, E., J. Timisjarvi, M. Nieminen, V. Ojutkangas, J. Leppaluoto, and O. Vakkuri. Seasonal and daily patterns in melatonin secretion in female reindeer and their calves. *Endocrinology*. 130(3): 1645-1652.
- Freestone, P. P. E., R. D. Haigh, P. H. Williams, and M. Lyte. 1999. Stimulation of bacterial growth by heat-stable, norepinephrine-induced autoinducers. *FEMS Microbiol. Letters*. 172: 53-60.
- Freestone, P. P. E., P. H. Williams, R. D. Haigh, A. F. Maggs, C. P. Neal, and M. Lyte. 2002. Growth stimulation of intestinal commensal *Escherichia coli* by catecholamines: A possible contributory factor in trauma-induced sepsis. *Shock*. 18(5): 465-470.

- Grauke, L. J., I. T. Kudva, J. W. Yoon, C. W. Hunt, C. J. Williams, and C. J. Hovde. 2002. Gastrointestinal tract location of *Escherichia coli* O157:H7 in ruminants. *Appl. Environ. Microbiol.* 68: 2269-2277.
- Hancock, D. D., T. E. Besser, D. H. Rice, D.E. Herriott, and P. I. Tarr. 1997. A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. *Epidemiol. Infect.* 118: 193-195.
- Laven, R. A., A. Ashmore, and C. S. Stewart. 2003. *Escherichia coli* in the rumen and colon of slaughter cattle, with particular reference to *E. coli* O157. *The Vet. J.* 165: 78-83.
- Lyte, M., C. D. Frank, B. T. Green. 1996. Production of an autoinducer of growth by norepinephrine cultured *Escherichia coli* O157:H7. *FEMS Microbiol. Letters.* 139: 155-159.
- Miller, J. J., B. W. Beasley, L. J. Yanke, F. J. Larney, T. A. McAllister, B. M. Olsen, L. B. Selinger, D. S. Chanasyk, and P. Hasselback. 2003. Bedding and seasonal effects on chemical and bacterial properties of feedlot cattle manure. *J. Environ. Qual.* 32: 1887-1894.
- Miller, M. B., and B. L. Bassler. 2001. Quorum sensing in bacteria. *Annu. Rev. Microbiol.* 55: 165-199.
- Plumb, B. C. 1995. Lab values. Page 749 in *Veterinary Drug Handbook*. 2<sup>nd</sup> ed. Iowa State Univ. Press, Ames, IA.
- Tan, D., L. C. Manchester, R. J. Reiter, W. Qi, M. Karbownik, and J. R. Calvo. 2000. Significance of melatonin in antioxidative defense system: Reactions and products. *Biol. Signals Recept.* 9: 137-159.
- Zinn, S. A., L. T. Chapin, W. J. Enright, A. L. Schroeder, E. P. Stanisiewski, and H. A. Tucker. 1988. Growth, carcass composition and plasma melatonin in postpubertal beef heifers fed melatonin. *J. Anim. Sci.* 66: 21-27.

# EFFECT OF FLUNIXIN MEGGLUMINE ON EARLY EMBRYONIC MORTALITY IN STRESSED BEEF FEMALES

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**ABSTRACT:** The objective of these studies was to determine if a treatment of 1.1 mg/kg BW of flunixin meglumine (FM) would reduce early embryonic mortality in stressed (TS) or non-stressed (NTS) beef females. Heifers (n = 259) or cows (n = 127) were assigned to one of four treatments in a 2x2 factorial design. Treatments were: control (CON), control with FM (CONFM), transportation stressed (S), and transportation stressed with FM (SFM). Treatments were applied to heifers and cows approximately 14 d following AI. Rectal temperatures were recorded and blood samples collected from all females before treatment, after ~2.5 h of treatment, and at the end of treatment (except final temperature not recorded in cows). Females receiving NTS treatment (CON and CONFM) remained at the ranch while TS treated females (S and SFM) were transported for 5-6 h. After ~ 2.5 h of transportation stress, TS females were unloaded from trucks, handled for temperature and blood collection, and loaded onto trucks again. Females were not exposed to clean-up bulls until after treatment. Transrectal ultrasonography was used to determine AI pregnancy status 33-35 d (heifers) or 55-57 d (cows) post-AI. In both heifers and cows, serum cortisol concentrations were similar ( $P > 0.10$ ) at the initial blood sampling, increased ( $P < 0.01$ ) at the intermediate blood sampling and decreased ( $P < 0.01$ ) below pretreatment levels in TS compared to NTS females at the final blood sampling. Among pooled data, AI pregnancy rates of TS females (62%) were not different ( $P > 0.10$ ) than NTS females (64%), however, AI pregnancy rates of FM treated females (69%) were higher ( $P = 0.03$ ) than NFM females (59%). Final pregnancy rates did not differ ( $P > 0.10$ ) among treatments. We conclude that FM administration ~14 d post-AI decreased embryonic mortality in beef females, and the magnitude of that decrease was similar in both transportation stressed (10%) and non-stressed (11%) females.

**Key Words:** Pregnancy Rate, Embryonic Mortality, Flunixin Meglumine

## Introduction

The greatest production loss for commercial cow-calf producers results from cows not becoming pregnant by the end of the breeding season (Bellows and Short, 1994). Many studies have been conducted on

factors contributing to reproductive performance in beef cattle; including nutrition, disease, chromosomal abnormalities, and physiological imbalances. However, relatively few studies have focused on the effects of postbreeding management to maintain pregnancies. Animals experiencing early embryonic loss, may repeatedly calve late resulting in younger and lighter calves, or fail to become pregnant. Harrington et al. (1995) indicated that transporting during early pregnancy increased embryonic losses. In that study, 74%, 62%, and 65% of heifers transported via tractor-trailer for 7 h at 1 to 4, 8 to 12, or 29 to 33 d post-AI were pregnant to AI, suggesting d 8 to 12 and d 29 to 33 after AI were critical time periods within gestation when transportation stress might affect pregnancy maintenance. Losses occurring around the time of maternal recognition of pregnancy may be occurring because certain embryos are unable to inhibit secretion of PGF<sub>2α</sub> (Thatcher et al., 2001).

Flunixin meglumine is a non-steroidal anti-inflammatory drug that inhibits the cyclooxygenase-2 enzyme, preventing the conversion of arachidonic acid to PGF<sub>2α</sub>. The suppressive effects of flunixin meglumine on PGF<sub>2α</sub> begin within 30 min of administration and persist for 6-24 h (Aiumlamai et al., 1990; Anderson et al., 1990). The objective of these studies was to determine if a single administration of flunixin meglumine would reduce early embryonic mortality in stressed or non-stressed beef females.

## Materials and Methods

In Exp. 1, 259 yearling, Angus-cross heifers (BCS  $5.15 \pm 0.34$ ) were utilized to determine the effects of flunixin meglumine on early embryonic mortality and serum cortisol concentration. All heifers were synchronized with an EAZI-BREED CIDR (Pfizer Animal Health, Kalamazoo, MI) for 7 d and PGF<sub>2α</sub> (25 mg, i. m.; Pfizer Animal Health, Kalamazoo, MI) at CIDR removal. Heifers had received GnRH (100 µg, i.m.; Intervet, Inc., Millsboro, DE) or no GnRH at CIDR insertion and were assigned to AI either 12 h following estrous detection up to 84 h after PGF<sub>2α</sub> or by appointment at 64 h following PGF<sub>2α</sub>. Heifers bred by appointment and heifers not exhibiting estrus by 72 h post PGF<sub>2α</sub> received GnRH (100 µg) at timed AI. Heifers were blocked by synchronization protocol before assignment to stress and flunixin meglumine treatments.

In Exp. 2, 127 multiparous, Angus-cross cows (BW  $681 \pm 4.5$  kg; mean age 5.4 yr) were utilized to test the effect of flunixin meglumine on early embryonic mortality and serum cortisol concentration. Cows received MGA (0.5 mg/d) for 14 d followed by an injection of PGF<sub>2 $\alpha$</sub>  19 d later to synchronize estrus, and were artificially inseminated approximately 12 h after onset of estrus for 5 d following PGF<sub>2 $\alpha$</sub> .

Approximately 14 d following AI, females in both experiments were randomly assigned to treatments: control (CON), control with flunixin meglumine (CONFM), induced stress (S), and induced stress with flunixin meglumine (SFM). The heifers or cows receiving CON (n=65 or 32, respectively) and CONFM (n=65 or 31, respectively) remained at the ranch and were provided access to water but no feed for 5 to 6 h (NTS). Heifers or cows receiving S (n=65 or 32, respectively) and SFM (n=64 or 32, respectively) were transported via semi-truck for approximately 5 h (TS). Before transportation-induced stress, SFM and CONFM treated females received flunixin meglumine (1.1 mg/kg, i.m.). Before treatment, rectal temperatures were recorded, and blood samples collected (coccygeal venipuncture) from all females for measurements of serum cortisol concentration. A second blood sample and temperatures was collected from all females after approximately 2.5 h of transportation stress (or NTS treatment) and TS females were reloaded onto semi-truck and received another 2.5 h of transportation stress. Blood samples were collected a third time after transportation stress was completed. All blood samples were placed on ice for approximately 24 h to allow clot formation. Serum was obtained by centrifugation at  $1800 \times g$  for 20 min, transferred to new tubes, and stored at  $-20^{\circ}$  C until analyzed. Females were exposed to clean-up bulls beginning the day after treatment for 30 d. Transrectal ultrasonography was used to determine AI pregnancy status for heifers at 33-35 d or for cows at 55-57 d post-AI. Final pregnancy rates were determined approximately 90 d after AI by transrectal ultrasonography for heifers and transrectal palpation for cows.

Serum cortisol concentrations were determined by a commercially available RIA (Kit TKC05; Diagnostic Products Corporation, Los Angeles, CA). Sensitivity of the assay was 2.2 ng/mL and inter- and intra-assay CV were 5.8% and 6.3% for high and 2.2% and 1.8% for low in-house controls, respectively.

A 2 x 2 factorial design of induced transportation stress (TS) or no transportation stress (NTS) and receiving an injection of flunixin meglumine (FM) or no flunixin meglumine (NFM) was used for analyzing differences in absolute serum cortisol concentration, absolute temperature, change in serum cortisol concentrations, change in temperature and AI and final pregnancy rates. When no transportation stress by flunixin meglumine interaction was present ( $P > 0.10$ ), only main effects are discussed.

## Results and Discussion

In Exp. 1 (Figure 1), no interaction ( $P > 0.10$ ) was detected in the initial serum cortisol concentration between treatments, so only main effects will be discussed. Heifers that did not receive an injection of flunixin meglumine (NFM) had lower ( $P < 0.05$ ) pre-treatment serum cortisol concentrations (34.51 ng/mL) than FM heifers (39.57 ng/mL). In the second blood sample, there was an interaction ( $P < 0.05$ ) of induced stress and flunixin meglumine on serum cortisol concentrations. Serum cortisol concentration of SFM heifers (48.5 ng/mL) was greater ( $P < 0.05$ ) than S, CON, and CONFM heifers (40.6, 37.0, and 35.6 ng/mL, respectively). In the final blood samples, there was no interaction ( $P > 0.10$ ) of induced stress by flunixin meglumine on serum cortisol concentrations. However, there was a decrease ( $P < 0.01$ ) in serum cortisol concentration for the transportation stressed heifers (TS; 19.39 ng/mL) heifers compared to NTS (33.77 ng/mL) heifers. The TS heifers may have adjusted to the situation with decreased serum cortisol concentrations as a defense mechanism for additional stimuli (Friend, 1991). These results are supported by others who have reported that an increase in serum cortisol concentrations was due to loading and that animals recovered as transportation continued (Wariss et al., 1995 as reported by Dixit et al., 2001).

In Exp. 2 (Figure 2), no interaction or main effect differences ( $P > 0.10$ ) were detected at the initial blood sampling for serum cortisol concentrations. In the second blood sample, no interaction ( $P > 0.10$ ) was detected. However, the concentrations of serum cortisol were higher ( $P < 0.01$ ) in TS cows (28.02 ng/mL) compared to NTS cows (15.15). No interaction ( $P > 0.10$ ) was detected in serum cortisol concentrations of cows following treatment. Concentrations of serum cortisol were lower ( $P < 0.01$ ) for TS cows (4.91 ng/mL) than for NTS cows (13.44 ng/mL). Becker et al. (1985) reported a similar response of increased cortisol concentrations followed by decreased serum cortisol concentrations in gilts after several hours of being tethered to stalls. Serum cortisol concentration was also lower ( $P < 0.05$ ) at the final blood sampling for NFM cows (8.01 ng/mL) than FM cows (10.34 ng/mL). Because serum cortisol concentrations differed greatly between heifers and cows, these data were not pooled with respect to cortisol.

In Exp. 1, no interaction ( $P > 0.10$ ) was detected in heifer AI or final pregnancy rates. The FM heifers tended ( $P = 0.15$ ; 63%) to have higher AI pregnancy rates compared to NFM heifers (54%; Figure 3). No differences ( $P > 0.10$ ) existed in final pregnancy rates of heifers among treatments.

In Exp. 2, no interaction ( $P > 0.10$ ) was detected in AI or final pregnancy rates of cows. The FM cows had a higher ( $P < 0.08$ ) AI pregnancy rate (80%) compared to NFM cows (66%; Figure 4). Giri et al., (1991) reported similar differences between AI pregnancy rates in cows that were infused with endotoxins and had received saline compared to cows that received flunixin meglumine. The magnitude of difference in AI pregnancy rates were similar to those observed in FM and NFM heifers from

Exp. 1. No differences ( $P > 0.10$ ) were detected in final pregnancy rates of cows.

There were no female or induced stress by flunixin meglumine interactions ( $P > 0.10$ ), so heifers and cows were combined from the 2003 breeding season and re-analyzed. The AI pregnancy rates were higher ( $P < 0.05$ ) for FM females (68%) compared to NFM females (58%; Figure 5). The higher pregnancy rate of FM females when compared to NFM females has been similar throughout the experiments reported here, and data reported earlier (Merrill et al., 2003). These results suggest that flunixin meglumine has an effect on AI pregnancy rates that overcomes transportation stress induced embryonic loss.

### Implications

These data indicate that flunixin meglumine reduces early embryonic mortality during early pregnancy (12-14 d). We presume the mechanism is due to decreased uterine PGF secretion that may allow a developing conceptus extra time to secrete sufficient interferon tau to prevent uterine PGF secretion and luteolysis, since serum cortisol concentrations did not differ among females receiving flunixin meglumine. Future trials, containing an additional control group without handling until pregnancy diagnosis may need to be conducted to determine if animal handling for data collection impacts embryonic mortality.

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### Literature Cited

- Aiumlamai, S., K. Odensvik, G. Stabenfeldt, and H. Kindahl. 1990. Regulation of prostaglandin biosynthesis with flunixin meglumine in the bovine species. *J. Vet. Med. A*. 37: 16-22.
- Anderson, K.L., C.A. Neff-Davis, L.E. Davis, V.D. Bass. 1990. Pharmacokinetics of flunixin meglumine in lactating cattle after single and multiple intramuscular and intravenous administrations. *Am. J. Vet. Res.* 51:1464-1467.
- Becker, B.A., J.J. Ford, R.K. Christenson, R.C. Manak, G.L. Hahn, and J.A. Deshazer. 1985. Cortisol response of gilts in tether stalls. *J. Anim. Sci.* 60:264- 270.
- Bellows, R.A. and R.E. Short. 1994. Reproductive Losses in the Beef Industry. In: *Factors Affecting Calf Crop*. Page 109. Ed. 1. CRC Press. Boca Raton, FL
- Dixit, V.D., M. Marahrens, and N. Parizi. 2001. Transport stress modulates adrenocorticotropin secretion from peripheral bovine lymphocytes. *J. Anim. Sci.* 79:729-734.
- Friend, T.H. 1991. Symposium: Response of animals to stress: Behavioral aspects of stress. *J. Dairy. Sci.* 74: 292-303.
- Giri, S.N., G.H. Stabenfeldt, T.A. Moseley, T.W. Graham, M.L. Bruss, R.H. BonDurant, J.S. Cullor and B.I. Osburn. 1991. Role of eicosanoids in abortion and its prevention by treatment with flunixin meglumine in cows during the first trimester. *J. Vet. Med. A* 38: 445-459.
- Harrington, T.E., M.E. King, H.E. Mihura, D.G. LeFever, R. Hill, and K.G. Odde. 1995. Effect of transportation time on pregnancy rates of synchronized yearling beef heifers. Colorado State University Beef Program Report. Fort Collins, CO.
- Merrill, M.L., R.P. Ansotegui, N.E. Wamsley, P.D. Burns, and T.W. Geary. 2003. Effects of Flunixin Megumine on early embryonic mortality in stressed beef cows. *Proc. West. Sec. ASAS*. 54:53-56.
- SAS. 2001. SAS/STAT® User's Guide (8.02). SAS Inst. Inc., Cary NC.
- Thatcher, W.W., K.L. Macmillan, P.J. Hansen and F.W. Bazer. 1994. Embryonic Losses: Cause and Prevention. In: *Factors Affecting Calf Crop*. Page 136. Ed. 1. CRC Press. Boca Raton, FL.

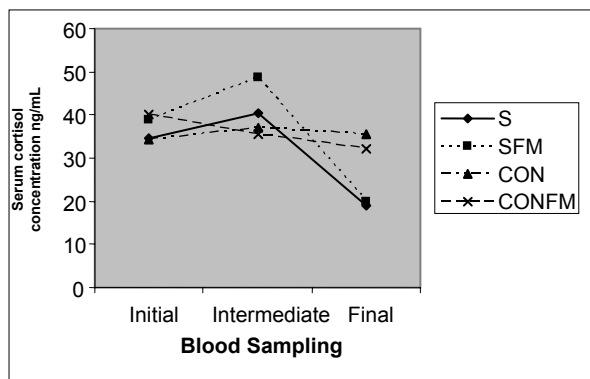


Figure 1. Mean serum cortisol concentrations of heifers for Exp. 1 at each of the three blood samples collected from control heifers (CON) and control heifers with flunixin meglumine (CONFM) and heifers that received 6 h transportation stress (S) or transportation stress with flunixin meglumine (SFM).

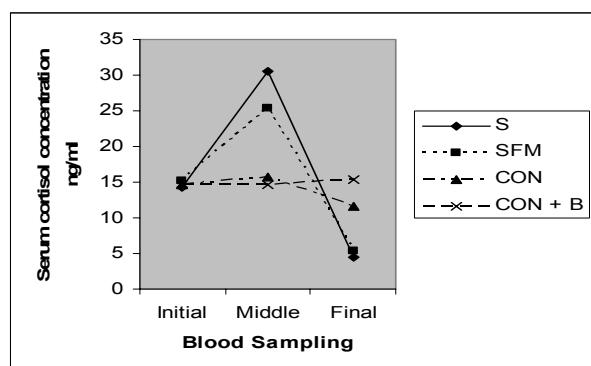


Figure 2. Mean cortisol concentrations for Exp. 2 at each of three blood samplings from control cows (CON) and control cows with flunixin meglumine (CONFM) and cows that received 5 h transportation stress (S) or transportation stress with flunixin meglumine (SFM).

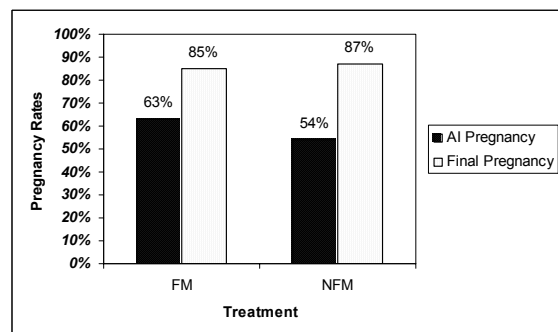


Figure 3. The AI and final pregnancy rates of Exp. 1 for heifers that received flunixin meglumine (FM) and heifers that did not receive flunixin meglumine (NFM). The AI pregnancy rates differ ( $P = 0.15$ ).

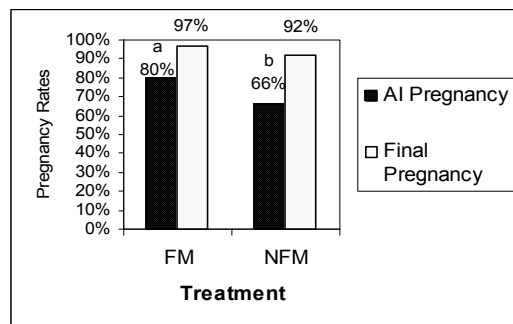


Figure 4. The AI and final pregnancy rates of Exp. 2 for cows that received flunixin meglumine (FM) and cows that did not receive flunixin meglumine (NFM). Bars that lack common superscripts within AI pregnancy rates differ ( $P = 0.08$ ).

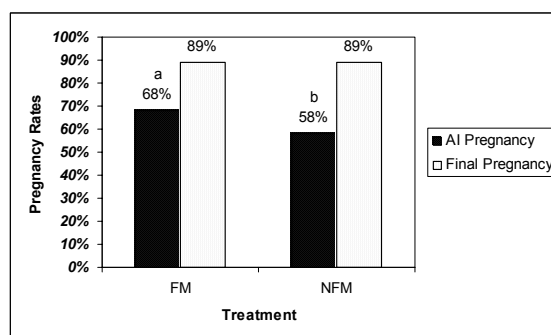


Figure 5. The AI and final pregnancy rates of Exp. 1 and 2 for females that received flunixin meglumine (FM) and females that did not receive flunixin meglumine (NFM). Bars that lack common superscripts within AI pregnancy rates differ ( $P = 0.03$ ).

## COMPARISON OF SOMATIC CELL COUNTS, MILK CONSTITUENTS AND WEANING WEIGHTS IN EWES WITH AND WITHOUT SUBCLINICAL MASTITIS

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**ABSTRACT:** Fifty-four multiparous Rambouillet ewes and their offspring were used to 1) assess the effectiveness of using flow cytometry (FC) relative to traditional somatic cell counts (SCC) and to determine the incidence of subclinical mastitis at weaning and 2) assess the relationships among FC, milk constituents, weaning weights and subclinical mastitis. At weaning, lambs were weighed and 15-mL milk samples were collected from each side of the ewe's udder and pooled. The cell counts between FC and traditional SCC procedures were positively correlated ( $r = .94$ ,  $P < .0001$ ). Multiple linear regression analyses revealed that the linear effects of milk fat, lamb adjusted weaning weight and days postpartum or their two-way interactions did not affect ( $P > 0.78$ ) the log<sub>10</sub> transformed FC. A second multiple linear regression revealed that 88% of the variation in log<sub>10</sub> transformed FC was accounted for by mastitis status, percentages of lactose and protein and the interaction between mastitis status x protein percentage. An interaction ( $P = .0001$ ) between mastitis and protein indicated that protein percentage increased with cell count in ewes with subclinical mastitis, whereas, protein percentage was not associated with FC in mastitis-free ewes. A total of 16.6% (10/54) of the ewes had FC associated with subclinical mastitis. Ewes that raised triplets, twins, and produced twins but raised a single had increased ( $P = .005$ ) cell counts ( $3.3$ ,  $2.8$  and  $3.0 \pm .28$  log<sub>10</sub> FC) than ewes producing single offspring ( $2.2 \pm .23$  log<sub>10</sub> FC). The adjusted weaning weights of lambs were not influenced ( $P > .28$ ) by mastitis status, type of rearing or milk quality. Flow cytometry is comparable to the traditional procedure for detecting SCC values as an indicator of subclinical mastitis. In addition, elevated milk protein percentages reflected increased FC and could be indicative of subclinical mastitis.

Key Words: Ewe, Mastitis, Flow Cytometry

### Introduction

Mastitis, which is defined as an inflammation of the mammary gland (Keisler et al., 1992; Forde et al.,

2003), is detrimental to the cattle and sheep industries. Mastitis decreases milk production, increases SCC and alters milk components in beef and dairy cattle (Paape et al., 2000). Mastitis infections are also seen in sheep resulting in significant economic losses through poor growth performance in lambs, reduction in milk quality and quantity, premature culling, and mortality (Keisler, et al., 1992; Forde et al., 2003; Torres-Hernandez and Hohenboken, 1979, 1980). Whether a producer ignores, treats, or culls a ewe with mastitis is dependent upon its effect on lamb performance and/or the cost and benefit of treatment (Keisler et al., 1992).

Overt mastitis can be diagnosed readily in clinical cases either visually or by palpation, but the only means of identifying subclinical mastitis is by measuring SCC (Keisler et al., 1992). Somatic cell counts have shown to be good indicators of subclinical mastitis infections in previous studies performed with cattle (Paape et al., 2000), sheep (Keisler et al., 1992), and goats (Hinckley, 1983). The traditional laboratory method for determination of SCC is reasonably effective, however, a newer flow cytometric method, may provide more definitive information including differentiation among the types of somatic cells in milk. Redelman et al. (1988) reported that five or more populations of cells could be identified in mastitic milk collected from dairy cows and this rapid and simple procedure identified subclinical mastitis during routine screening. Flow cytometric technology may also be a more effective and efficient means for determining mastitis in sheep and needs to be examined.

The objectives of this study were to 1) assess the effectiveness of using flow cytometry (FC) relative to traditional somatic cell counts (SCC) and to determine the incidence of subclinical mastitis at weaning using FC and 2) assess the relationships among FC, milk constituents, weaning weights and subclinical mastitis.

### Materials and Methods

*Animals.* Fifty-four multiparous Rambouillet ewes (3 to 6 yr old) and their 95 offspring were selected for this study. Ewes lambed within a 3-wk period with lambing starting the third week of March. At lambing,

birth weight, sex, and number of lambs born were recorded. The ewes were group-fed. Each ewe received alfalfa pellets (2.72 kg/d) and 0.23 kg corn/d for the first 30 d of lactation. Following the 30-d period, ewes and their offspring were moved to pasture and maintained there until lambs were weaned. Lambs received no creep feed, but had access to the ewe's diet the first 30 d of age. Each ewe's lambing status (triplet, twin or single births) and changes in the suckling status were recorded during the lactation period. Twelve ewes produced and raised singles, seven ewes produced twins and raised singles, 29 ewes produced and raised twins, and six ewes produced and raised triplets.

**Sampling Procedures.** At weaning ( $83 \pm 5.7$  d postpartum; mean SD), lambs were separated from the ewes and weighed. Milk samples were collected from each side of the ewe's udder at this time. Sampling consisted of cleaning the teat with 70% alcohol swabs and removing and discarding approximately 3-mL of milk. A 15-mL milk sample was obtained from each ewe's udder half. The samples from each udder half were pooled and gently mixed. After which, a 5-mL sub-sample from the pooled milk was collected and used for FC determination. The remaining portion of the pooled sample was sent to the Dairy Herd Improvement Association (DHIA) laboratory located in Fresno, CA and analyzed for percentages of protein, lactose, fat, and SCC.

**FC Analysis.** The number of cells in the milk on the day of sampling were quantified by flow cytometry after staining with a combination of SYBR Green I<sup>TM</sup> (SYBR I Molecular Probes, Eugene, OR) and propidium iodide (PI). This nucleic acid-specific dye combination stains living cells green (SYBR I), and dead cells red (PI). Each 5-ml sample was gently shaken and 0.5  $\mu$ L of milk was pipetted into 1.5-mL microcentrifuge tubes containing 450  $\mu$ L of the fluorescent dye solution and the contents mixed. After incubation at 37°C for 5 min, FC was quantified based on the logs of red and green fluorescence using a Coulter Epics Profile II<sup>®</sup> flow cytometer (Beckman Coulter, Fullerton, CA).

**SCC and Milk Constituents Analyses.** The SCC and milk constituents were determined using a Bentley 2000-Somacount 500 Combi<sup>®</sup> (Bentley Instruments, Chaska, MN). This instrument consists of a computer, an optical system, and an infrared (IR) filter system that can simultaneously determine SSC along with the percentages of milk protein, fat and lactose in milk.

**Statistical Procedure.** SAS software version 8.2 (SAS Corporation, Cary, NC) was used to perform all statistical analyses. The SAS PROC CORR of Pearson correlation coefficient was used to determine the relationship between the two methods (FC vs. traditional SCC) used to determine the cell count in the milk. The FC was transformed to the logarithm to the base of 10 in the regression analysis. A multiple linear regression

model with indicator variables and their interactions were fitted using the SAS PROC GLM to determine factors influencing the log transformation of FC. In the final regression model, only the statistically significant ( $P < .05$ ) terms were included in the model. Indicator variable mastitis status (Yes or No), and continuous predictor variables, percentages of milk protein, fat and lactose, days postpartum and lamb adjusted weaning weight were fitted in the multiple linear regression model. Mastitis status was determined by FC. Ewes with counts above 500,000 cells/mL were classified as having subclinical mastitis and ewes below that value to be mastitis free. A two (mastitis) x four (type of rearing) factorial arrangement was used to examine the effect of mastitis and type of rearing on log FC. Lamb adjusted weaning weight was added to the model as a covariate. All lamb weights were adjusted for age, sex of lamb, birth weight of lamb, age of dam and type of birth or rearing prior to analyses.

## Results and Discussion

The actual and log<sub>10</sub> transformed FC and traditional SCC were positively correlated ( $r = .94$ ,  $P < .0001$ ;  $r = .90$ ,  $P < .0001$ ), respectively. These data indicate that the total SCC obtained by flow cytometry are comparable to DHIA SCC estimates and accurately reflect the SCC population in ewe's milk.

The multiple linear regression model used to explain the variation for log<sub>10</sub> transformed FC consisted of mastitis status, percentages of milk protein, fat, and lactose, lamb adjusted weaning weight and days postpartum. Multiple linear regression analyses revealed that the linear effects of percentage of milk fat, lamb adjusted weaning weight and days postpartum and their two-way interactions did not affect the log<sub>10</sub> transformed FC. These variables were removed from the model and the data analyzed a second time. The refined multiple linear regression analysis revealed that 88% of the variation in the log<sub>10</sub> FC was accounted for by mastitis status, percentage of lactose and protein and the interaction between mastitis status x percentage of milk protein. Examination of the two-way interactions revealed no mastitis x percentage of lactose interaction ( $P = .13$ ) or percentages of protein x lactose interaction ( $P = .09$ ). The percentage of lactose did not influence ( $P = .06$ ) the regression model. A mastitis x percentage of protein interaction for log<sub>10</sub> transformed FC was noted ( $P = .0001$ ) and is shown in Figure 1.

In this study, milk protein percentages increased as FC increased in ewes considered to have subclinical mastitis (Figure 1). The percentage of protein in milk, however, was not affected by FC in ewes considered to be mastitis-free. Similarly, Keisler et al. (1992) found milk protein percentage increased ( $r = .38$ ,  $P < .01$ ) as SCC increased, but this study did not compare ewes with and without mastitis. Also, Torres-Hernandez and

Hohenboken (1979) reported ewes free of mastitis had lower milk protein percentages than infected ewes and suggested that these differences were due to the normal milk protein plus additional protein associated with the increase in white blood cells. Our data also indicated that ewe milk with FC below 500,000/mL reflect milk protein only, and above that value show an increase in percentage of protein, as FC increases. Our results suggest that values above a certain protein threshold level might be indicative of subclinical mastitis.

On an individual animal basis, 3 % of the ewes (2/54) and 14.8 % (8/54) exceeded the 500,000 and 1,000,000 FC/mL with a total of 17.8% (10/54) having counts indicative of subclinical mastitis. Keisler et al. (1992) stated it was difficult to identify all ewes within a flock with subclinical mastitis based on a single-sample measurement because the severity of the subclinical mastitis in a ewe may change considerably during lactation as seen in dairy cattle (Schalm et al., 1971; Smith and Schultz, 1967). McFarland (2000), however, reported that when mastitis-infected ewes were collected at 20, 30, 60, and 90 d postpartum, the FC did fluctuate but remained above the mastitis FC threshold for at least three of the four sampling times and that at weaning, 75% of the ewes tested positive that were positive before weaning. Although the ewes in our study were sampled only at weaning, McFarland's (2000) study would suggest that at least  $\frac{3}{4}$  of the ewes that had elevated cell counts had subclinical mastitis.

When effect of mastitis status and type of rearing were examined on log<sub>10</sub> FC values, a mastitis x type of rearing interaction ( $P = .004$ ) was detected, but this interaction may not be biologically significant. Ewes classified with mastitis had an increased FC ( $3.7 \pm .12$  log<sub>10</sub> FC;  $P = .0001$ ) than ewes with no mastitis ( $2.0 \pm .05$  log<sub>10</sub>). Flow cytometric cell counts were influenced by type of rearing status. Ewes that raised triplets, twins, and produced twins but raised a single had increased ( $P = .005$ ) cell counts ( $3.3, 2.8$  and  $3.0 \pm .28$  log<sub>10</sub> FC) than ewes that produced single offspring ( $2.2 \pm .23$  log<sub>10</sub> FC). Gonzalo et al. (1994) also reported SCC was high in ewes raising twins compared to a single birth.

Lambs adjusted weaning weights were not influenced ( $P > .28$ ) by mastitis status, type of rearing or milk quality. Keisler et al. (1992) reported similar results and concluded that growth performance of lambs having access to supplemental feed are not influenced by either milk quality or the presence of subclinical mastitis. Although lambs in our study did not receive supplemental feed, they did have access to the ewe's diet during their first 30 d of age and pasture until day of weaning.

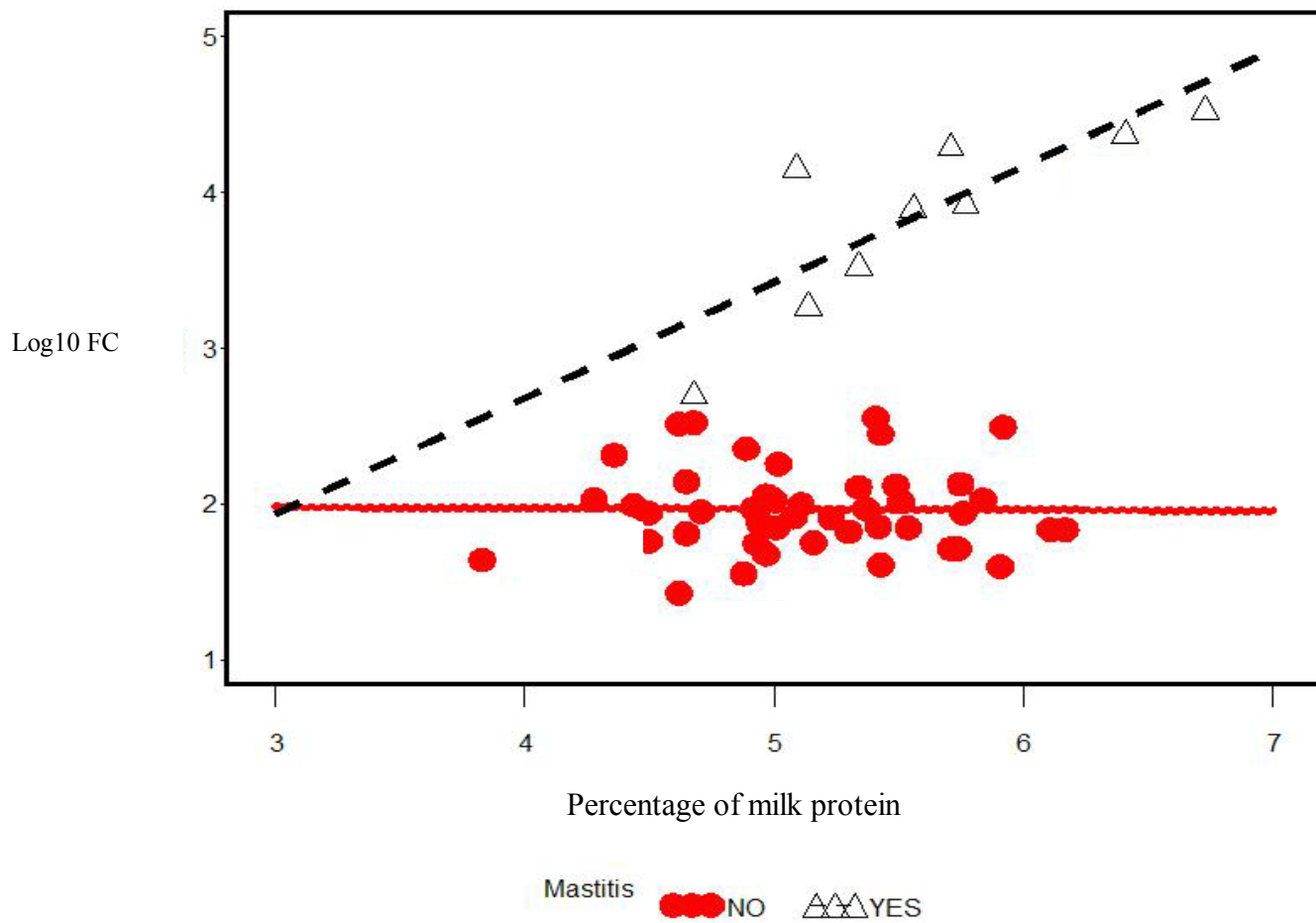
### Implications

Our results indicate that flow cytometry provides comparable somatic cell values to traditional SSC as used

in dairy cattle and that the resultant data can be used to determine incidence of subclinical mastitis in sheep. These data also show that elevated milk protein percentages may reflect increased SCC suggesting that elevated milk protein levels could be indicative of subclinical mastitis provided a threshold value could be established. Finally, subclinical mastitis did not affect growth performance in lambs' which have access to the ewe's diet and pasture.

### Literature Cited

- Forde, K.N., B.J. McCluskey, and K.S. Morgan. 2003. An investigation into the risk factors associated with clinical mastitis in Colorado sheep. *Sheep & Goat Research J.* 18:44-46.
- Fthenakis, G.C., E. T. S. El-Masannat, and J. M. Booth and J.E.T. Jones. 1991. Somatic cell counts of ewe's milk. *Br. Vet. J.* 147:575-581.
- Gonzalo, C., J.A. Carriedo, J.A. Baro and F. San Primitivo. 1994. Factors influencing variation of test day milk yield, somatic cell count, fat, and protein in dairy sheep. *J. Dairy Sci.* 77:1527-1542.
- Hinckley, L.S. 1983. Somatic cell count in relation to caprine mastitis. *VM.SAC* 78:1267-1271.
- Keisler, D. H., M. L. Andrews, and R. J. Moffatt. 1992. Subclinical mastitis in ewes and its effect on lamb performance. *J. Anim. Sci.* 70:1677-1681.
- McFarland, M. 2000. Quantification of mastitis in sheep. University of Nevada, M.S. Thesis.
- Paape, M. J, M. I Dueanas, R.P. Wettemann and L. W. Douglass. 2000. Effects of intramammary infection and parity on calf weaning weight and milk quality in beef cows. *J. Anim. Sci.* 78:2508-2514.
- Redelman, D., S. Butler, J.D. Robison, and D.L. Garner. 1988. Identification of inflammatory cells in bovine milk by flow cytometry. *Cytometry* 9:463-468.
- Schalm, O.W., E.J. Carroll, and N.C. Jain. 1971. *Bovine Mastitis*. Lea & Febiger, Philadelphia.
- Smith, J.W., and W.D. Schultze. 1967. Variation of cell content of milk associated with time of sample collection. *J. Dairy Sci.* 50:1083-1087.
- Torres-Hernandez, G., and W. Hohenboken. 1979. Genetic and environmental effects on milk production, milk composition, and mastitis incidence in crossbred ewes. *J. Anim. Sci.* 49:410-417.
- Torres-Hernandez, G., and W. Hohenboken. 1980. Relationships between ewe milk production and composition and preweaning lamb weight gain. *J. Anim. Sci.* 50:597-603.



**Figure 1.** The effect of mastitis status x percentage of milk protein interaction ( $P=.0001$ ) on the log10 transformed flow cytometric somatic cell count (FC). The regression model differed ( $P=.0002$ ) between milk from normal ewes ( $\hat{y}=2.91-0.0173_{\text{protein}}-0.1675_{\text{lactose}}$ ) and those exhibiting subclinical mastitis ( $\hat{y}=0.5528+0.7167_{\text{protein}}-0.1675_{\text{lactose}}$ ); FC  $\geq 500,000$  cell/mL.

## EFFECTS OF PROGESTERONE THERAPY ON EMBRYONIC SURVIVAL AND PREGNANCY RATES IN EWES

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**ABSTRACT:** This study was conducted to determine the effect of supplemental progesterone ( $P_4$ ) on embryonic survival and pregnancy rates in multi- and nulliparous ewes. Multi- and nulliparous ewes ( $n=84$  and  $17$ , respectively) were randomly assigned to four treatments (control, Time 4, Time 6, and Time 8). Animals were group fed with ad libitum access to water and mineral. Three vasectomized rams were penned with the multiparous ewes and nulliparous ewes were exposed to one vasectomized ram. Ewes were bred via natural service after estrus (d 0) detection using vasectomized rams and the HeatWatch® system. Treatments Time 4, 6, and 8 correspond to day post-mating when  $P_4$  therapy was initiated using a controlled internal drug release device. Therapy continued until d 18. Ewes assigned to the control treatment did not receive  $P_4$  therapy. Ovulation rate was determined (mid-ventral laparotomy between d 4 to 8) using 10 randomly selected ewes from each multiparous treatment and all nulliparous ewes. Blood samples were collected (jugular venipuncture) from all ewes from d 0 to 20 at 0600 on 2-d intervals. Serum  $P_4$  concentrations were determined by RIA. Multiparous ewes did not exhibit a time x treatment interaction but nulliparous ewes experienced an interaction ( $P > 0.41$  and  $P < 0.009$ , respectively). Multiparous ewes given  $P_4$  therapy had higher  $P_4$  concentrations than controls ( $P < 0.001$ ). Nulliparous ewes in treatments Time 4 and 6 had higher ( $P < 0.05$ )  $P_4$  concentrations than multiparous ewes of the same treatment at the first sampling after  $P_4$  therapy was initiated. Corpora lutea present was similar ( $P > 0.75$  and  $P > 0.68$ ) across treatments for multi- and nulliparous ewes, respectively. Additionally, lambing rates were similar ( $P > 0.50$ ) across treatments for multi- or nulliparous ewes. In summary,  $P_4$  therapy initiated on d 4, 6, and 8 post estrus has no effect on embryonic survival or pregnancy rate in multi- and nulliparous ewes when compared to controls.

Keywords: progesterone, ovulation, embryonic survival, pregnancy rate

### Introduction

Early embryonic success is influenced by progesterone (Parr, 1992). Efforts to explain the physiological effects on pregnancy have been inconsistent. Between 20 and 30 % of fertilized ova are lost in the first weeks of pregnancy (Edey, 1969). Faris et al. (2003) administered exogenous progesterone ( $P_4$ ) via a controlled internal drug release (CIDR) device to increase embryonic survival by supplying additional  $P_4$  to the uterus until corpora lutea (CL) were fully functional. Some of the literature (Thomas et al.,

1984; Parr et al., 1987; Ashworth et al., 1989) has focused on nutrition having detrimental effects on  $P_4$ ; however, results from Faris et al. (2003) showed no differences ( $P = 0.67$ ) in embryonic survival among nutritional treatments. Surprisingly, embryonic survival tended to decrease with  $P_4$  therapy administered on d 2 post-mating. Therefore, the objective of this study was to determine the effect of supplemental  $P_4$  (d 4, 6, and 8 post-mating) on embryonic survival and pregnancy rates in multi- and nulliparous ewes.

### Materials and Methods

On September 1, multi- and nulliparous, black-faced ewes ( $n = 84$  and  $17$ , respectively) were weighed (avg =  $71$  and  $72$  kg, respectively), scored on body condition (BCS; avg =  $2.5$  and  $3.0$ , respectively), and fitted with HeatWatch® transmitters to detect estrus. Ewes were then randomly assigned to four treatments using a completely randomized design. Multiparous ewes were housed together along with three vasectomized rams in a  $21 \times 32$  m pen with ad libitum access to water and mineral blocks. Nulliparous ewes were housed together with one vasectomized ram in a  $21 \times 12$  m pen with ad libitum access to water and mineral blocks. Ewes were fed chopped alfalfa ( $1.81$  kg/ewe; TDN =  $58\%$ , CP =  $13\%$ , as fed) in a bunk-line feeder. HeatWatch® was used to determine estrus at 0600 and ewes were gathered for breeding. Ewes that exhibited estrus were sorted for hand-mating. Three fertile rams were used to mate ewes exhibiting estrus and allowed one ejaculate per ewe. After the morning mating, ewes were returned to their pen for feeding. HeatWatch® was checked again at 1800 to determine ewes that were still in estrus or beginning estrus. All ewes were serviced one additional time 12 h after the first service. Time 4, Time 6, and Time 8 correspond to day post-mating (d 0 = mating) when  $P_4$  therapy was initiated to three of the four treatment groups and continued until d 20 via a CIDR (  $0.3$  g  $P_4$ , Pharmacia and Upjohn Ltd. Co, Auckland, NZ.). Ovulation rate was determined (mid-ventral laparotomy between d 4 to 8) using 10 randomly selected ewes from each multiparous treatment and all nulliparous ewes. Blood samples were collected (jugular venipuncture) using glass serum separator tubes from all ewes from d 0 to 20 at 0600 on 2-d intervals. Ewes that showed initial signs of estrus in the afternoon were sampled for  $P_4$  determination the following morning at 0600. Blood samples were collected just prior to CIDR application and removal for ewes undergoing  $P_4$  therapy. Blood was allowed to clot for 30 min at room temperature and centrifuged ( $1500 \times g$ ) for 25 min. Serum was immediately decanted after centrifugation and kept frozen at  $-20^\circ\text{C}$  until

assayed for P<sub>4</sub>. Serum P<sub>4</sub> concentrations were quantified using a commercial RIA kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). Eleven multiparous and two nulliparous ewes were removed from the data set. Reasons for removal include lost CIDR and two ewes died. All procedures were approved by the Institutional Animal Care and Use Committee.

Serum P<sub>4</sub> concentrations were analyzed with Proc GLM as a split-plot design (SAS Inst., Inc., Cary, NC, Version 8.0). Animal within treatment was used to determine treatment effects while the residual was used to test time effects and time related interactions. Categorical data were analyzed using chi-square option of the frequency procedure of SAS (SAS Inst., Inc., Cary, NC, Version 8.0).

## Results and Discussion

Faris et al. (2003) demonstrated serum progesterone concentration was increased ( $P < 0.01$ ) by an average of 2 ng/mL for ewes receiving P<sub>4</sub> therapy beginning 60 h after CIDR insertion. Progesterone levels remained higher ( $P < 0.01$ ) through d 11 and again at d 20 ( $P < 0.03$ ). Diskin and Niswender (1989) used progesterone-impregnated silastic implants to increase ( $P < 0.001$ ) overall serum concentrations in treated ewes compared to controls ( $3.50 \pm 0.06$  vs  $2.65 \pm 0.05$  ng/mL, respectively) from d 6 to 50 after mating. In order to determine the effects P<sub>4</sub> has on the embryo, we must have a good method of delivery. The previously mentioned studies have shown that P<sub>4</sub> can be delivered in a therapeutic manner using the CIDR.

Consistent with our previous study (Faris et al., 2003), serum P<sub>4</sub> concentrations were higher ( $P < 0.001$ ) in multiparous ewes receiving therapy than controls (Table 1). However, no treatment x time interaction ( $P = 0.41$ ) was detected. All P<sub>4</sub> concentrations became similar ( $P = 0.80$ ) after therapy was discontinued for all ewes.

Nulliparous ewes experienced a treatment x time interaction ( $P < 0.001$ ; Table 2). Ewes beginning therapy at Time 4 had a higher ( $P < 0.05$ ) P<sub>4</sub> concentration on d 6, 10, 16, and 18 when compared to controls. Additionally, concentrations were higher ( $P < 0.05$ ) than other treatments for d 6, 16, and 18. Naturally, a difference would be expected on d 6 as these are the only ewes undergoing therapy. However, we do not have an explanation for the difference occurring on d 16 and 18. Ewes receiving therapy at Time 6 had a higher ( $P < 0.05$ ) P<sub>4</sub> concentration than other treatments on d 10. Progesterone concentrations were similar ( $P > 0.80$ ) on d 8 and 12 for these two groups. A larger group of ewes may help determine what some of the treatment effects may be since we did not see an increased P<sub>4</sub> concentration for nulliparous ewes receiving therapy.

Table 1. Least squares means of serum progesterone concentrations (ng/mL) over time for multiparous black-faced ewes.<sup>a</sup>

Day <sup>c</sup>	Treatment <sup>b</sup>				SE <sup>d</sup>
	Control	Time 4	Time 6	Time 8	
4	2.4	2.9	3.0	3.3	0.9
6	4.3	6.6	4.7	5.4	0.8
8	4.9	7.9	8.2	6.6	0.7
10	5.7	7.4	8.4	9.3	0.9
12	7.3	8.6	8.5	8.8	0.6
14	6.5	8.6	8.5	8.9	0.6
16	6.7	8.1	9.4	9.0	1.0
18	7.0	9.5	8.2	9.1	0.9
20	6.1	6.5	6.6	6.4	0.5
Avg	5.6 <sup>e</sup>	7.4 <sup>f</sup>	7.3 <sup>f</sup>	7.4 <sup>f</sup>	0.4

<sup>a</sup> Data were analyzed as a split-plot design. No treatment x time interaction ( $P = 0.41$ ) was detected. Therefore, only overall treatment effects were compared..

<sup>b</sup> Progesterone treatments: Control (n = 14), Time 4 (n = 20), Time 6 (n = 20), and Time 8 (n = 19) represent time progesterone therapy was initiated.

<sup>c</sup>Day 0 = mating and all samples were collected at 0600 h.

<sup>d</sup>Most conservative standard error reported (n=126; 14 ewes and 9 samples).

<sup>e,f</sup>Row means with different superscripts differ ( $P < 0.05$ ).

Table 2. Least squares means of serum progesterone concentrations (ng/mL) over time for nulliparous black-faced ewes.<sup>a</sup>

Day <sup>c</sup>	Treatment <sup>b</sup>				SE <sup>d</sup>
	Control	Time 4	Time 6	Time 8	
4	2.5 <sup>e</sup>	3.0 <sup>e</sup>	2.6 <sup>e</sup>	1.6 <sup>f</sup>	0.3
6	4.6 <sup>e</sup>	10.0 <sup>f</sup>	4.9 <sup>e</sup>	4.3 <sup>e</sup>	0.8
8	6.0 <sup>e</sup>	8.5 <sup>e,f</sup>	11.9 <sup>f</sup>	7.2 <sup>e</sup>	1.6
10	5.8 <sup>e</sup>	9.3 <sup>e</sup>	12.2 <sup>f</sup>	9.1 <sup>e</sup>	1.9
12	6.7 <sup>e</sup>	10.2 <sup>f,g</sup>	10.0 <sup>f,g</sup>	9.1 <sup>e,g</sup>	1.0
14	8.4	11.4	9.3	10.4	1.3
16	7.8 <sup>e</sup>	13.7 <sup>f</sup>	9.2 <sup>e</sup>	9.5 <sup>e</sup>	1.2
18	7.8 <sup>e</sup>	12.4 <sup>f</sup>	8.4 <sup>e</sup>	9.5 <sup>e</sup>	1.4
20	6.8	8.3	6.5	6.9	1.0

<sup>a</sup> Data were analyzed as a split-plot design. A treatment x time interaction ( $P < 0.001$ ) was detected. Therefore, only the interactive means were compared..

<sup>b</sup> Progesterone treatments: Control (n = 4), Time 4 (n = 4), Time 6 (n = 3), and Time 8 (n = 4) represent time progesterone therapy was initiated.

<sup>c</sup>Day 0 = mating and all samples were collected at 0600 h.

<sup>d</sup>Most conservative standard error reported (n=27, 3 ewes and 9 samples).

<sup>e,f,g</sup>Row means with different superscripts differ ( $P < 0.05$ ).

Ovulation rates did not differ among treatments for multiparous and nulliparous ewes ( $P = 0.75$  and  $0.68$ , respectively). In terms of ovulation rates and lambs born, P<sub>4</sub> therapy had no effect ( $P > 0.50$ ) on reproductive wastage in multiparous or nulliparous ewes.

The hypothesis, in our previous experiment (Faris et al., 2003), was to supplement the uterus starting on d 2 (24 h post estrus) with P<sub>4</sub> until the CL became fully functional. Surprisingly, ewes receiving P<sub>4</sub> therapy had a lower incidence ( $P = 0.06$ ) of multiple births than controls. However, in the current study, we did not observe change in embryonic survival or lambing rates. Additionally, the number of ewes failing to establish a pregnancy was similar among P<sub>4</sub> groups. These results are consistent with results of other studies (Diskin and Sreenan, 1986; Diskin, 1987; Diskin and Niswender, 1989), using highly fertile dairy cattle, in which no effect of P<sub>4</sub> supplementation on embryonic survival was observed. However, in heifers with lower fertility, embryonic survival rate was increased by P<sub>4</sub> supplementation. The present data from nulliparous ewes contradicts these findings in that nulliparous ewes did not respond to P<sub>4</sub> therapy. Therefore, luteal insufficiency does not appear to be a cause of embryonic mortality in highly fertile ewes. Embryonic survival rates for multiparous ewes (Control, Time 4, 6, and 8) were 65, 88, 68, and 75%, respectively. Embryonic survival rates for nulliparous ewes (Control, d 4, d 6, and d 8) were 78, 63, 71, 86%, respectively. In summary, P<sub>4</sub> therapy initiated on d 4, 6, and 8 post estrus has no effect on embryonic survival or pregnancy rate in multi- and nulliparous ewes when compared to controls.

### Implications

Future studies will be conducted using a modified super ovulation program in order to determine if P<sub>4</sub> supplementation can increase embryonic survival in ewes. Additionally, progesterone and nutritional effects will be further investigated to determine how reproductive wastage can be reduced.

### Literature Cited

- Ashworth, C. J., D. I. Sales, and I. Wilmut. 1989. Evidence of an association between the survival of embryos and the periovulatory plasma progesterone concentration in the ewe. *J. Reprod. Fertil.* 87:23.
- Diskin, M. G. 1987. Studies related to embryonic mortality in the cow. Ph.D. Diss. National Univ. of Ireland, Dublin.
- Diskin, M. G., and G. D. Niswender. 1989. Effect of progesterone supplementation on pregnancy and embryo survival in ewes. *J. Anim. Sci.* 67:1559-1563.
- Diskin, M. G., and J. M. Sreenan. 1986. Progesterone and embryo survival in the cows. In: J. M. Sreenan and M. G. Diskin (Ed.) *Embryonic Mortality in Farm Animals*. pp. 142-158. Martinus Nijhoff for CEC.
- Edey, T. N. 1969. Prenatal mortality in sheep: A review. *Anim. Breed. Abstr.* 2:173.
- Faris, B. R., J. E. Otero, T. T. Ross, A. S. Carman, R. W. Montgomery, L. A. Terrazas, and D. M. Hallford. 2003. Effects of nutrition and progesterone therapy on ovulation, embryonic survival, and pregnancy rates in ewes. *Proc., W. Sect., Amer. Soc. Anim. Sci.* 54:247-250.
- Parr, R. A. 1992. Nutrition-progesterone interactions during early pregnancy in sheep. *Reprod. Fertil. Dev.* 4:297.
- Parr, R. A., I. F. Davis, R. J. Fairclough, and M. A. Moss. 1987. Overfeeding during early pregnancy reduces peripheral progesterone concentration and pregnancy rate in sheep. *J. Reprod. Fertil.* 80:317.
- Thomas, D. L., J. L. Goodyear, A. R. Cobb, J. M. Stookey, and P. J. Dzuik. 1984. Ovulation rate of ewes provided supplemental grain, Phenobarbital or mineral oil prior to estrus. *J. Anim. Sci.* 59(Suppl.1):118(Abstr.).

## BODY TEMPERATURE AND HORMONAL RESPONSES OF SHEEP UNDERGOING RUMEN CANNULATION

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**ABSTRACT:** Stress associated with surgery in humans results in physiological changes (hypothermia, hyperglycemia) that may be factors in development of infections. Many large animal studies require surgery to prepare animals for data collection. This study evaluated animal responses to a surgical procedure to determine if similar changes occur in ruminants. Rumen cannulations were performed on three Rambouillet wethers (8 mo, 53 kg) under general anesthesia (ketamine/xylazine). Rectal temperature (RT) measurements and serum were collected 24 h before surgery, and values from this period served as controls for subsequent samples. Additional RT and samples were collected during preoperative procedures (preop, clipping, washing, etc.), at the time of skin incision, at the beginning of muscle dissection, at the end of surgery, and at 0.5, 2.5, 5, and 24 h after surgery. Serum was analyzed for glucose, insulin, and cortisol. The RT was 38.8 °C 24 h before surgery and remained constant ( $P > 0.10$ ) through the end of the procedure. At 30 min after surgery, RT ( $37.1 \pm 0.2$  °C) was lower ( $P < 0.01$ ) than the pre-surgery value. At 5 ( $39.6 \pm 0.2$  °C) and 24 ( $39.2 \pm 0.2$  °C) h after the cannulations were completed, RT tended ( $P < 0.10$ ) to be greater than pre-surgery values. Serum glucose was greater ( $P < 0.05$ ) at all times through 2.5 h (87 mg/dL) after surgery than at 24 h before the procedure began (60 mg/dL). The highest glucose value was observed at the time the surgery ended ( $123 \pm 8$  mg/dL,  $P < 0.01$ ). Although serum insulin did not differ ( $P > 0.05$ ) after surgery compared with the 24-h pre-treatment sample ( $2.4 \pm 0.2$  ng/mL), values were numerically greater after surgery and tended ( $P = 0.09$ ) to be elevated at 5 h post surgery ( $3.2 \pm 0.2$  ng/mL). Serum cortisol was 17 ng/mL 1 d before surgery compared with  $38 \pm 5$  ng/mL when the skin was incised ( $P = 0.05$ ) and  $75 \pm 7$  ng/mL 24 h after surgery ended ( $P < 0.01$ ). Surgery resulted in transient hypothermia followed by a rise in body temperature along with hyperglycemia without a corresponding rise in serum insulin.

Key words: Stress, Surgery, Health

### Introduction

Nosocomial infections (infections not present prior to hospitalization, Eggimann and Pittet, 2001) are relatively common in human medicine and at least one third can be prevented by maintaining normothermia and controlling acute hyperglycemia (Burke, 2003). Anesthetics tend to inhibit thermoregulation thus making temperature of anesthetized patients dependent on the environment (Sessler, 1997). If the body temperature deviates substantially from normal, metabolic functions generally

deteriorate (Sessler, 1997). Immune functions such as phagocytosis, chemotaxis, and production of antibodies are impaired by mild hypothermia (Kurz, 1996). This impairment of the immune system due to thermoregulatory vasoconstriction lowers resistance to infection. Stress as well as preoperative fasting associated with surgical procedures can easily induce acute hyperglycemia. The immune system can be affected by hyperglycemia. Kwoun et al. (1997) found that hyperglycemia decreased the respiratory burst of alveolar macrophages. In contrast, hyperglycemia enhanced phagocytosis in alveolar macrophages of rats infused with 30% dextrose to maintain plasma glucose concentrations of 300 mg/dL (Kwoun et al., 1997). The primary antimicrobial agent in phagocytes is superoxide radical and superoxide production may be impaired by high glucose concentrations (Perner et al., 2003). Surgical stress enhances the production of catabolic hormones which stimulate gluconeogenesis (Schricker, 2001). In addition to hyperglycemia, insulin resistance may also be observed. Insulin resistance results in decreased glucose uptake by peripheral tissues, primarily muscle and fat (Yuchen et al., 2003). The objective of our study was to determine if the effects of surgical stress observed in humans also occur in ruminants.

### Materials and Methods

#### Animals

Three Rambouillet wethers (8 mo, 53 kg) undergoing surgical insertion of rumen cannulae were observed for surgical stress associated with hypothermia and hyperglycemia. All procedures were carried out in accordance with the guidelines set forth in the New Mexico State University Guidelines for Animal Surgery in Research and Teaching and were approved by the Institutional Animal Care and Use Committee.

#### Surgical Procedure

Sheep were fasted for a period of 18 h before surgery. On d 0 (day of surgery), wethers were premedicated with 91 mg ketamine (Vedco Inc., St. Joseph, MO) and 9 mg xylazine (Lloyd Labs, Shenandoah, IA) i.v. and prepared for surgery by shearing and cleansing the surgical site in the left paralumbar fossa. After preparation, animals were transferred to the surgery room, positioned in lateral recumbency with their right side down, and fitted with an indwelling jugular catheter set to deliver physiological saline at a rate of 25 mL/min. The left paralumbar fossa as well as the circular area where the cannula was to be placed was injected with 20 mL of 2% lidocaine (Vedco Inc., St. Joseph, MO; both s.c. and i.m.). During the 20 to 30 min procedure, wethers received an additional 100 to 200 mg

ketamine (i.v.) as needed. The skin was removed from the circular area and blunt dissection of the underlying muscle layers was performed. The peritoneum was then punctured and the rumen was anchored to the skin with numerous mattress sutures (Braunamid, Jorgensen Labs, Loveland, CO). The protruding rumen wall was then excised and the cannula (Bar Diamond, Parma, ID, number 8C) was inserted. Post operative care included cleaning with antimicrobial disinfectants (daily for 7 d) and administration of penicillin (300,000 units/d for 3 d, s.c.).

### Sampling

Body temperature measurements and serum samples were collected during the experiment. Temperature was obtained via rectal thermometry. Blood samples were collected by jugular venipuncture into 10-mL vacuum tubes (Corvac, Kendall, St. Louis, MO). Rectal temperature measurements and serum were collected 24 h before surgery, and values from this period served as controls for subsequent samples. Additional rectal temperature measurements and serum samples were collected during preoperative procedures (pre-op; clipping, washing, etc.), at the time of skin incision, at the beginning of muscle dissection, at the end of surgery, and at 0.5, 2.5, 5, and 24 h after surgery (post-op). Blood was allowed to clot at room temperature for 30 min after which serum was separated by centrifugation at 1500 x g and stored frozen until analyzed.

### Analytical and Statistical Procedures

All serum samples were analyzed for glucose by the glucose oxidase method (Sigma, St. Louis, MO). In addition, serum insulin (Reimers et al., 1982) and cortisol were quantified by RIA using components from commercial kits produced by Diagnostic Products Corp. (Los Angeles, CA). Both hormones were determined in single assays having within assay coefficients of variation of 12% or less. Rectal temperature and serum glucose, insulin, and cortisol were analyzed by analysis of variance in which values at each time period were compared to values obtained 24 h before surgical preparation was initiated. Analyses were computed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC).

### Results and Discussion

Body temperature 24 h before surgery was 38.8°C and remained relatively constant ( $P > 0.10$ ) through the end of the surgical procedure (Figure 1). At the end of surgery, rectal temperature ( $37.1 \pm 0.2^\circ\text{C}$ ) was lower ( $P < 0.01$ ) than 24 h before surgery. Temperature then rose above the value observed 24 h prior to surgery reaching a peak ( $39.6 \pm 0.2^\circ\text{C}$ ,  $P = 0.09$ ) 5 h after the surgery. Twenty-four hours after surgery ended, rectal temperature ( $39.2^\circ\text{C}$ ,  $P = 0.07$ ) remained elevated above the pre-surgery value. The fall in body temperature to  $37.1^\circ\text{C}$  demonstrates possible hypothermia; however, the rise in body temperature to  $39.7^\circ\text{C}$  may indicate the response of the immune system to possible surgical infection.

Serum glucose 24 h pre-op measured  $60 \pm 4$  mg/dL (Figure 1). After the 18-h fast, the glucose concentration rose to 75

$\pm 4$  mg/dL ( $P = 0.05$ ). Serum glucose concentration continued to increase resulting in values of  $119 \pm 4$ ,  $121 \pm 6$ , and  $123 \pm 7$  mg/dL at the time of first incision, muscle dissection, and the end of surgery, respectively (vs 24 h pre-op value,  $P < 0.01$ ). At 0.5 and 2.5 h after completion of the procedure, serum glucose declined to  $119 \pm 10$  and  $87 \pm 6$  mg/dL but values were still greater ( $P < 0.05$ ) than the 24 h pre-op value. Five ( $69 \pm 5$  mg/dL) and 24 ( $60 \pm 4$  mg/dL) h after surgery, serum glucose concentration was similar ( $P > 0.20$ ) to the 24 h pre-op value (60 mg/dL). The rise in serum glucose concentration suggests hyperglycemia induced by surgical stress.

The 24-h pre-op serum insulin value was  $2.4 \pm 0.2$  ng/mL (Figure 1). This value remained relatively constant ( $P > 0.20$ ) during and for 2.5 h after surgery. However, serum insulin tended to be greater ( $P = 0.09$ ) at 5 h post-op ( $3.2 \pm 0.2$  ng/mL) than the 24-h pre-op value. Examination of the serum insulin profile depicted in Figure 1 suggests a rising pattern for insulin after completion of the surgery. This rise in insulin would be expected in response to the elevation in serum glucose.

Serum cortisol concentration was  $17 \pm 11$  ng/mL 24 h before surgery began compared with  $38.0 \pm 5$  ng/mL at the first incision ( $P = 0.05$ ). As shown in Figure 1, cortisol then declined until the end of surgery and then began a gradual increase during the post-op period to a peak value of  $93 \pm 12$  ng/mL ( $P = 0.02$ ) at 5 h after surgery ended. The increase in serum concentration of cortisol after the end of surgery likely indicates surgical stress to the animal and is the probable cause of the elevated glucose discussed previously. In addition, the elevated cortisol may have attenuated the expected insulin rise in response to the elevated serum glucose.

The incidence of nosocomial infections in humans due to physiological changes induced by stress associated with surgery and anesthesia is a major issue (Burke, 2003). There is little research of such complications and changes in ruminants, even though many ruminants used in research undergo specific types of surgeries to prepare them for data collection. In this study, the wethers experienced a change in body temperature of  $2^\circ\text{C}$  below normal thereby confirming hypothermia. The entire surgical procedure took only about 20 to 30 min; however, this time appeared sufficient to influence thermoregulation. The elevation in serum glucose concentration observed in this study demonstrates acute hyperglycemia induced from surgical fasting and/or stress. Metabolism can also be altered by fasting and may stimulate glucose production and impair glucose utilization resulting in hyperglycemia. Surgical stress enhances production of catabolic hormones such as cortisol which stimulate gluconeogenesis (Schricker, 2001). Insulin is the primary suppressor of hepatic glucose output as well as hepatic gluconeogenesis. Therefore under insulin resistance, the liver can increase hepatic glucose output thus contributing to hyperglycemia. It is possible that the stress induced elevation in cortisol may have resulted in insulin insensitivity.

## Implications

We hypothesized that ruminants experience physiological changes in response to surgery and anesthesia similar to those observed in human patients. Data reported herein confirm that wethers develop hypothermia and hyperglycemia during and shortly after surgery. These similarities suggest that the increased incidence of wound infections observed in humans could also occur in ruminants thus delaying recovery time. It is possible that similar precautions taken in humans could be beneficial to research animals such as maintaining normothermia and preventing hyperglycemia. Finally, these findings may have implications for dealing with other forms of stress especially those associated with shipping and the receiving period for feedlot animals.

## Acknowledgments

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## Literature Cited

Burke, J.P. 2003. Infection control: A problem for patient safety. *N. Engl. J. Med.* 348:651-656.

Eggimann, P., and D. Pittet. 2001. Infection control in ICU. *Chest* 120:2059-2093.

Kurz, A., D.I. Sessler, and R. Lenhardt. 1996. Perioperative normothermia to reduce the incidence of surgical-wound infection and shorten hospitalization. *N. Engl. J. Med.* 334:1209-1215.

Kwoun, M.O, P.R. Ling, E. Lydon, A. Imrich, Z. Qu, J. Palombo, and B.R. Bistrian. 1997. Immunologic effects of acute hyperglycemia in nondiabetic rats. *J. Parent. Enter. Nutr.* 21:91-95.

Perner, A., S.E. Neilsen, and J. Rask-Madsen. 2003. High glucose impairs superoxide production from isolated blood neutrophils. *Intens. Care Med.* March 2003 (epub ahead of print).

Schricker, T. 2001. The catabolic response to surgery: How can it be modified by the anesthesiologist? *Can. J. Anesth.* 48(6):R1-R5.

Sessler, D.I. 1997. Mild perioperative hypothermia. *N. Engl. J. Med.* 336:1730-1737.

Yuchen, M., P. Wang, J.F. Kuebler, I.H. Chaudry, and J.L. Messina. 2003. Hemorrhage induces the rapid development of hepatic insulin resistance. *AJP-Gastrointest. Liver Physiol.* 284:G107-G115.

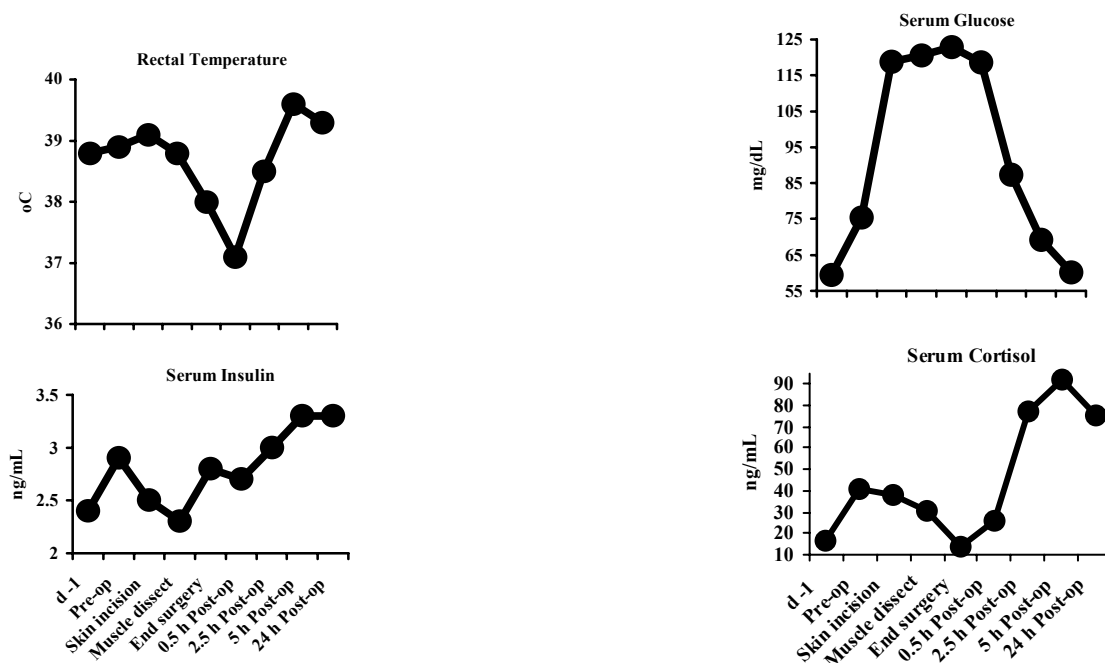


Figure 1. Rectal temperature and serum glucose, insulin, and cortisol in wethers before, during, and after surgical insertion of ruminal cannulae. Temperature measurements and serum samples were collected 24 h before and at various times during and after the surgical procedure. See text for statistical comparisons between the d-1 values and values at other times during and after surgery.

# RELATIVE AMOUNTS OF mRNA ENCODING ORNITHINE DECARBOXYLASE AND SPERMIDINE/SPERMINE N1-ACETYLTRANSFERASE IN THE BOVINE CORPUS LUTEUM

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**ABSTRACT:** Polyamines (PA) are low molecular weight, positively charged, organic molecules required for cell growth and differentiation. Concentrations of PA are highly regulated and change rapidly in response to a wide variety of external stimuli. The rate-limiting enzymes in the PA biosynthetic and catabolic pathways are ornithine decarboxylase (ODC) and spermidine/spermine N1-acetyltransferase (SSAT), respectively. The objective of this study was to evaluate changes in mRNA encoding ODC and SSAT in the bovine corpus luteum (CL) during the early and mid-luteal stages, and in response to PGF<sub>2α</sub>. Bovine CL were collected via ovariectomy during early-luteal (d 5; early; n = 4; d 0 = estrus), mid-luteal (d 12; mid; n = 6), mid-luteal, 6 h after prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) injection on d 12 (mid-6; n = 5), and mid-luteal, 24 h after PGF<sub>2α</sub> injection on d 12 (mid-24; n = 5). Total RNA was extracted from each CL and amplified in duplicate with real-time RT-PCR to determine relative amounts of mRNA encoding ODC and SSAT. Early CL had greater amounts ( $P < 0.01$ ) of mRNA encoding ODC than those from mid-luteal cows ( $7.0 \times 10^4$  vs  $1.9 \times 10^4 \pm 5.1 \times 10^3$  arbitrary units, early and mid, respectively) or than mid-luteal CL following PGF<sub>2α</sub> ( $2.4 \times 10^4$ , and  $1.7 \times 10^4$  arbitrary units, mid-6 and mid-24, respectively). Mid CL did not differ ( $P = 0.46$ ) in mRNA encoding ODC when compared to mid CL collected 6 and 12 h after PGF<sub>2α</sub>. No differences were observed in mRNA encoding SSAT in early vs mid CL ( $P = 0.70$ ) or mid CL following PGF<sub>2α</sub> treatment ( $P = 0.39$ ). Differences were also not observed in amounts of mRNA encoding SSAT in mid vs mid-6 and mid-24 CL ( $P > 0.38$ ). During the early-luteal phase of the bovine CL, a period of rapid luteal cell growth and differentiation, relative amounts of mRNA encoding ODC were markedly elevated compared to the mid-luteal phase and during PGF<sub>2α</sub>-induced luteolysis when cell growth and differentiation are stabilized and/or declining. These data suggest that PA synthesis may be altered during the luteal phase by changes in mRNA encoding the rate-limiting enzyme of the PA biosynthetic pathway, ODC.

**Key Words:** Corpus Luteum, Ornithine Decarboxylase, Spermidine/Spermine N1-Acetyltransferase,

## Introduction

Polyamines (PA) are ubiquitously distributed molecules required for optimal cellular growth and differentiation (Tabor and Tabor, 1984; Pegg, 1988). The three most abundant PA in mammalian cells are putrescine, spermidine and spermine (Pegg and McCann, 1982).

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Polyamines alter a multitude of cellular functions including transcription, translation, hormone receptor-ligand binding, second messenger levels, and enzymatic activities (Tabor and Tabor, 1984; Thomas and Thomas, 2001). Polyamine metabolism and transport are intricately controlled processes, which suggest that they serve as regulatory molecules within cells. As a result, PA may serve purposes specific to ovarian function, including corpus luteum (CL) growth, maintenance, and luteolysis. Polyamines have been shown to influence steroidogenesis in granulosa cells (Veldhuis and Hammond, 1979) and are critical for synthesis and secretion of progesterone in the murine CL (Bastida et al., 2002). Moreover, PA may play a role in angiogenesis (Auvinen, 1997), a vital component in formation of the early CL (Redmer and Reynolds, 1996).

The rate-limiting enzymes in the PA biosynthetic and catabolic pathways are ornithine decarboxylase (ODC) and spermidine/spermine N1-acetyltransferase (SSAT), respectively (Figure 1). These enzymes have short half-lives and are highly inducible by a variety of stimuli (Scalabrino and Lorenzini, 1991; Pegg et al., 1994; Fogel-Petrovic et al., 1996; McCloskey et al., 1999). As key enzymes in synthesis and catabolism of PA, regulation of transcription, translation and/or activity of ODC and SSAT would provide a mechanism for the potential role of PA in luteal growth, differentiation and regression. Therefore, the present study was designed to determine the relative amounts of mRNA encoding bovine ODC and SSAT by real-time RT-PCR during the early and mid-luteal stages and in response to prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) during the mid-luteal stage.

## Materials and Methods

Cross-bred beef cows were synchronized with two injections of PGF<sub>2α</sub> (25 mg, i.m., Lutalyse®; Pharmacia & UpJohn Co., Kalamazoo, MI) 9 d apart and randomly assigned to one of four treatments. Corpora lutea were collected via ovariectomy during early-luteal (d 5; **early**; estrus = d 0; n = 4), mid-luteal (d 12; **mid**; n = 6), mid-luteal, 6 h after PGF<sub>2α</sub> injection on d 12 (**mid-6**; n = 5), and mid-luteal, 24 h after PGF<sub>2α</sub> injection on d 12 (**mid-24**; n = 5). Upon ovariectomy, CL were immediately snap-frozen in liquid nitrogen and stored at -80°C.

Total RNA was extracted from each CL using TriReagent® (Molecular Research Center, Cincinnati, OH), according to manufacturer instructions. Total RNA was amplified in duplicate by real-time RT-PCR to determine relative amounts of mRNA encoding ODC and SSAT and 18s rRNA. Oligonucleotide primers and TaqMan probes for ODC and SSAT were designed using the software Primer Express (Applied Biosystems, Foster City, CA). Real-time RT-PCR for ODC and SSAT was carried out in

96-well optical reaction plates using 1 µg total RNA in a total volume of 25 µL of TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA). All reactions occurred in an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA).

To correct for inherent variation, real-time RT-PCR was performed on 18s rRNA using 0.5 µg of total RNA for each sample amplified for ODC and SSAT mRNA. A standard curve was generated from pooled bovine CL total RNA to establish relative amounts mRNA encoding ODC and SSAT and 18s rRNA.

All relative levels of mRNA encoding were expressed as arbitrary units. Concentrations of ODC and SSAT mRNA and 18s rRNA-amplified product were analyzed by analysis of variance for a completely random design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). When differences were detected, pair-wise comparisons of least squares means were performed. No differences were detected in 18s rRNA co-amplified with ODC and SSAT mRNA ( $P > 0.05$ ). Therefore, no corrections were necessary and actual values for mRNA encoding ODC and SSAT are reported.

## Results and Discussion

Optimal cell growth and differentiation are dependent on the interactions between PA, nucleic acids, and proteins. Polyamines have been shown to alter a variety of cellular functions, including cell cycle progression, gene expression, and translation (Fredlund et al., 1995; Thomas et al., 1999; Iwata et al., 2000), in a tissue-specific manner (Seiler, 1990; Casero and Pegg, 1993). Inhibitors of PA and(or) alterations in PA concentrations block cell growth and induce apoptosis (Ha et al., 1997; Xie, et al., 1997; Ha et al., 1998). As a result, PA may serve purposes specific to ovarian function, including CL growth, maintenance, and luteolysis. Ornithine decarboxylase and SSAT, the rate limiting enzymes in PA biosynthesis and catabolism, respectively, have a short half-life and are tightly regulated by a variety of stimuli (Scalabrino and Lorenzini, 1991; Pegg et al., 1994; Fogel-Petrovic et al., 1996; McCloskey et al., 1999), thereby enabling cells to rapidly and sensitively control intracellular PA pools.

The objective of this study was to determine relative concentrations of mRNA encoding ODC and SSAT in early- and mid-luteal bovine CL and in response to PGF<sub>2α</sub> during the mid-luteal stage. Corpora lutea collected in the early-luteal stage had markedly greater amounts ( $P < 0.01$ ) of mRNA encoding ODC than those from mid-luteal cows or than mid-luteal CL collected following treatment of cows with PGF<sub>2α</sub> (mid-6 and mid-24, pooled; Figure 2). Relative amounts of mRNA encoding ODC were similar ( $P = 0.46$ ) in mid-luteal CL and mid-luteal CL collected after PGF<sub>2α</sub> injection (mid-6 and mid-24, pooled). In addition, no differences were observed ( $P = 0.57$ ) in mRNA encoding ODC in mid-luteal CL collected 6 h vs 12 h after PGF<sub>2α</sub> (Figure 2). These findings are in agreement with previous data from our lab, which indicate that concentrations of PA are greatest in the early ovine CL and decrease as the luteal phase progresses and in response to PGF<sub>2α</sub> (Raymond, 1996).

Degradation of PA occurs via the action of the rate-limiting enzyme of the PA catabolic pathway, SSAT. No differences were observed in mRNA encoding SSAT in early vs mid CL ( $P = 0.70$ ; Figure 3) nor in early CL compared to mid-luteal CL collected from PGF<sub>2α</sub>-treated cows (mid-6 and mid-24, pooled;  $P = 0.35$ ). Moreover, amounts of mRNA encoding SSAT were similar in mid CL compared to mid CL collected after PGF<sub>2α</sub> treatment ( $P = 0.39$ ) and in mid CL collected 6 h vs 12 h after cows were treated with PGF<sub>2α</sub> ( $P = 1.0$ ; Figure 3). These data suggest that alterations in the transcription of mRNA encoding SSAT may not be a regulatory mechanism in the PA pathway in the bovine CL. Therefore, enhanced transcription of mRNA encoding ODC, rather than decreased SSAT mRNA transcription, may lead to the increased PA observed previously in the ovine early CL (Raymond, 1996) when compared to the mid-luteal CL or the mid-luteal CL following treatment with PGF<sub>2α</sub>.

Collectively, these results and those reported by Raymond (1996) suggest that during the early-luteal phase, a period of rapid cell growth and differentiation, relative amounts of mRNA encoding ODC, as well as PA concentrations, are elevated. This increase in PA may contribute to angiogenesis (Auvinen, 1997), a vital component in formation of the early CL (Redmer and Reynolds, 1996), as well as early synthesis and secretion of progesterone (Velhuis and Hammond, 1979; Bastida et al., 2002). As the CL progresses through the mid-luteal phase and luteolysis in response to PGF<sub>2α</sub>, PA concentrations may decline due to decreased transcription of mRNA encoding ODC.

## Implications

Polyamines may play a role in the growth and differentiation of the early-luteal phase CL. Regulation of PA in the CL may occur via changes in amount of mRNA encoding the rate-limiting enzyme of the PA biosynthetic pathway, ODC.

## Literature Cited

- Auvinen, M. 1997. Cell transformation, invasion and angiogenesis: A regulatory role for ornithine decarboxylase and polyamines? J. Natl. Cancer Inst. 89:533-537.
- Bastida, C. M., F. Tejada, A. Cremades, and R. Penafiel. 2002. The preovulatory rise of ovarian ornithine decarboxylase is required for progesterone secretion by the corpus luteum. Biochem. Biophys. Res. Comm. 293:106-111.
- Casero, R. A., and A. E. Pegg. 1993. Spermidine/Spermine N1-acetyltransferase – the turning point in polyamine metabolism. FASEB J. 7:653-661.
- Fredlund, J. O., M. C. Johansson, E. Dahlberg, and S. M. Oredsson. 1995. Ornithine decarboxylase and S-adenosylmethionine decarboxylase expression during the cell cycle of Chinese hamster ovary cells. Exp. Cell Res. 216:86-92.
- Fogel-Petrovic, M., S. Vujcic, P. J. Brown, M. K. Haddox, and C. W. Porter. 1996. Effects of polyamines,

polyamine analogs, and inhibitors of protein synthesis on spermidine-spermine N1-acetyltransferase gene expression. *Biochemistry* 35: 14436-14444.

- Ha, H. C., P. M. Woster, and R. A. Casero, Jr. 1998. Unsymmetrically substituted polyamine analogue induces caspase-independent programmed cell death in Bcl-2-overexpressing cells. *Cancer Res.* 58:2711-2714.
- Ha, H. C., P. M. Woster, J. D. Yager, and R. A. Casero, Jr. 1997. The role of polyamine catabolism in polyamine analogue-induced programmed cell death. *Proc. Natl. Acad. Sci. USA* 94:11557-11562.
- Iwata, M., M. Izawa, N. Sasaki, Y. Nagumo, H. Sasabe, and Y. Hayashizaki. 2000. T7 RNA polymerase activation and improvement of the transcriptional sequencing by polyamines. *Bioorg. Med. Chem.* 8:2185-2194.
- McCloskey, D. E., C. S. Coleman, and A. E. Pegg. 1999. Properties and regulation of human spermidine/spermine N1-acetyltransferase stably expressed in Chinese hamster ovary cells. *J. Biol. Chem.* 274:6175-6182.
- Pegg, A. E. 1988. Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. *Cancer Res.* 48:759-774.
- Pegg, A. E., and P. P. McCann. 1982. Polyamine metabolism and function. *Am. J. Physiol.* 243:C212-C221.
- Pegg, A. E., L. M. Shantz, and C. S. Coleman. 1994. Ornithine decarboxylase: Structure, function and translational regulation. *Biochem. Soc. Trans.* 22:846-852.
- Raymond, S. 1996. Polyamines alter progesterone production by ovine mixed luteal cell cultures. Ph.D. Diss., New Mexico State University, Las Cruces.
- Redmer, D. A., and L. P. Reynolds. 1996. Angiogenesis in the ovary. *Rev. Reprod.* 1:182-192.
- Scalabrino, G., and E. C. Lorenzini. 1991. Polyamines and mammalian hormones. Part II: Paracrine signals and intracellular regulators. *Mol. Cell. Endocrinol.* 77:37-56.
- Seiler, N. 1990. Polyamine metabolism. *Digestion* 46:319-330.
- Tabor, C. W., and H. Tabor. 1984. Polyamines. *Ann. Rev. Biochem.* 53:749-790.
- Thomas, T., S. Balabhadrapathruni, C. R. Gardner, J. Hong, C. A. Faaland, and T. J. Thomas. 1999. Effects of epidermal growth factor on MDA-MB-468 breast cancer cells: Alterations in polyamine biosynthesis and expression of p21/CIP1/WAF1. *J. Cell. Physiol.* 179:257-266.
- Thomas, T., and T. J. Thomas. 2001. Polyamines in cell growth and cell death: Molecular mechanisms and therapeutic applications. *Cell. Mol. Life. Sci.* 58:244-258.
- Veldhuis, J. D., and J. M. Hammond. 1979. Role of ornithine decarboxylase in granulosa-cell replication and steroidogenesis in vitro. *Biochem. Biophys. Res. Comm.* 91:770-777.
- Xie, X., M. E. Tome, and E. W. Gerner. 1997. Loss of intracellular putrescine pool-size regulation induces apoptosis. *Exp. Cell Res.* 230:386-392.

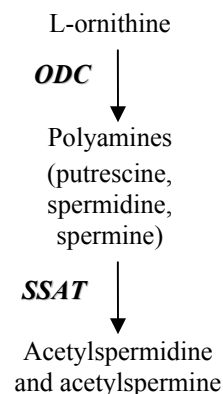


Figure 1. The rate-limiting steps in polyamine biosynthesis and catabolism are catalyzed by ornithine decarboxylase (ODC) and spermidine/spermine N1-acetyltransferase (SSAT), respectively.

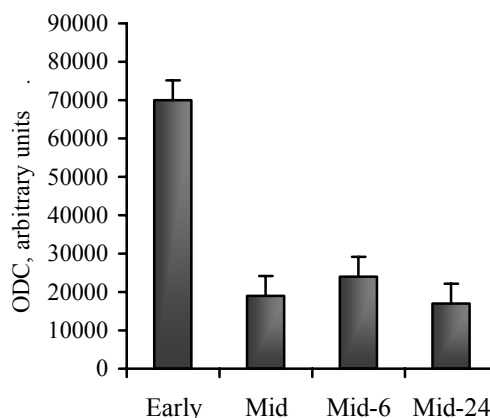


Figure 2. Relative levels of mRNA encoding ODC during the early-luteal stage (d 5; estrus = d 0; n = 4) of the bovine CL were greater ( $P < 0.01$ ;  $SE = 5.1 \times 10^3$  arbitrary units) than those in mid-luteal CL (d 12; n = 6) and in mid-luteal CL following treatment with  $PGF_{2\alpha}$  (mid-6, n = 5, and mid-24, n = 5). No differences were observed in mRNA encoding ODC in mid CL when compared to all CL collected following  $PGF_{2\alpha}$  treatment (mid-6 and mid-24 pooled;  $P = 0.46$ ) nor in mid CL collected 6 hr after  $PGF_{2\alpha}$  compared to 24 hr after  $PGF_{2\alpha}$  ( $P = 0.57$ ).

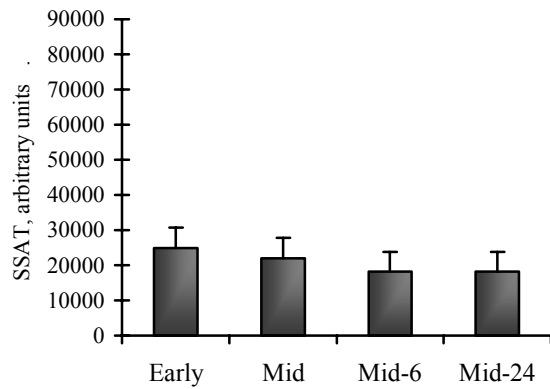


Figure 3. Relative levels of mRNA encoding SSAT during early luteal stage (d 5, early,  $n = 4$ ), mid-luteal (d 12; mid;  $n = 6$ ), mid-luteal, 6 h after  $\text{PGF}_{2\alpha}$  injection on d 12 (mid-6;  $n = 5$ ), and mid-luteal, 24 h after  $\text{PGF}_{2\alpha}$  injection on d 12 (mid-24;  $n = 5$ ) from the bovine CL (estrus = d 0). No differences were observed among early vs mid ( $P = 0.70$ ;  $\text{SE} = 5.8 \times 10^3$  arbitrary units) or early vs CL collected following treatment with  $\text{PGF}_{2\alpha}$  (mid-6 and mid-24 pooled;  $P = 0.35$ ). In addition, no differences were observed in mRNA encoding SSAT in mid CL when compared to CL collected following  $\text{PGF}_{2\alpha}$  treatment (mid-6 and mid-24 pooled;  $P = 0.39$ ) nor in mid CL collected 6 hr after  $\text{PGF}_{2\alpha}$  compared to 24 hr after  $\text{PGF}_{2\alpha}$  ( $P = 1.0$ ).

## COLOSTRUM IMMUNOGLOBULINS TRANSFERENCE IN HOLSTEIN CATTLE ACCORDING THE AGE OF THE DAM

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**ABSTRACTS:** The objective of the present study was to determine the effect of the age of the cow on colostral immunoglobulin levels (CIL) of Holstein cows and colostral immunoglobulins transference (CIT) to Holstein calves. Sixty-eight Holstein cows calved in a dairy herd in the desert region of Baja California, Mexico, were grouped according their lactation number in first (n=7); second (n=21); third (n=18), and fourth or more lactations (n= 22). The CIL levels were measured using a colostrometer during the first four milkings postpartum. Blood samples were collected from the jugular vein of calves at birth, 24 and 48 h after partum in order to measure CIT using the ELISA procedure. Calves consumed 2 L of colostrum at 6 and 12 h after birth and then 4 L of whole milk daily until 60 d of age. Calf starter was offered to all calves from the first week of age. Statistical analysis was performed using linear models through analysis of variance. Cows of first (103.43 ± 9.8 mg/ml), second (87.86 ± 5.7 mg/ml) and third lactation (98.05 ± 6.12 mg/ml) lactation in the first milking postpartum had higher (P<.05) CIL than cows of four or more lactations (78.64 ± 5.33 mg/ml). In the fourth milking postpartum, cows of first lactation had higher (P<.05) CIL (22.86 ± 4.32 mg/ml) than cows of second (8.10 ± 2.5 mg/ml), third (11.94 ± 2.69 mg/ml), and fourth or more lactations (10.0 ± 2.49 mg/ml). Age of the dam did not (P>.05) increases as age of the cow increases, but colostrum immunoglobulins transference did not differ according to age of cow.

Key Words: Holstein calves, Colostrum quality, Mexico.

### INTRODUCTION

Transference of passive immunity through colostrums is essential for the calf health during the first weeks of age (Perino y Wittum, 1995). Recently born calves present the condition known as agamaglobulinemic as a normal condition (Tizard, 1992). Some studies have determined the relationship among age of the calf measured in hours at the moment when colostrum is taken, CIL concentration, colostrum consumed and the efficiency of the CIT in calves (Besser et al., 1991). The time between partum and the moment of first colostrum consumption is very important since the mechanism of pinocytosis through which the rumen mucous is able to absorb the CIL

diminishes gradually until it almost disappears at 36 h of age (Besser et al., 1991). Quality of colostrums has a strong relationship with CIL concentration, because the more CIL concentration the more quality of colostrum (Fleenor and Stott 1980). The most abundant CIL in the rumen colostrum are the Ig G, followed by Ig A and Ig M (Tizard, 1992). There is a relationship between colostrum density and the concentration of immunoglobulins, so that density is an indicative of quality of colostrum (Fleenor and Stott 1980). Among the causes producing a reduction of immunoglobulins concentration in colostrum are: mastitis, number of milking after partum, lactation number of the cow, infections in the cow, metabolic diseases, poor nutrition and season of the year (Fleenor and Stott 1980; Medina, 1994; Perino and Wittum 1995). In cows, the total amount of colostrum produced and the concentration of colostrum immunoglobulins raise with the lactation number. Therefore, calves from first calve cows will get less immunoglobulins than calves birth from mature cows (Kruse, 1970; Oyeniyi and Hunter, 1978; Stott et al., 1981). The objective of the present study was to evaluate the concentration of immunoglobulins in colostrum in order to determine the CIT to Holstein calves during the first 48 h of age and to determine the protein concentration in colostrum and blood of calves.

### Material and Methods

The study was carried out at the Experimental Dairy Herd Unit of the Instituto de Ciencias Agrícolas of the UABC, located at the Ejido Nuevo Leon, 42 km SW of the city of Mexicali. Sixty-eight cows from august, 2001 to February, 2002 were used. Animals were assigned to 4 groups according to their lactation number: first (n=7); second (n=21); third (n=18), and fourth or more lactations (n=22). Quality of colostral immunoglobulins was measured using a colostrometer<sup>®</sup> during the first four milkings after partum, and the protein concentration was evaluated from the first to the fourth milking after partum through Dumas Method (Leco FP-528). Calves were assigned to the group correspondent to their respective dam. Blood samples were collected from the jugular vein of calves at birth, 24 and 48 h after partum in order to measure CIT using the ELISA procedure and the protein concentration was evaluated using the Dumas Method (Leco FP-528). All

calves were given 2 L of colostrum from their dam during the first 6 to 12 h after birth. From the second day postpartum, calves were given 4 L of whole milk divided in two portions of 2 L (am and pm) until they reach 60 d of age. Calf starter (18% of PC) and water were given from the first week of age. The statistical analysis was performed using the GLM (General Linear Models) procedure of the SAS system (1991).

## Results and Discussion

Table 1 shows the concentration of immunoglobulins in colostrum during the first milkings according to the age of the cow. Immunoglobulins concentration was higher ( $P < .05$ ) in cows of first, second and third lactation at first milking ( $103.43 \pm 9.80$ ;  $87.86 \pm 5.70$ ; and  $98.05 \pm 6.12$  mg/ml respectively) compared with cows of four or more lactations ( $78.64 \pm 5.53$  mg/ml). In the third milking, CIL of cows of first and third lactation ( $37.86 \pm 5.57$  and  $28.33 \pm 3.58$  mg/ml respectively) was higher ( $P < .05$ ) than cows of second and fourth or more lactations ( $24.29 \pm 3.32$  and  $23.57 \pm 3.32$  mg/ml respectively). In the fourth milking, the CIL was higher ( $P < .05$ ) in cows of first lactation ( $22.86 \pm 4.32$  mg/ml) than cows in second, third and fourth or more lactations ( $8.10 \pm 2.50$ ;  $11.94 \pm 2.69$  and  $10.00 \pm 2.49$  mg/ml respectively). Protein percent did not differ ( $P > .05$ ) among lactation number. In the present study, the CIL in colostrum of first lactation cows was higher than cows of more lactations, results that are contradictory to the reported in the literature, where the CIL rises with the number of lactations (Devery-Pocius and Larson, 1993; Donovan et al., 1986). Results obtained in cows of first lactation could be due to climatic factors more than physiologic factors, because calvings occurred when the weather conditions of the Mexicali Valley were more comfortable for the animals (fall and winter seasons). Table 2 shows CIT in calf's blood according to the age of cow. There were no significant differences ( $P > .05$ ) among lactation number; average CIT was of  $8.27 \pm 0.67$ ;  $7.42 \pm 0.41$ ;  $8.04 \pm 0.37$  y  $8.05 \pm 0.36$  mg/ml, for first, second, third and fourth or more lactations, respectively. The percentage of protein in blood at birth was also similar ( $P > .05$ ) at 24 to 48 h of age, and there were no significant differences ( $P > .05$ ) at any lactation number. In this study, the range of CIT levels is considered low (7.42 to 8.27 mg/ml), because other studies consider concentrations below 10 mg/ml of CIT as presence of failures on the transference of passive immunity (ITF) and this may be a cause of high morbidity and mortality in calves (Besser et al., 1991). However, there is a controversy on the risk level of ITF associated to low levels of CIL (Pijoan, 1997), situation that support the results of the present study since the calves used in this study did not show health problems.

## Implications

CIL, CIT, and percent of protein in blood did not vary according to the age of cow, and they were higher in cows of first lactation. The quality of colostrum diminishes at the same time the number of milking raises.

## Literature cited

- Besser, T. E., C. C. Gay and C. P. Lori. 1991. Comparison of three methods of feeding colostrums to dairy calves. JAVMA. 198:3 419-422.
- Devery-Pocius, J.E. and B. L. Larson. 1983. Age and Previous Lactations as Factors in the Amount of Bovine Colostral Immunoglobulins. J. Dairy Sci. 66:221-226.
- Donovan G. A., L. Badinga., R. J. Collier., C. J. Wilcox and R. K. Braun. 1986 Factors Influencing Passive Transfer in Dairy Calves. J. Dairy Sci. 69:754-759.
- Fleenor, W. A and G. H. Stott. 1980. Hydrometer test for estimation of immunoglobulin concentration in bovine colostrums. J. Dairy. Sci. 63:973-977.
- Kruse, V: 1970. Yield of colostrums and immunoglobulin in cattle at the first milking after parturition. Animal Prod. 12:619-626.
- Medina, C. M. 1994. Medicina Productiva en la Crianza de Becerras Lecheras (1ra. Ed). Ed. LIMUSA. México, D. F.
- Oyenyi, O. O. and A. G. Hunter. 1978. Colostral constituents including immunoglobulins in the first three milkings postpartum. J. Dairy Sci. 61:44.
- Perino, L. J. and T. E. Wittum. 1995. Effects of various risk factors on plasma protein and serum immunoglobulin concentration of calves at postpartum hours 10 and 24. Am. J. Vet. Res. 56:114-118.
- Pijoan P. 1997. Niveles de inmunoglobulinas calostrales en becerras lecheras de la región de Tijuana y su efecto en la sobrevivencia y desarrollo de la cría durante la lactancia. Vet. Mex. 28(3):203-208.
- SAS. 1991. SAS/STAT Users guide: Statistics (ver 7.) SAS Inst. Inc. Cary NC.
- Stott, G. H., W. A. Fleenor and W. C. Kleese. 1981. Colostral immunoglobulin concentration in two fractions of first milking postpartum and five additional milkings. J. Dairy Sci. 64:459.
- Tizard. I. R. 1996. Inmunología Veterinaria en: Inmunidad en el feto y el neonato. (5ta. Edición). Ed. McGraw-Hill. México, D.F.

Table 1. Concentration of immunoglobulin (mg/ml) in colostrums during first milkings according to the number of lactation.

VARIABLES	LACTATION NUMBER			
	First	Second	Third	Fourth or more
Animals	7	21	18	22
1 <sup>st</sup> Milking	103.43 ± 9.80 <sup>a</sup>	87.86 ± 5.70 <sup>ab</sup>	98.05 ± 6.12 <sup>a</sup>	78.64 ± 5.53 <sup>b</sup>
2 <sup>nd</sup> Milking	60.71 ± 8.70	54.76 ± 5.01	57.50 ± 5.41	50.50 ± 5.01
3 <sup>rd</sup> Milking	37.86 ± 5.57 <sup>a</sup>	24.29 ± 3.32 <sup>bc</sup>	28.33 ± 3.58 <sup>ac</sup>	23.57 ± 3.32 <sup>bc</sup>
4 <sup>th</sup> Milking	22.86 ± 4.32 <sup>a</sup>	8.10 ± 2.50 <sup>b</sup>	11.94 ± 2.69 <sup>b</sup>	10.00 ± 2.49 <sup>b</sup>
Protein (%)	19.98 ± 1.40	17.69 ± 0.80	19.12 ± 0.78	17.60 ± 0.79

a. b. c. Different letters in rows indicate a significant difference ( $P < .05$ )

Table 2. CIT (mg/ml) and percentage of protein in blood syrup of calves according to the number of lactation of the dam.

VARIABLES	LACTATION NUMBER*			
	First	Second	Third	Fourth or more
Animals	7	21	18	22
Birth	8.16 ± 1.39	8.19 ± 0.86	9.40 ± 0.78	8.20 ± 0.75
24 h	8.50 ± 0.82	6.70 ± 0.51	7.54 ± 0.46	7.91 ± 0.44
48 h	8.16 ± 0.76	7.10 ± 0.47	7.18 ± 0.43	8.05 ± 0.41
Average of CIT	8.27 ± 0.67	7.42 ± 0.41	8.04 ± 0.37	8.05 ± 0.36
% of Protein				
Birth	5.90 ± 0.70	5.86 ± 0.43	6.00 ± 0.40	5.93 ± 0.40
24 h	7.00 ± 0.62	7.05 ± 0.40	7.00 ± 0.35	7.11 ± 0.34
48 h	6.80 ± 0.70	6.66 ± 0.42	7.17 ± 0.40	6.72 ± 0.37

\*There were no found any significant difference ( $P > .05$ )

# EFFECTS OF SHORT-TERM FASTING ON METABOLIC MEDIATORS OF REPRODUCTION IN BEEF COWS

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**ABSTRACT:** The mechanism by which short-term nutrient deprivation affects reproduction during the midluteal phase of the bovine estrous cycle has not previously been determined. Glucose, GH, and IGF-I, metabolic mediators of reproduction, are known to be responsive to nutrient status. To evaluate metabolic consequences of acute nutrient deprivation, mature beef cows were randomly assigned to one of three treatments: control (CONT; n = 8), controlled internal drug release device (CIDR; n = 9), or fasted (FAST; n = 9). Control and CIDR cows received 10 kg alfalfa hay daily while FAST were withheld from feed from d 10 through 14 of the estrous cycle (ovulation = d 0). CIDR cows received an intravaginal CIDR (InterAg, Hamilton, NZ) containing 1.38 g progesterone on d 11, which was removed on d 15. Blood was collected once daily on d 10 through 18 of the estrous cycle. Samples collected from d 10 through 18 were analyzed for IGF-I, while glucose and GH were analyzed in serum samples collected d 10 through 14. Data were analyzed using the MIXED procedure of SAS. To control for inherent variability, basal values collected on d 10 were set at 100% and subsequent samples were expressed as a percentage of basal concentrations. Glucose concentrations were similar ( $P = 0.69$ ) among treatments on d 11 through 14 (98.0, 102.6, 92.6  $\pm$  8.6% for CONT, CIDR, and FAST, respectively). Concentrations of GH on d 11 through 14 were similar ( $P = 0.77$ ) among treatments (102.6, 119.9, 120.5  $\pm$  20.1% for CONT, CIDR, and FAST, respectively). IGF-I concentrations of FAST cows were lower (81.6  $\pm$  16.5%;  $P < 0.05$ ) than CONT and CIDR cows (139.5 and 153.3  $\pm$  16.5%). Our previous data reported a substantial delay in time of ovulation post PGF<sub>2 $\alpha$</sub>  in FAST cows compared to CONT or CIDR (96, 101, 255  $\pm$  55 h for CONT, CIDR, and FAST, respectively;  $P \leq 0.05$ ). The decline in IGF-I concentrations may be responsible for the impairment in ovulation in cows fasted for 5 d during the luteal phase of the estrous cycle.

Key Words: Fasting, Metabolism, Reproduction

## Introduction

Reproduction and nutritional status have long been closely linked, as adequate nutrition is requisite for reproductive processes to occur. Inadequate nutrition

impairs all elements of reproduction (Dunn and Moss, 1992). Acute nutrient restriction decreases the growth rate and maximum diameter of dominate follicles (Mackey et al., 2000), reduces the amplitude of, and delays, the preovulatory LH surge, and delays ovulation (McCann and Hansel, 1986; Schillo 1992; Ramos et al., 2003). The exact mechanism of this relationship is poorly understood.

Both GH and IGF-I are responsive to nutrient status and are involved in ovarian function. Growth hormone may mediate effects directly or indirectly on reproduction through regulation of IGF-I synthesis (Gong, 2002). Growth hormone and IGF-I serum concentrations are positively coupled in ruminants in positive energy balance (Cohick and Clemmons, 1993). During periods of nutrient stress, however, GH is elevated and IGF-I is markedly decreased (McGuire et al., 1992), thereby providing a possible nutritional mechanism for regulation of reproduction via uncoupling of the GH/IGF-I axis.

The IGF-I system is influenced by glucose availability (Schillo, 1992). Kiyama et al. (2001) and Spicer et al. (1992) reported decreased serum IGF-I concentrations in ruminant females in response to fasting. Fasting did not, however, affect intraovarian IGF-I concentrations. Nutritional effects on ovarian functions appear mediated by serum IGF-I rather than by intraovarian IGF-I (Diskin et al., 2003).

Decreased IGF-I concentrations in nutrient-restricted animals decreases amplitude of LH pulses and delays ovulation (McCann and Hansel, 1986; Schillo 1992). The objective of this study was to characterize the response of glucose, GH and IGF-I in beef cows subjected to short-term fasting during the luteal phase.

## Materials and Methods

Twenty-six normally cycling crossbred Angus-Hereford cows (body condition score = 4.5  $\pm$  0.5, BW 475  $\pm$  28 kg) were randomly assigned to one of three treatments: control (CONT; n = 8), controlled internal release device (CIDR; n = 9), or fasted (FAST; n = 9).

Estrus was synchronized with two injections of prostaglandin F<sub>2 $\alpha$</sub>  (PGF<sub>2 $\alpha$</sub> ; 25 mg, i.m., Lutalyse®, Pharmacia & UpJohn Co., Kalamazoo, MI) 11 d apart. Day of ovulation was defined by disappearance of the largest follicle following the second PGF<sub>2 $\alpha$</sub>  injection. Ultrasonography (Aloka 500v console and 7.5 mHz transducer; Corometrics Medical Products; North

Wallingford, CT) performed daily per rectum was utilized to determine d of ovulation ( $d = 0$ ).

Control and CIDR cows were fed 10 kg ground alfalfa daily throughout the experiment. CIDR cows received an intravaginal CIDR (InterAg, Hamilton, NZ) containing 1.38 g progesterone on d 11. Previously in fasted ewes, Kiyama et al. (2001) reported that progesterone remained elevated after  $\text{PGF}_{2\alpha}$ . Therefore, to provide an appropriate control, CIDR's were utilized to sustain progesterone after injection of  $\text{PGF}_{2\alpha}$ . Fasted cows were withheld from feed from d 10 through 14 of the first estrous cycle following synchronization. Ad libitum water, salt, and minerals were available to all cows.

At 0600 on d 15 all cows received  $\text{PGF}_{2\alpha}$  (25 mg, i.m., Lutalyse®, Pharmacia & UpJohn Co., Kalamazoo, MI) to initiate luteolysis, and FAST cows were returned to ad libitum feed. Intravaginal CIDR's were removed 12 h after administration of  $\text{PGF}_{2\alpha}$ .

Blood was collected at 0600 on d 10 through 18. Indwelling jugular catheters using 14 g x 13.5-cm catheters (Jorgensen Laboratories, Inc.; Loveland CO) with removable caps were used for blood collection. Serum was separated via centrifugation at  $1300 \times g$  for 20 min at  $4^\circ\text{C}$  and stored at  $-20^\circ\text{C}$ .

Serum glucose levels were determined in samples collected d 10 through 14 using the glucose modified endpoint protocol (ThermoDMA Infinity Glucose Hexokinase Reagent, Thermo DMA, Arlington, TX) with inter- and intraassay CV of 7%. Volumes were modified to allow detection with a 96-well plate reader. Serum GH and IGF-I concentrations were determined via RIA, as described by Hoeffler and Hallford (1987) and Berrie et al. (1995), respectively. Growth hormone analysis was performed for samples collected d 10 through 14 with an intraassay CV of 8%, while IGF-I analysis was performed on samples collected d 10 through 18 with interassay and intraassay CV of 4% and 6%, respectively.

**Statistical Analysis.** Differences in glucose, GH, and IGF-I concentrations among treatment groups were determined using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). To control for inherent variability among animals, basal values collected on d 10 were set at 100% and subsequent samples were expressed as a percentage of basal concentrations.

## Results and Discussion

Acute nutrient restriction in ruminant females decreases follicular growth rate, maximum follicle diameter, and preovulatory LH surge amplitude, while delaying the preovulatory LH surge and ovulation (McCann and Hansel, 1986; Schillo 1992; Mackey et al., 2000; Kiyama et al., 2001; Ramos et al., 2003). The mechanism by which these effects are manifested is poorly understood. The objective of this study was to characterize the response of glucose, GH and IGF-I in beef cows subjected to short term fasting during the luteal phase.

Serum glucose and GH concentrations across d 10 through 14 did not differ among treatment groups. Glucose concentrations were  $98.0$ ,  $102.6$ , and  $92.6 \pm 8.6\%$  for

CONT, CIDR, and FAST, respectively ( $P = 0.69$ ). Growth hormone concentrations were  $102.6$ ,  $119.9$ , and  $120.5 \pm 20.1\%$  for CONT, CIDR, and FAST, respectively ( $P = 0.77$ ). The failure of glucose and GH concentrations to differ in response to 5 d of fasting may be due to the short duration of the fasting period, as glucose concentrations tend to remain relatively stable in ruminants (Dhuyvetter and Caton, 1996). McCann and Hansel (1986) reported lower glucose concentrations in heifers fasted d 8 through 16 of the estrous cycle. The 8 d of fasting utilized by these authors, compared to 5 d of fasting in the present study, corresponds to the time required for complete emptying of the rumen (8 to 10 d; Balch, 1950).

Concentrations of serum IGF-I across d 10 through 18 differed between treatment groups ( $139.5$ ,  $153.3$ , and  $81.6 \pm 16.5\%$  for CONT, CIDR, and FAST, respectively;  $P < 0.05$ ). Specifically, IGF-I concentrations of CONT and CIDR were similar ( $P = 0.50$ ), while FAST were lower than CIDR and CONT ( $P = 0.002$  and  $P = 0.014$ , respectively). Thus, fasting mature cows for 5 d during the luteal phase of the estrous cycle caused a reduction in serum IGF-I. Spicer et al. (1992) and Kiyama et al. (2001) also reported decreased serum IGF-I concentrations in heifers fasted up to 48 h and ewes fasted 3 d, respectively.

Ramos et al. (2003) showed a marked delay in ovulation in cows fasted during the luteal phase ( $P < 0.05$ ) with time from  $\text{PGF}_{2\alpha}$  to ovulation doubled in FAST compared to CONT and CIDR cows. In addition, although no differences were observed ( $P = 0.18$ ), FAST cows tended to have an increased interval between the onset of the LH surge and ovulation compared to CONT and CIDR cows ( $41$  vs  $24$  and  $16 \pm 9$  h, for FAST, CONT, and CIDR, respectively). These data are in agreement with Mackay et al. (1999) who reported an inability of 60% of heifers to ovulate two successive dominate follicles following acute fasting. Reduced IGF-I concentrations, such as those observed in fasted cows in the present study and in fasted ewes (Kiyama et al., 2001), may delay onset of the preovulatory LH surge.

Under the conditions of this study, serum IGF-I was suppressed in response to short-term fasting while GH and glucose did not respond. Short-term fasting during the luteal phase resulted in uncoupling of the GH/IGF-I axis, which may explain the previously reported delay in ovulation in FAST cows.

## Implications

Nutritional deficiencies commonly occur in grazing ruminant females and often result in reduced pregnancy rates. Strategies that prevent uncoupling of the GH/IGF-I axis may improve reproductive rates in cows facing acute nutrient restriction.

## Literature Cited

Balch, C. C. 1950. Factors affecting the utilization of food by dairy cows: The rate of passage of food through the digestive tract. *Br. J. Nutr.* 4:361-388.

- Berrie, R. A., D. M. Hallford, and M. L. Galyean. 1995. Effects of zinc source and level on performance, carcass characteristics, and metabolic hormone concentrations of growing and finishing lambs. *Prof. Anim. Sci.* 11:149-156.
- Cohick, W. S., and D. R. Clemmons. 1993. The insulin-like growth factors. *Ann. Rev. Physiol.* 55:131-153.
- Dhuyvetter, D. V., and J. S. Caton. 1996. Manipulation of reproduction and lactation with supplementation in beef cattle. In: *Proc. Graz. Livest. Nutr. Conf., Custer SD.* pp 83-93.
- Diskin, M. G., D. R. Mackey, J. F. Roche, and J. M. Sreenan. 2003. Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. *Anim. Reprod. Sci.* 78:345-370.
- Dunn, T. G., and G. E. Moss. 1992. Effects of nutrient deficiencies and excesses on reproductive efficiency of livestock. *J. Anim. Sci.* 70: 1580-1593.
- Gong, J. G., W. J. Lee, P. C. Garnsworthy, and R. Webb. 2002. Effect of dietary-induced increases in circulating insulin concentrations during the early post-partum period on reproductive function in dairy cows. *Reproduction* 123:419-427.
- Hoefler, W. C., and D. M. Hallford. 1987. Influence of suckling status and type of birth on serum hormone profiles and return to estrus in early-postpartum, spring-lambing ewes. *Theriogenology* 27:887-895.
- Kiyama, Z., E. A. Van Kirk, W. J. Murdoch, and G. E. Moss. 2001. Effects of feed restriction on ovulation and reproductive hormones in ewes. *Proc. West. Sec. Am. Soc. Anim. Sci.* 52:418-421.
- Mackey, D. R., A. R. G. Wylie, J. M. Sreenan, J. F. Roche, and M. G. Diskin. 2000. The effect of acute nutritional change on follicle wave turnover, gonadotropin, and steroid concentration in beef heifers. *J. Anim. Sci.* 78:429-442.
- McCann, J. P., and W. Hansel. 1986. Relationships between insulin and glucose metabolism and pituitary-ovarian functions in fasted heifers. *Biol. Reprod.* 34:630-641.
- McGuire, M. A., J. L. Vicini, D. E. Bauman, and J. J. Veenhuizen. 1992. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. *J. Anim. Sci.* 70:2901-2910.
- Ramos III, A., K. K. Kane, D. E. Hawkins, W. D. Bryant, D. M. Hallford, G. E. Moss, and R. S. Kelling. 2003. Effects of short-term fasting on reproductive function in beef cows. *Proc. West. Sec. Am. Soc. Anim. Sci.* 54:285-287.
- Schillo, K. K. 1992. Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. *J. Anim. Sci.* 70:1271-1282.
- Spicer, L. J., M. A. Crowe, D. J. Prendiville, D. Goulding, and W. J. Enright. 1992. Systemic but not intraovarian concentrations of insulin-like growth factor-I are affected by short-term fasting. *Biol. Reprod.* 46:920-925.
- Spicer, L. J., E. Alpizar, and S. E. Echternkamp. 1993. Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and (or) insulin-like growth factor I production in vitro. *J. Anim. Sci.* 71:1232-1241.

## SUPPLEMENT INTAKE VARIATION IN GRAZING BEEF COWS

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**ABSTRACT:** One hundred twenty-one pregnant Angus cross cows (avg. wt  $636 \pm 50$  kg) grazing native range pastures (*Agropyron spicatum*, *Festuca idahoensis*) were used in a  $2 \times 7$  factorial design with individual animal as the experimental unit to determine effects of herd size (large = 76 cows and small = 45 cows) and cow age (3 to 9 yr) on individual intake of hand-fed supplement. The study was conducted at the Montana State University Red Bluff Research Ranch near Norris, MT from October 14, 2002 to December 13, 2002. Each herd was assigned to one of two native range pastures to achieve equal stocking rates (0.4 AU/ha). Titanium dioxide was added to the supplement at 1% as an external marker to estimate individual supplement intake. Forage intake was calculated using estimates of fecal output obtained using chromium boluses and *in situ* 48 h DM digestibility. Individual fecal samples were collected on d 23, 36, 38, 40, and 61 and analyzed for Ti to give five estimates of supplement intake per cow. Forage intake was higher ( $P < 0.001$ ) for cows in the large herd vs. cows in the small herd (19.8 vs. 18.3 kg.cow<sup>-1</sup>.d<sup>-1</sup>). Herd size did not affect ADG ( $P = 0.11$ ; avg. 1.06 kg/d); however, ADG was lowest ( $P < 0.001$ ) for 3-year-old cows (0.72 kg/d) and highest for 7-yr-olds (1.33 kg/d). Supplement DMI was higher ( $P = 0.03$ ) for the large herd compared to the small herd on d 36 (0.99 vs. 0.82 kg.cow<sup>-1</sup>.d<sup>-1</sup>, respectively); however, herd size did not affect ( $P > 0.10$ ) supplement DMI for the other four sampling dates. Average DM supplement intake was 33% lower ( $P < 0.001$ ) for 3- and 4-yr-old cows compared to 8- and 9-yr-olds (0.72 vs. 1.07 kg/d, respectively). Supplement DMI CV was not different ( $P = 0.35$ ) between herds (avg. 21%). Results of this study indicate that cow age may have more influence on individual supplement intake than herd size.

Key Words: Cow age, Supplement intake, Forage intake, Native range

### Introduction

Providing supplements to range cattle during times of low forage quality can improve animal performance and provide increased economic returns (DelCurto et al., 2000).

Protein is often the first-limiting nutrient on low quality native range pastures (Lusby, 1990). The positive effects of protein supplementation have been well documented, and include increased forage intake and digestibility, increased gain and BCS, and increased reproductive efficiency. However, the success of supplementation programs is often limited by the inability

to ensure that each animal is consuming recommended supplement intake levels. Using a hand-fed supplement delivery method allows for increased control of amount of supplement given to each animal; however, variation from the target amount may occur due to competition between animals (Bowman and Sowell, 1997).

Most of the research on hand-fed supplementation of beef cows has focused on factors such as timing, amounts, sources, and frequency of supplementation. Little is known about factors that influence individual supplement intake variation of cattle fed in groups. Therefore, the objectives of our study were to determine the effects of herd size and cow age on individual supplement intake, variation in supplement intake, individual forage intake, and performance. A further objective was to determine the correlation of fecal titanium concentration with individual supplement intake to investigate the effectiveness of titanium dioxide as a supplement intake marker.

### Materials and Methods

The study was conducted at the Montana State University Red Bluff Research Ranch near Norris, MT from October 14, 2002 to December 13, 2002. One hundred twenty-one pregnant, mixed-age, Angus cross cows (3 to 9 years old; avg. wt  $636 \text{ kg} \pm 50 \text{ kg}$ ) grazing native range were assigned by weight and age to one of two herds. Both herds were supplemented every other day with a commercially available pelleted protein supplement.

Treatment was herd size, with seventy-six cows assigned to the First Feeder (257 ha), and forty-five cows assigned to the Second Feeder (158 ha). Herd size was determined by setting equal and moderate stocking rates for each pasture. Cows were considered 1.4 AU, and stocking rates were set at 0.4 AU/ha. Cows remained on their assigned pasture for the duration of the study.

The supplement used in the study was a hand-fed, commercial pelleted protein supplement (20% CP, United Agri-Products, Billings, MT) with target intake of 0.91 kg.cow<sup>-1</sup>.d<sup>-1</sup>. Titanium dioxide was mechanically mixed into the supplement at 1% as an external marker to estimate supplement intake. Supplement was fed using a pickup and cake feeder at approximately 1300 h every other day. Cows had ad-libitum access to water, mineral, and white salt. Target intake of mineral supplement was 3 oz.hd<sup>-1</sup>.d<sup>-1</sup>, and mineral was composed of 6.5 to 7.5% Ca, 8% P, 4.6% Mg, 4.6% K, 0.23% Cu, 0.00352% Se, 0.86% Zn, 90720 IU/kg Vit. A, 22680 IU/kg Vit. D, and 90.72 IU/kg Vit. E.

Upland vegetation was typical of a foothill bunchgrass type. Pastures were dominated by Idaho fescue (*Festuca idahoensis*) and bluebunch wheatgrass (*Agropyron*

*spicatum*). Average temperature over the study period was 1° C, with a low of -25° C and a high of 20° C.

Cows were weighed at the beginning and end of the study, and fecal grab samples were collected on d 23, 36, 38, 40, and 60. All cows were dosed with sustained release Cr<sub>2</sub>O<sub>3</sub> boluses (Captec LTD, Manurewa, Auckland, New Zealand) on d 23 of the trial to administer chromium oxide as an external marker to estimate fecal output. The bolus release rate as stated by Captec LTD was 1.50 g chromium sesquioxide per day. Because release rate may differ due to feed type, four ruminally cannulated cows were used to determine the actual release rate by assessing the disappearance of the matrix over time. The average release rate for our trial site and feeding conditions was 1.35 g Cr/d.

Individual fecal samples from the five collection dates were analyzed separately for DM and Ti (Meyers et al., 2004). In addition, fecal grab samples collected on d 36, 38, and 40 were oven-dried, ground through a 1 mm screen in a Wiley mill, and composited on an equal-weight basis by cow. Composite fecal samples were analyzed for DM, OM, CP (AOAC, 2000), NDF, ADF (Van Soest et al., 1991), and Cr by inductively coupled plasma emission spectroscopy (Fassel, 1978).

Supplement samples were collected every two weeks, composited, and analyzed for DM, OM, CP, starch (AOAC, 2000), ADF (Van Soest et al., 1991), and Ti (Myers et al., 2004). Forty frames (0.25 m<sup>2</sup>) per pasture were estimated and ten frames per pasture were clipped at the beginning and end of the study to determine forage quality and production. Clip and extrusa samples were ground to pass a 5 mm screen. Nylon bags containing approximately 5 g of sample were incubated in the rumen of cannulated cows for 48 and 72 h. One blank bag was included as a method to measure microbial contamination and entry of particulate material into bags. After removal from the rumen, bags were hand-washed in cold water until the rinse water ran clear. Bags were dried in a forced air oven at 60° C for 48 h, and *in situ* DM digestibility was calculated. Remaining clip sample from both sampling dates was ground through a 1 mm screen and analyzed for DM, OM, CP (AOAC, 2000), NDF, and ADF (Van Soest et al., 1991). Estimates of individual total fecal output, forage intake, and individual supplement intake were obtained using the following equations:

$$FO = \text{Cr intake (g)} / \text{fecal Cr concentration (g)}$$

$$\text{Forage intake} = FO / (1 - 48 \text{ h DM digestibility})$$

$$\text{Supplement intake} = (\text{Fecal Ti concentration} * FO) / \text{Supplement Ti concentration}$$

Estimates of fecal output were not available for the beginning and end of the study; therefore, a regression equation was developed to predict supplement intake for d 23 and 61 based on 110 observations of the relationship between avg. fecal Ti concentration and supplement intake for d 36, 38, and 40. The regression equation was as follows:  $y = 0.749x + 0.016$  ( $R^2 = 0.86$ ,  $P < 0.001$ ).

Data were analyzed using the GLM procedure of SAS for a completely randomized design with individual animal as the experimental unit. Significant treatment means ( $P < 0.05$ ) were separated using the LSD test. Least square means and  $P$ -values are reported. Supplement intake distribution was analyzed as a 2 x 7 factorial, with herd size and cow age as the main factors. Each age group within a pasture was used as the experimental unit for calculation of the CV for individual supplement intake and the proportion of cows consuming low, target, high, and excessive amounts of supplement.

## Results and Discussion

Forage quality and production were consistent between pastures (Table 1). Forage of both pastures would be considered low quality (avg 4.6% CP, 72.4% NDF, and 41.5% ADF). These forages would be deficient in meeting the CP requirement of mature, gestating beef cows during the study time period (6.59%; NRC, 1996).

Estimates of forage and supplement intake for nine cows in the large herd and two cows in the small herd were not included in statistical analysis due to high variation in fecal Cr content. No age x treatment interactions were seen for any variables; therefore, main effects of age and treatment are presented. Cows in Herd 1 consumed more forage and had a higher forage intake as % body weight than cows in Herd 2 (Table 2). However, weight change during the 61 d trial did not differ ( $P > 0.10$ ) between treatments (avg. ADG 1.06 kg/d). Three-yr-olds gained the least amount of weight ( $P < 0.001$ ) compared to all other age groups (avg. 0.72 vs. 1.12 kg/d, respectively; Table 2).

The target amount of supplement was 0.91 kg.cow<sup>-1</sup>.d<sup>-1</sup> (as-fed). All individual fecal samples across all five sampling dates contained Ti, indicating that every cow consumed some level of supplement. Size of herd did not affect ( $P > 0.10$ ) supplement DM intake on d 23, 38, 40, or 61; however, the large herd had higher ( $P = 0.03$ ) supplement intake than the small herd on d 36 (avg. 0.99 vs. 0.82 kg/d, respectively; Table 2). Average supplement DMI was lowest ( $P < 0.001$ ) for 3-year-old cows and highest for 8- and 9-year-old cows (avg. 0.68 vs. 1.07 kg/d, respectively). This agrees with Daniels et al. (1998), who reported greater intake of liquid supplement for 4-, 5-, and 6- yr old cows compared to 2- yr old cows.

Supplement intake distribution is presented in Table 2. The range in supplement intake was smaller for the small herd compared to the large herd (0.63 to 1.10 kg.cow<sup>-1</sup>.d<sup>-1</sup> vs. 0.70 to 1.24 kg.cow<sup>-1</sup>.d<sup>-1</sup>, respectively); however the CV for supplement DMI was similar ( $P = 0.35$ ) between herds (avg. 21%). Nearly twice as many cows in the large herd were classified as excessive consumers compared to the small herd (31.2 vs. 16.7%). This could be explained by an increased opportunity for an individual animal in the large herd to consume excess amounts of supplement due to a larger total supplement allowance. More than 50% of 3-yr-olds were classified as low consumers compared to 0% of 8- and 9-yr-olds. In contrast, 65% of 8-yr-olds and 47% of 9-yr-olds were

classified as excessive consumers, while there were no excessive consumers in the 3- and 4-yr-olds.

### Implications

This is the first study to investigate the effects of herd size on supplement intake by a mixed-age group of cows. Supplement intake between herds was statistically different for only one out of five sampling dates; however, three-year-old cows consumed the least supplement of all age groups for all sampling dates. The results of this trial suggest that it may be beneficial to manage 3-year-old cows separately from older cows in order to obtain maximum benefit from supplementation. The CV for hand-fed supplement in this trial (avg. 21%) was lower than reports of CV's found in the literature for self-fed supplements, indicating that hand-fed supplements may minimize variation in supplement intake. Titanium dioxide is an economical alternative to other intake markers such as Yb, and could be used for large-scale supplementation studies in commercial production situations. Further research is needed to assess diurnal variation and marker recovery of Ti.

### Literature Cited

AOAC. 2000. Official Methods of Analysis of AOAC International (17<sup>th</sup> Ed.) AOAC International, Gaithersburg, MD.

Bowman, J.G.P., and B. F. Sowell. 1997. Delivery method and supplement consumption by grazing ruminants: A review. *J. Anim. Sci.* 75:543-550.

Daniels, T. K., J.G.P. Bowman, B. F. Sowell, E. E. Grings, and M. D. MacNeil. 1998. The effects of cow age and supplement delivery method on forage and liquid supplement intake. *Proc. West. Sec. Am. Soc. Anim. Sci.* 49:145-148.

DelCurto, T., B. W. Hess, J. E. Huston, and K. C. Olson. 2000. Optimum supplementation strategies for beef cattle consuming low-quality roughages in the western United States. *Proc. Am. Soc. Anim. Sci.*, 1999.

Fassel, T. W. 1978. Quantitative elemental analysis by plasma emission spectrophotometry. *Science*. 202:183.

Lusby, K. S. 1990. Supplementation of cattle on rangeland. *Proc. of the 46<sup>th</sup> Southern Pasture and Forage Crop Improvement Conference*. p. 64-71.

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. *J. Anim. Sci.* 82:179-183.

NRC. 1996. Nutrient Requirements of Beef Cattle. National Academy Press, Washington, D. C.

Suverly, N. A., T. DelCurto, J. R. Jaeger, and M. R. Keller. 2000. Influence of cow age on consumption of hand-fed supplements and subsequent performance of beef cows winter grazing stockpiled forage. *Proc. West. Sec. Am. Soc. Anim. Sci.* 51:281-285.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.

Table 1. Pasture size, forage production, composition and in situ DM disappearance of clipped samples from native range pastures grazed by beef cows in one of two mixed-age herds and supplemented with a 20% CP hand-fed supplement

Sampling date	Pasture 1		Pasture 2	
	10/18/02	12/18/02	10/18/02	12/18/02
Size of pasture, ha	257	-	158	-
Production, kg/ha	464	229	349	256
Composition, %				
DM	96.17	92.42	96.21	93.42
OM	91.76	94.62	93.72	91.91
CP	4.88	4.64	4.50	4.22
NDF	69.20	77.21	70.65	72.53
ADF	39.12	42.93	45.33	38.87
DM disappearance, %				
48 h	58.88	40.64	55.06	61.37
72 h	69.94	-	65.87	-
DOM:CP	11.1	8.3	11.5	13.4

Table 2. Performance, forage DMI, supplement DMI, and distribution of supplement intake of beef cows grazing native range in one of two mixed-age herds supplemented with a 20% CP hand-fed supplement

Item	Herd Size		SE	Cow Age							SE	P-value	
	Large	Small		3	4	5	6	7	8	9		Trt	Age
No. cows	76	45	-	28	19	26	17	16	7	8	-	-	-
Weight, kg													
Initial	644	649	6.3	585 <sup>a</sup>	644 <sup>b</sup>	636 <sup>b</sup>	658 <sup>bc</sup>	658 <sup>bc</sup>	681 <sup>c</sup>	662 <sup>bc</sup>	11.5	0.63	<0.001
Ending	712	710	6.0	629 <sup>a</sup>	709 <sup>b</sup>	707 <sup>b</sup>	717 <sup>bc</sup>	740 <sup>c</sup>	744 <sup>c</sup>	732 <sup>bc</sup>	10.9	0.74	<0.001
Wt change	68	61	3.1	44 <sup>a</sup>	65 <sup>bc</sup>	71 <sup>cd</sup>	59 <sup>b</sup>	82 <sup>d</sup>	64 <sup>bc</sup>	69 <sup>bd</sup>	5.7	0.11	<0.001
ADG, kg	1.12	1.00	0.051	0.72 <sup>a</sup>	1.06 <sup>bc</sup>	1.16 <sup>cd</sup>	0.97 <sup>b</sup>	1.33 <sup>d</sup>	1.04 <sup>bc</sup>	1.13 <sup>bd</sup>	0.094	0.11	<0.001
Forage Intake													
DM, kg	19.8	18.3	0.30	17.6 <sup>a</sup>	19.2 <sup>bc</sup>	18.5 <sup>ab</sup>	19.2 <sup>bc</sup>	20.3 <sup>c</sup>	19.3 <sup>bc</sup>	19.5 <sup>bc</sup>	0.55	<0.001	0.003
DM, g/kg BW	29	27	0.5	29	29	28	28	29	27	28	0.87	0.001	0.51
Supplement DMI, kg													
Nov. 11, 2002 (d 23) <sup>x</sup>	0.92	0.88	0.044	0.61 <sup>a</sup>	0.70 <sup>a</sup>	0.91 <sup>b</sup>	0.99 <sup>b</sup>	0.93 <sup>b</sup>	1.08 <sup>b</sup>	1.09 <sup>b</sup>	0.080	0.488	< 0.001
Nov. 18, 2002 (d 36)	0.99	0.82	0.054	0.68 <sup>a</sup>	0.70 <sup>a</sup>	0.94 <sup>b</sup>	0.87 <sup>b</sup>	0.94 <sup>b</sup>	0.99 <sup>bc</sup>	1.19 <sup>c</sup>	0.098	0.025	0.007
Nov. 20, 2002 (d 38)	0.95	0.89	0.028	0.72 <sup>a</sup>	0.78 <sup>ab</sup>	0.84 <sup>b</sup>	0.95 <sup>c</sup>	0.96 <sup>c</sup>	1.12 <sup>d</sup>	1.04 <sup>cd</sup>	0.052	0.139	<0.001
Nov. 22, 2002 (d 40)	0.90	0.86	0.025	0.65 <sup>a</sup>	0.76 <sup>b</sup>	0.85 <sup>c</sup>	0.91 <sup>cd</sup>	0.94 <sup>d</sup>	1.10 <sup>e</sup>	0.94 <sup>cd</sup>	0.045	0.279	<0.001
Dec. 13, 2002 (d 61) <sup>x</sup>	0.87	0.89	0.031	0.72 <sup>a</sup>	0.77 <sup>a</sup>	0.89 <sup>b</sup>	1.00 <sup>c</sup>	0.88 <sup>b</sup>	0.94 <sup>bc</sup>	0.97 <sup>bc</sup>	0.180	0.659	<0.001
Avg. supplement DMI <sup>y</sup>	0.94	0.86	0.028	0.68 <sup>a</sup>	0.75 <sup>a</sup>	0.88 <sup>b</sup>	0.91 <sup>b</sup>	0.95 <sup>bc</sup>	1.07 <sup>c</sup>	1.06 <sup>c</sup>	0.050	0.028	<0.001
Supplement DMI Distribution													
Min., kg	0.70	0.63	0.031	0.52 <sup>a</sup>	0.57 <sup>a</sup>	0.67 <sup>a</sup>	0.61 <sup>a</sup>	0.63 <sup>a</sup>	0.83 <sup>b</sup>	0.85 <sup>b</sup>	0.058	0.16	0.039
Max., kg	1.24	1.10	0.058	0.91	0.96	1.11	1.25	1.41	1.33	1.22	0.109	0.13	0.108
Mean, kg	0.95	0.86	0.029	0.69 <sup>a</sup>	0.75 <sup>ab</sup>	0.88 <sup>bc</sup>	0.91 <sup>c</sup>	0.96 <sup>cd</sup>	1.09 <sup>d</sup>	1.06 <sup>d</sup>	0.055	0.07	0.014
Supplement DMI CV, %	19	23	2.8	20	18	14	25	26	26	17	5.3	0.35	0.579
Proportion of cows within consumption group:													
Low (< 75% of mean)	13.4	23.2	4.62	51.3 <sup>c</sup>	38.2 <sup>cb</sup>	5.0 <sup>a</sup>	16.6 <sup>ab</sup>	16.6 <sup>ab</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	8.63	0.18	0.032
Target (76 – 100% of mean)	33.8	33.2	7.90	40.6	47.3	48.4	31.6	15.0	25.0	26.7	14.77	0.95	0.658
High (101 – 125% of mean)	21.6	27.0	4.54	8.1 <sup>a</sup>	14.5 <sup>a</sup>	42.5 <sup>bc</sup>	18.4 <sup>a</sup>	50.0 <sup>c</sup>	10.0 <sup>a</sup>	26.7 <sup>ab</sup>	8.49	0.43	0.069
Excessive (> 125% of mean)	31.2	16.7	3.97	0.0 <sup>a</sup>	0.0 <sup>a</sup>	4.1 <sup>a</sup>	33.4 <sup>bc</sup>	18.4 <sup>ab</sup>	65.0 <sup>d</sup>	46.6 <sup>cd</sup>	7.44	0.04	0.004

<sup>x</sup> Determined using regression equation

<sup>y</sup> Average of d 36, 38, and 40

## EFFECTS OF CORN CONDENSED DISTILLERS SOLUBLE SUPPLEMENTATION ON INTAKE AND DIGESTION IN BEEF STEERS CONSUMING LOW-QUALITY HAY

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**ABSTRACT:** Four ruminally and duodenally cannulated steers ( $561 \pm 52.8$  kg initial BW) were used in a  $4 \times 4$  Latin square to evaluate effects of corn condensed distillers solubles (CCDS) supplementation on intake and site of digestion in beef steers fed low-quality hay. Steers were fed ad libitum at 0700 and 1900 daily and were allowed free access to water. Switchgrass hay (*Panicum virgatum* L.) offered was 5.1% CP, 40.3% ADF, and 70.7% NDF. Composition of CCDS was 28.1% DM, 19.3% CP, and 4.7% fat (DM basis). Treatments included: 1) control, no CCDS; 2) 5% CCDS; 3) 10% CCDS; and 4) 15% CCDS supplementation (DM basis). Experimental periods consisted of a 9 d of diet adaptation and 5 d of collection. During collections, fecal output was measured, and duodenal samples taken twice daily from all steers as follows: d 2, 0630 and 1230; d 3, 0800 and 1400; d 4 0930 and 1530; and d 5, 1100 and 1700. Ruminant, postruminal, and total tract OM digestibilities were not affected by treatment ( $P \geq 0.21$ ). Crude protein intake ( $326, 450, 537$ , and  $666 \pm 48$  g/d), and total tract CP digestibility ( $34.3, 51.4, 52.3$ , and  $57.3 \pm 4.82$  %) increased linearly ( $P \leq 0.002$ ) and microbial protein synthesis ( $238, 279, 328$ , and  $302 \pm 24$  g/d) tended to increase linearly ( $P = 0.11$ ) with level of CCDS supplementation. However, microbial efficiency ( $11.6, 9.9, 13.0$ , and  $12.0 \pm 2.66$  g of N/kg OM truly fermented) was not affected by treatment ( $P = 0.38$ ). Acetate molar proportion ( $73.9, 71.0, 67.7$ , and  $64.5 \pm 0.64$ ), and acetate to propionate ratio ( $4.7, 4.4, 4.0$ , and  $3.7 \pm 0.18$ ) decreased linearly ( $P \leq 0.001$ ), and butyrate molar proportion ( $6.4, 9.1, 10.9$ , and  $13.0 \pm 0.29$ ) increased linearly ( $P \geq 0.001$ ) with level of CCDS supplementation. Supplementation of up to 15% CCDS to low-quality hay-based diets increased CP intake digestibility, and tended to increase total DM and OM intakes without affecting hay DMI, or OM, ADF, and NDF digestibilities. These results suggest CCDS supplementation to steers consuming low-quality hay improves total nutrient availability without altering hay utilization.

Four ruminally and duodenally cannulated steers ( $561 \pm 52.8$  kg initial BW) were used in a  $4 \times 4$  Latin square to evaluate effects of CCDS supplementation on intake and site of digestion in beef steers fed low-quality hay. Steers were fed switchgrass hay ad libitum and CCDS at 0700 and 1900 daily and were allowed free access to water. First, CCDS was offered and if any was left, then was placed in the rumen via the rumen cannula. Then the hay was offered. Switchgrass hay (*Panicum virgatum* L.) offered was 5.1% CP, 40.3% ADF, and 70.7% NDF. Corn condensed distillers solubles composition was 28.1% DM, and 19.3% CP (DM basis). Treatments consisted of: 1)

Key Words: Corn Condensed Distillers Solubles, Digestibility, Cattle, Fermentation

### Introduction

The ethanol industry is expanding rapidly in the upper midwest. Consequently, availability of ethanol byproducts is also expanding. Interest among cow calf producers in using corn condensed distillers solubles (CCDS) is growing. Corn condensed distillers solubles are high in protein (20 to 30%; DM basis), which makes the product an attractive supplement for low quality forages. Protein supplements may increase forage digestibility, forage intake, or both, resulting in increased animal performance and lowered production costs (McCollum and Galyean 1985; Caton and Dhuyvetter, 1997). Also, CCDS are high in fat. Even though, fat increases NE of diets (Zinn 1992; Krehbiel et al., 1995), high levels of fats in ruminant diets can reduce fiber digestibility several ways. One is by physically coating the fiber particles in ruminant diets (MacLeod and Buchanan-Smith, 1972). This can impair or impede colonization of fiber by ruminal microorganisms. Some fatty acids, especially unsaturated fatty acids, are reportedly toxic to certain classes of ruminal microorganisms (Henderson, 1973; Chalupa et al., 1984). In most cases, added fat is generally limited to 3 to 4 percent of diet dry matter (Chalupa et al., 1984). At these levels, the above mentioned problems with fat are generally not encountered. Above these levels, fats may have negative effects on fiber digestibility.

Currently, little is known about optimum levels of condensed distillers solubles in low-quality forage based diets and subsequent effects on ruminal fermentation, digestion, and ruminal metabolism. Therefore, objectives of this study were to evaluate the influence of CCDS supplementation on intake and site of digestion in beef steers fed low-quality grass hay.

### Experimental Procedures

control, no CCDS; 2) 5% CCDS; 3) 10% CCDS; and 4) 15% CCDS supplementation (DM basis). The experimental period consisted of a 9-d treatment adjustment period followed by a 5-d collection period. During collections, fecal output was measured by total collection, and duodenal samples were taken twice daily from all steers as follows: d 2, 0630 and 1230; d 3, 0800 and 1400; d 4 0930 and 1530; and d 5, 1100 and 1700. Ruminal fluid samples were taken at 0, 2, 4, 6, 8, 10, and 12 h post-feeding, via the ruminal cannula using a suction strainer, for pH measurement and VFA, Co, and ammonia analysis. Samples were subjected to all or part of the following analysis: DM (oven drying at

105°C until no further weight loss); ash, Kjeldahl N, ammonia N (AOAC, 1997); ADF and NDF (Robertson and Van Soest, 1991); purines (Zinn and Owens, 1986); VFA concentration of ruminal fluid (Goetsch and Galyean, 1983); and chromic oxide (Fenton and Fenton, 1979). In addition, cobalt concentrations were determined in ruminal fluid with an air-plus-acetylene flame using atomic absorption spectroscopy (Uden et al., 1980).

Data were analyzed as a 4 x 4 Latin square using Mixed procedures of SAS. The model included CCDS level and period as fixed effects, and steer as random effects. Ruminal data over time were analyzed as a repeated measured design using Mixed procedures of SAS. The model included CCDS level, period, time, and the interaction CCDS level x time as fixed effects; and steer nested within period x CCDS level as random effects. Orthogonal contrasts for linear, quadratic, and cubic effects of CCDS level are discussed when a significant ( $P < 0.10$ ) treatment F-test was detected.

## Results and Discussion

Effects of CCDS level on DMI, and fluid dilution rate in beef steers consuming low-quality hay are shown in Table 1. Hay DM intake was not affected by level of CCDS supplementation. By design, consumption of CCDS increased linearly ( $P > 0.001$ ), as a result total DMI tended to increase linearly ( $P = 0.11$ ) when expressed as kg/d, however when expressed as % of BW, no effects were detected ( $P = 0.20$ ).

Treatment effects on OM digestibility are presented in Table 2. Organic matter intake ( $P = 0.13$ ), total OM flowing to small intestine ( $P = 0.15$ ), and microbial OM flowing to the small intestine ( $P = 0.14$ ) tended to increase linearly with increasing CCDS level. However, there was no effect on ruminal, post ruminal, or total tract OM digestibility.

Crude protein intake ( $495 \pm 48$  g/d), and total tract CP digestibility ( $48.8 \pm 4.82$  %) increased linearly ( $P \leq 0.002$ ) and microbial protein synthesis ( $287 \pm 24$  g/d) tended to increase linearly ( $P = 0.11$ ) with increasing level of CCDS supplementation (Table 3). However, microbial efficiency ( $11.6 \pm 2.66$  g of N/kg OM truly fermented) was not affected by treatment ( $P = 0.38$ ). Treatment did not affect ADF or NDF digestibilities ( $P \geq 0.39$ ; data not shown). Although fat supplementation (3 to 5% of DM) has been reported to decrease fiber digestibility (Chalupa et al., 1984), negative effects on fiber digestion were not expected. Detrimental effects on fiber digestion were not expected because for the 15 % CCDS treatment, only 0.71% of diet was calculated to be fat supplied by the supplement.

Treatment effects on ruminal pH, ammonia concentration, and VFA molar proportions are shown in Table 4. Levels of CCDS did not affect pH, ruminal ammonia, or VFA concentration. However, acetate molar proportion, and acetate to propionate ratio decreased linearly ( $P \leq 0.001$ ), and butyrate molar proportion increased linearly ( $P \leq 0.001$ ) with level of CCDS supplementation.

Failure to observe an increase in hay DM intake suggests that the increase in total tract CP digestibility, was

due to higher digestibility of CCDS protein than hay protein and not to a improvement in hay CP digestibility. In summary, supplemental CCDS for cattle consuming low quality hay (5% CP), increased total DMI, CP intake and total tract digestibility. However, effects were not observed on hay intake or utilization.

## Implications

The results of this study suggest that CCDS is a suitable ingredient for supplementation of cattle consuming low-quality hay. Supplementation of CCDS improved the availability of nutrients of steers consuming low-quality hay. Even though CCDS supplementation did not improve hay utilization, fiber digestibility was not depressed due to the relatively high fat content of CCDS.

## Literature Cited

- AOAC. 1997. Official Methods of Analysis. 16th ed. Assoc. Offic. Anal. Chem., Arlington, VA.
- Caton, J.S., D.V. Dhuyvetter. 1997. Influence of energy supplementation on grazing ruminants: Requirements and responses. *J. Anim. Sci.* 75:533-542.
- Chalupa, W., B. Rickabaugh, D. S. Kronfeld and D. Sklan. 1984. Rumen fermentation in vitro as influenced by long chain fatty acids. *J. dairy Sci.* 67:1439-1444.
- Fenton, T.W. and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. *Can. J. Anim. Sci.* 59:631-634.
- Goetsch, A. L. and M. L. Galyean. 1983. Influence of feeding frequency on passage of fluid and particulate markers in steers fed a concentrate diet. *Can. J. Anim. Sci.* 63:727-730.
- Henderson, C. 1973. The effect of fatty acids on pure culture of rumen bacteria. *J. Agric. Sci. (Camb.)* 81:107-112.
- Krehbiel, C. R., R. A. McCoy, R. A. Stock, T. J. Klopfenstein, D. H. Shain and R. P. Huffman. 1995. Influences of grain type, tallow level, and tallow feeding system on feedlot cattle performance. *J. anim sci.* 73:2916-2921.
- McLeod, G. L. K., and Buchanan-Smith., 1972. Digestibility of hydrogenated tallow, saturated fatty acids and soybean oil-supplemented diets by sheep. *J. Anim. Sci.* 35:890-895.
- Robertson, J. B. and P. J. Van Soest. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.
- Uden, P., P. E. Colucci, and P. J. Van Soest. 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *J. Sci. Food Agr.* 31:625-632.
- Zinn, R. A. 1992. Comparative feeding value of supplemental fat in steam flaked corn and steam-flaked wheat-based finishing diets for feedlot steers. *J. Anim. Sci.* 70:2959-2969.

Zinn, R. A. and F. N. Owens. 1986. A rapid procedure for purine measurements and its use for estimating net ruminal protein synthesis. Can. J. Anim. 66:157-166.

Table 1. Effect of CCDS level on DMI, ruminal fill, and fluid dilution rate in beef steers consuming low-quality hay

Item	CCDS level (%)				SEM <sup>b</sup>	P-value	Contrast <sup>a</sup>		
	0	5	10	15			L	Q	C
Hay intake, (DM basis)									
g/d	6,148	6,893	6,681	6,809	807	0.70	0.44	0.55	0.57
% of BW	1.19	1.31	1.26	1.24	0.11	0.88	0.86	0.54	0.68
CCDS intake, g/d (DM basis)	0	444	954	1,610	52	0.001	0.001	0.06	0.72
Total Intake									
g/d	6,148	7,335	7,640	8,415	844	0.11	0.03	0.70	0.58
% of BW	1.19	1.39	1.44	1.54	0.11	0.20	0.05	0.65	0.65
Fluid dilution rate, %/h	2.17	2.30	2.27	1.97	0.17	0.58	0.46	0.26	0.90

<sup>a</sup>Probabilities for contrasts: linear(L), quadratic (Q), and cubic (C).

<sup>b</sup> n = 4.

Table 2. Effect of CCDS level on OM digestion in beef steers consuming low-quality hay

Item	CCDS (%)				SEM <sup>b</sup>	P-value	Contrast <sup>a</sup>		
	0	5	10	15			L	Q	C
OMI, (g/d)	5,709	6,789	7,045	7,719	782	0.13	0.03	0.68	0.58
Duodenal OM flow									
Total, g/d	2,810	3,042	3,067	3,234	133	0.15	0.04	0.78	0.50
Microbial, g/d	507	595	678	617	50	0.14	0.08	0.13	0.48
Non-Microbial, g/d	2,300	2,449	2,383	2,622	119	0.27	0.10	0.69	0.31
Fecal OM flow, g/d	2,693	2,744	2,876	2,903	107	0.25	0.07	0.88	0.60
Digestion (% intake)									
Apparent ruminal	49.3	55.5	53.5	55.2	5.40	0.59	0.35	0.55	0.47
True ruminal	57.9	64.2	63.5	64.4	4.53	0.30	0.14	0.31	0.47
Postruminal	1.7	4.5	3.2	4.8	1.31	0.30	0.17	0.62	0.22
Total tract	51.0	60.1	56.5	60.1	4.63	0.21	0.13	0.39	0.19

<sup>a</sup>Probabilities for contrasts: linear(L), quadratic (Q), and cubic (C).

<sup>b</sup> n = 4.

Table 3. Effect of CCDS level on CP digestion in beef steers consuming low-quality hay

Item	CCDS (%)				SEM <sup>b</sup>	P-value	Contrast <sup>a</sup>		
	0	5	10	15			L	Q	C
Crude protein									
Intake									
Total, g/d	326	450	537	666	48	0.002	0.001	0.93	0.56
Hay, g/d	325	364	352	357	40	0.67	0.46	0.48	0.53
CDS, g/d	0	86	184	310	10	0.001	0.001	0.06	0.72
Duodenal CP flow									
Total, g/d	494	448	631	596	57	0.18	0.10	0.93	0.13
Microbial, g/d	238	279	328	302	24	0.11	0.05	0.16	0.40
Non-microbial, g/d	255	170	303	295	63	0.42	0.37	0.53	0.23
Fecal CP output, g/d	205	223	244	270	11	0.001	0.001	0.45	0.89
CP digestion (% intake)									
Apparent ruminal	-58.3	3.4	-32.2	4.2	23.4	0.23	0.17	0.57	0.14
True ruminal	14.3	65.5	30.3	52.6	20.4	0.27	0.34	0.43	0.13
Postruminal	91.6	49.3	85.7	52.0	19.3	0.33	0.36	0.82	0.14
Total tract	34.3	51.4	52.3	57.3	4.82	0.03	0.009	0.16	0.24
Microbial efficiency <sup>c</sup>	11.6	9.9	13.0	12.0	2.66	0.38	0.71	0.89	0.46

<sup>a</sup>Probabilities for contrasts: linear(L), quadratic (Q), and cubic (C).<sup>b</sup> n = 4.<sup>c</sup>Grams microbial N per kg OM truly fermented.

Table 4. Effect of level of CCDS on rumina; pH, ammonia concentration, and VFA concentration in beef steers consuming low quality hay

Item	CCDS (%)				SEM <sup>b</sup>	P-value	Contrast <sup>a</sup>		
	0	5	10	15			L	Q	C
pH	6.31	6.47	6.39	6.46	0.10	0.68	0.45	0.67	0.41
Ammonia (mM)	2.07	1.21	1.39	1.21	0.36	0.34	0.17	0.37	0.41
VFA									
Total (mM)	34.9	36.6	39.4	34.9	2.46	0.55	0.79	0.24	0.46
	-----mol/100mol-----								
Acetate	73.9	71.0	67.6	64.5	0.64	0.0001	0.0001	0.91	0.83
Propionate	16.5	16.4	17.2	17.8	0.63	0.40	0.13	0.64	0.65
Butyrate	6.4	9.1	10.9	13.0	0.29	0.0001	0.0001	0.32	0.37
Acetate:Propionate ratio	4.7	4.4	4.0	3.7	0.18	0.02	0.003	0.87	0.68

<sup>a</sup>Probabilities for contrasts: linear(L), quadratic (Q), and cubic (C).<sup>b</sup> n = 4.

# EFFECTS OF CANOLA SEED SUPPLEMENTATION ON STEERS FED LOW-QUALITY HAY

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**ABSTRACT:** Fourteen Holstein steers ( $446 \pm 4.4$  kg initial BW) with ruminal, duodenal, and ileal cannulae were used in a completely randomized design to evaluate effects of whole or ground canola seed (39.6% fat) on intake, digestion, duodenal protein supply, and microbial efficiency in steers fed forage based diets. Basal diet consisted of switchgrass hay (6% CP) offered ad libitum with free access to water. Treatments consisted of: hay only (CON); hay plus whole canola (8% of diet DM; WC); or hay plus ground canola (8% of diet DM; GC). Steers were adapted to diets for 14 d followed by a 7-d collection period. Hay DMI, OM intake, and OM digestibility were not affected ( $P > 0.37$ ) by treatment. Likewise, no differences ( $P > 0.70$ ) were observed for NDF or ADF total tract digestion. Bacterial OM at duodenum increased ( $P < 0.01$ ) in steers consuming canola compared with CON and increased ( $P = 0.08$ ) in steers consuming GC compared with WC. Apparent ruminal CP and true ruminal CP digestibilities were increased ( $P < 0.04$ ) with canola supplementation compared to CON. Canola supplementation decreased ruminal pH ( $P = 0.03$ ) compared with CON. Acetate molar proportion tended ( $P = 0.10$ ) to decrease with canola supplementation. Ground canola decreased acetate ( $P < 0.01$ ) and increased propionate ( $P < 0.01$ ) proportions compared with WC. In situ disappearance (%/h) of hay DM, NDF, and ADF were not altered by treatment. In situ disappearance (%/h) of canola DM, NDF, and ADF were increased ( $P < 0.01$ ) with GC compared with WC. Likewise, GC increased ( $P < 0.01$ ) soluble CP fraction and CP degradation rate (%/h) of canola compared to WC. No treatment effects were observed with ruminal fill, fluid dilution rate, or microbial efficiency. Canola supplementation at 8% of diet DM had little impact on forage intake and digestibility. Results suggest canola processing enhances ruminal fermentation and digestion rates.

Key Words: Digestion, Ground Canola, Low-Quality Hay, Steers, Whole Canola

## Introduction

Canola seed supplementation is gaining popularity in the feed industry. This due to abundance of canola in North Dakota and its ability to increase energy density of diets. Canola seed increases lipid and protein content of the diet while minimizing management problems associated with handling fat. Canola seed contains approximately 40% fat. This fat is composed of oleic acid (51%), linoleic acid (25%), and linolenic acid (14%; Khorasani et al., 1991). Data suggests whole canola seed is relatively resistant to digestion in the rumen and intestine unless processed (Khorasani et al., 1992; Hussein et al., 1995). By processing canola seed,

contents are accessible to intestinal enzymatic degradation and absorption when fed forage based diets (Aldrich et al., 1997). Moore et al. (1986) reported fat levels greater than 5% in forage based diets result in decreased DMI and DE. They also reported a decrease in ADF, DM, OM, and GE digestibility. Cracking canola seed may slow the release of unsaturated fatty acids thereby minimizing detrimental effects in rumen function (Hussein et al., 1995). Therefore, our objectives were to investigate effects of whole and processed canola seed on intake, digestion, ruminal fermentation, duodenal protein supply, and microbial efficiency in steers fed low-quality forage.

## Materials and Methods

### Animals and diets

Fourteen ruminally, duodenally, and ileally cannulated Holstein steers ( $446 \pm 44.4$  kg initial BW) were used in a completely randomized design. All animal care and handling techniques followed protocols approved by the North Dakota State University Institutional Animal Care and Use Committee. All steers were fed at 0700 daily. Diets consisted of switchgrass hay (*Panicum virgatum*; 5.84% CP, 77.7% NDF, and 47% ADF) offered ad libitum, free access to water, and either whole canola or ground canola offered at 8% of intake. Treatments consisted of: hay only (CON); hay plus whole canola (WC); or hay plus ground canola (GC).

### Sample Collection

Steers were housed in an enclosed, climate controlled room in individual pens during the 14-d adaptation period and individual tie stalls during each 7-d collection period. Diet samples were collected weekly and composited by period. Ort samples (10% of total) were collected daily throughout collection period. Five days prior to and throughout collections, 8 g of chromic oxide was dosed ruminally twice daily at 0600 and 1800 via gelatin capsule and used as a flow marker. Total fecal output was collected using fecal trays. Fecal subsamples (10% of output) were composited within steer by period. Subsamples were stored (20° C) until mixed in a rotary mixer where another subsample was taken and frozen (-20° C) until analysis. Duodenal and ileal fluid samples (200 ml) were taken over 4 d in a manner that allowed for every other hour in a 24-h period to be sampled. Samples were taken on d 3 at 0800, 1400, 2000; d 4 at 0200, 1000, 1600, 2200; d 5 at 0400, 1200, 1800, 2400; and d 6 at 0600 of each collection period. Samples were composited within steer and frozen (-20° C) until analysis. In situ DM, CP, NDF, and ADF disappearance was determined using dacron bags (Ankom, Fairport, NY: 10 x 20 cm;  $53 \pm 10$   $\mu$ m

pore size) containing 5 g of either hay, WC, or GC. Hay was ground in a Wiley mill (Arthur H. Thomas, Philadelphia, PA) to pass through a 2-mm screen. Bags were incubated in the rumen for 98, 72, 48, 36, 24, 14, 9, 5, 2, and 0 h. After incubation, all bags were removed and rinsed with a hose to remove large particulate matter. Bags were then rinsed using a top-loading washing machine (General Electric, Louisville, KY) on delicate cycle. Bags were agitated for one minute, drained, and spun for two minutes. Cycle was repeated until water was clear. Bags were dried in a forced-air oven (55°C; The Grieve Corporation, Round Lake, IL) for 48 h and stored at room temperature until analysis.

Liquid dilution rate was determined by dosing 200 ml Co-EDTA (Uden, et al., 1980) intraruminally 2 h prior to feeding on d 6 of each collection period. Ruminal fluid samples were collected with a suction strainer at 0, 2, 4, 6, 8, 10, and 12 h post-feeding. After collection, pH was determined and then sample (200 ml) was acidified with 6.0 N HCl at 1 mL/100 mL ruminal fluid. Samples were then frozen (-20°C) until ammonia and cobalt analysis. Another ruminal sample (3 ml) was collected and added to 0.75 ml metaphosphoric acid and frozen (-20°C) until analyzed for VFA.

On d 21 of each period, ruminal evacuations were conducted to determine ruminal fill. Ruminal contents were removed, weighed and sub-sampled. A grab sample was taken for analysis of DM, OM, ADF, and NDF. A second ruminal content sample (4 kg) was taken and 2 L of formalin/saline solution (3.7% formaldehyde/0.9% NaCl) was added (Zinn and Owens, 1986) for isolation of bacterial cells which were later analyzed for DM, ash, N, and purine. Samples were stored frozen (-20°C) until analysis.

#### *Laboratory Analysis*

Diet, ort, and fecal samples were dried using a forced-air oven (55°C; The Grieve Corporation, Round Lake, IL) for 48 h. Dried samples were ground in a Wiley mill to pass a 2-mm screen. Duodenal and ileal samples were lyophilized (Virtis Genesis 25LL; The Virtis Company, Inc., Gardiner, NY) and ground with Wiley mill to pass a 1-mm screen.

Diet, ort, duodenal, ileal, and fecal samples were analyzed for DM, OM, ash, CP, N (AOAC, 1997), ADF (Goering and Van Soest, 1970), and NDF (Robertson and Van Soest, 1991) using an Ankom 200 Fiber Analyzer (Ankom Co., Fairport, NY). Chromium concentrations were analyzed in duodenal, ileal, and fecal samples by the spectrophotometric method (Fenton and Fenton, 1979). In situ residue was analyzed for DM, CP, N (AOAC, 1997), ADF (Goering and Van Soest, 1970), NDF (Robertson and Van Soest, 1991), and purines (Zinn and Owens, 1986).

Ruminal fluid samples were centrifuged at 20,000 x g for 20 minutes for analysis of ammonia (Broderick and Kang, 1980) and VFA (Goetsch and Gaylean, 1983) quantified by gas chromatography (Hewlett Packard 5890A Series II GC, Wilmington, DE) using a capillary column. Cobalt was analyzed by methods described by Uden et al. (1980) by an air-plus acetylen flame using atomic absorption spectroscopy (Model: 3030B, PerkinElmer, Inc., Wellesley,

MA).

Ruminal content was analyzed for DM, OM, ash, and N (AOAC, 1997). A Waring blender (Model: 37BL19 CB6, Waring Products, New Hartford, CT) was used to blend ruminal content. Sample was blended on high speed for 1 minute, and mixture strained through four layers of cheesecloth. Feed particles and protozoa were removed via centrifugation at 1,000 x g for 10 min. Bacteria were separated from supernatant by centrifuging at 20,000 x g for 20 min. Isolated bacterial cells and duodenal contents were analyzed for purines (Zinn and Owens, 1986) as microbial markers.

#### *Statistical Analysis*

Data was analyzed as a completely randomized design using GLM procedures of SAS (SAS Inst., Cary, NC). The model included diet and canola level as fixed effects and steer as random effects. Data over time was analyzed as a repeated measures design using mixed procedures of SAS (SAS Inst., Cary, NC). The model included animal, diet, time, diet x time, and animal x diet.

### **Results and Discussion**

All diets consisted of primarily switchgrass hay (5.8% CP, 77.7% NDF, and 47.0% ADF). Canola seed averaged 23.3% CP, 51.8% NDF, 36.9% ADF, and 39.6% fat. Canola was supplemented at 8% of diet DM.

Hay DMI and total DMI were not affected by treatment (Table 1). These findings are in agreement with Ferlay et al. (1992), Hussein et al. (1995), and Aldrich et al. (1997b) when cattle were fed forage based diets with fat inclusion of 5 to 10% of diet DM.

Intake of hay OM and total OM was not different across treatments which was similar to Hussein et al. (1995) and Aldrich et al. (1997a) when supplementing cattle with canola on forage based diets. Bacterial OM at duodenum was increased ( $P < 0.01$ ) with canola supplementation compared to CON and increased ( $P = 0.08$ ) with GC compared with WC. Others have reported no differences in duodenal OM flow resulting from canola seed supplementation (Ferley et al., 1992; Aldrich et al., 1997a). In regards to OM digestibility, no differences were observed. Hussein et al. (1995) reported no differences for apparent and true ruminal OM digestibility, but total tract OM was decreased by canola supplementation which they attributed to low digestibility of the seed coat. Aldrich et al. (1997a) reported ruminal and intestinal OM digestibilities were similar, but total tract OM digestibility was decreased with canola.

Hay CP intake was not affected by treatment (Table 2). As expected, total CP intake was increased ( $P = 0.05$ ) with canola supplementation compared with controls. Supplementation with canola increased ( $P = 0.03$ ) apparent ruminal digestibility and decreased ( $P \leq 0.04$ ) true ruminal and small intestinal digestibilities. Neither total tract CP digestibility or microbial efficiency was different ( $P \geq 0.21$ ) among treatments. Aldrich et al. (1997a) observed no differences with microbial efficiency when steers were supplemented canola at 10% of diet DM.

Ruminal fermentation data are presented in Table 3. Ruminal pH decreased ( $P = 0.03$ ) with canola supplementation. Other studies have reported no difference in pH with fat supplementation through seeds (Hussein et al., 1996; Khorasani and Kennelly, 1998) or oil (Ferlay and Doreau, 1992). Ruminal ammonia concentration was not affected ( $P = 0.27$ ) nor was total VFA concentration ( $P = 0.52$ ) across treatments which is in agreement with other studies (Hussein et al., 1995; Aldrich et al., 1997; Khorasani and Kennelly, 1998). Contrary to Hussein et al. (1995) and Petit et al. (1997) who reported no differences in molar proportions of acetate, propionate, and butyrate, we found decreased ( $P = 0.10$ ) acetate with canola supplementation compared with CON and an increase ( $P < 0.01$ ) with WC supplementation compared with GC. Propionate, on the other hand, decreased ( $P < 0.01$ ) with WC vs GC. This trend of decreased acetate and increased propionate was similar to findings of Ferlay and Doreau (1992) with supplementation of canola oil. This shift in molar proportions they attributed to the decrease in ruminal fiber digestion. However in our study, we did not observe a decrease in ruminal fiber digestion with fat supplemented steers. Butyrate was not affected by treatment.

In situ data indicated no differences (data not shown) in rate of hay DM, NDF, or ADF ruminal disappearance. As expected, rate of canola DM, NDF, and ADF were increased ( $P \leq 0.01$ ) with GC compared to WC due to grinding process of the seedcoat (data not shown). Ruminal fill and fluid dilution rate was not affected ( $P \geq 0.60$ ) by treatment.

### Implications

Supplementation with ground or whole canola at 8% diet DM had no effect on intake or fiber digestion. These results suggest canola seeds, fed in either form, may be added to increase energy density of low-quality forage.

### Literature Cited

- Aldrich, C. G., N. R. Merchen, J. K. Drackley, S. S. Gonzalez, G. C. Fahey, Jr., and L. L. Berger. 1997a. The effects of chemical treatment of whole canola seed on lipid protein digestion by steers. *J. Anim. Sci.* 75:502-511.
- Aldrich, C. G., N. R. Merchen, J. K. Drackley, G. C. Fahey, Jr., and L. L. Berger. 1997b. The effects of chemical treatment of whole canola seed on intake, nutrient digestibilities, milk production, and milk fatty acids of Holstein cows. *J. Anim. Sci.* 75:512-521.
- AOAC. 1997. Official Methods of Analysis. Vol. I (16<sup>th</sup> Ed.). Association of Official Analytical Chemists, Arlington, VA.
- Broderick G. A. and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63:64-75.
- Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. *Can. J. Anim. Sci.* 59:631-634.
- Ferlay, A., F. Legay, D. Bauchart, C. Poncet, and M. Doreau. 1992. Effect of a supply of raw or extruded rapeseeds on digestion in dairy cows. *J. Anim. Sci.* 70:915-923.
- Ferlay, A. and M. Doreau. 1992. Influence of method of administration of rapeseed oil in dairy cows. 1. Digestion of nonlipid components. *J. Dairy Sci.* 75:3020-3027.
- Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analysis. U.S. Dept. Of Agric. Handbook, No. 379. Washington, D.C.
- Goetsch A. L. and M. L. Galyean. 1983. Influence of feeding frequency on passage of fluid and particulate markers in steers fed a concentrate diet. *Can. J. Anim. Sci.* 63:727-730.
- Hussein, H. S., N. R. Merchen, and G. C. Fahey, Jr. 1995. Effects of forage level and canola seed supplementation on site and extent of digestion of organic matter, carbohydrates and energy by steers. *J. Anim. Sci.* 73:2458-2468.
- Hussein, H. S., N. R. Merchen, and G. C. Fahey, Jr. 1996. Effects of forage percentage and canola seed on ruminal protein metabolism and duodenal flows of amino acid in steers. *J. Dairy Sci.* 79:98-104.
- Khorasani, G. R., G. de Boer, P. H. Robinson, and J. J. Kennelly. 1992. Effect of canola fat on ruminal and total tract digestion, plasma hormones, and metabolites in lactating dairy cows. *J. Dairy Sci.* 75:492-501.
- Khorasani, G. R. and J. J. Kennelly. 1998. Effect of added dietary fat on performance, rumen characteristics, and plasma metabolites of midlactation dairy cows. *J. Dairy Sci.* 81:2459-2468.
- Khorasani, G. R., P. H. Robinson, G. de Boer, and J. J. Kennelly. 1991. Influence of canola fat on yield, fat percentage, fatty acid profile, and nitrogen fractions in Holstein milk. *J. Dairy Sci.* 74:1904-1911.
- Moore, J. A., R. S. Swingle, and W. H. Hale. 1986. Effects of whole cottonseed, cottonseed oil or animal fat on digestibility of wheat straw diets by steers. *J. Anim. Sci.* 63:1267-1273.
- Ørskov, E. R. and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* 92:499-503.
- Petit, H. V., R. Rioux, P. S. D'Oliveira, and I. N. do Prado. 1997. Performance of growing lambs fed grass silage with raw or extruded soybean or canola seeds. *Can. J. Anim. Sci.* 77:455-463.
- Robertson, J. B. and P. J. Van Soest. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.
- Uden, P., P. E. Colucci, and P. J. Van Soest. 1980. Investigation of chromium, cerium, and cobalt as

markers in digesta. Rate of passage studies. J. Sci. Food Agr. 31:625-632.

Zinn, R. A. and F. N. Owens. 1986. A rapid procedure for purine measurement and it's use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66: 157-166.

Table 1. Effect of canola supplementation on OM digestion in steers fed low-quality hay

Item	Treatment <sup>a</sup>			SE	P-Value	Contrasts <sup>b</sup>	
	Control	WC	GC			CON vs Canola	WC vs GC
Hay DMI, kg/d	8.53	9.07	8.93	1.25	0.94	0.74	0.94
g/kg BW	18.4	19.0	19.1	1.68	0.94	0.74	0.98
Total DMI, kg/d	8.53	9.44	9.31	1.28	0.84	0.57	0.94
g/kg BW	18.4	19.8	19.9	1.71	0.76	0.47	0.98
Organic Matter							
Intake, kg/d							
Hay	7.99	8.49	8.37	1.17	0.94	0.74	0.94
Total	7.99	9.27	9.16	1.23	0.68	0.39	0.95
Duodenal, kg/d							
Bacterial	0.66	0.77	0.91	0.05	0.01	0.01	0.08
Apparent feed	3.03	3.13	2.95	0.22	0.84	0.96	0.56
Total	3.68	3.90	3.86	0.24	0.78	0.49	0.91
Ileal, kg/d	3.29	3.52	3.49	0.23	0.71	0.41	0.92
Fecal, kg/d	2.96	3.22	3.17	0.21	0.61	0.33	0.87
Digestibility, % of intake							
Apparent ruminal	52.4	56.1	56.0	4.12	0.74	0.44	0.98
True ruminal	60.8	65.1	66.4	3.17	0.38	0.19	0.76
Small intestine	5.2	4.1	4.5	1.04	0.74	0.46	0.80
Large intestine	4.4	3.4	3.6	0.78	0.59	0.32	0.82
Total tract	62.0	63.7	64.1	3.01	0.85	0.59	0.91

<sup>a</sup>Control = hay only; WC = whole canola; GC = ground canola. Supplements were provided at 8% of diet DM.

<sup>b</sup>CON vs Canola = hay vs canola supplements; WC vs GC = whole canola vs ground canola supplemented.

Table 2. Effect of canola supplementation on crude protein digestion in steers fed low-quality hay

	Treatment <sup>a</sup>					Contrasts <sup>b</sup>	
Item	Control	WC	GC	SE	P-Value	CON vs Canola	WC vs GC
Crude Protein							
Intake, g/d							
Hay	484.5	516.3	506.1	72.6	0.94	0.75	0.92
Total	484.5	706	698	87.9	0.13	0.05	0.95
Duodenal, g/d							
Bacterial	322	347	373	28.6	0.40	0.26	0.52
Apparent feed	356	419	407	28.7	0.25	0.10	0.77
Total	677	766	780	50.5	0.27	0.12	0.84
Ileal, g/d	378	484	431	43.9	0.24	0.13	0.39
Fecal, g/d	267	348	240	44.4	0.22	0.60	0.10
Digestibility, % of intake							
Apparent ruminal	-43.4	-13.1	-16.2	10.1	0.08	0.03	0.82
True ruminal	24.3	38.9	39.2	5.65	0.11	0.04	0.97
Small intestine	63.4	43.0	50.9	5.37	0.04	0.02	0.30
Large intestine	23.3	18.4	26.5	7.29	0.72	0.92	0.42
Total tract	43.4	48.3	61.3	7.70	0.21	0.21	0.24
Microbial Efficiency <sup>c</sup>	11.0	10.0	10.4	1.54	0.88	0.66	0.82

<sup>a</sup>Control = hay only; WC = whole canola; GC = ground canola. Supplements were provided at 8% of diet DM.

<sup>b</sup>CON vs Canola = hay vs canola supplements; WC vs GC = whole canola vs ground canola supplemented.

<sup>c</sup>grams of microbial N per kg of OM truly fermented.

Table 3. Effect of canola supplementation on ruminal pH and VFA molar proportion in steers fed low-quality hay

Item	Treatment <sup>a</sup>			SE	P-Value	Contrasts <sup>b</sup>	
	Control	WC	GC			CON vs	WC vs GC
pH	6.81	6.55	6.63	0.08	0.07	0.03	0.43
Ammonia, mM	2.81	2.86	4.55	0.90	0.27	0.39	0.19
VFA							
Total, mM	56.9	60.5	60.3	2.64	0.52	0.26	0.94
----- mol/100 mol-----							
Acetate	74.6	74.7	72.3	0.55	0.01	0.10	0.01
Propionate	15.5	15.1	16.3	0.26	0.02	0.63	0.01
Butyrate	6.47	6.56	6.97	0.27	0.34	0.35	0.29
Acetate:Propionate	4.82	4.96	4.46	0.09	0.01	0.31	0.01

<sup>a</sup>Control = hay only; WC = whole canola; GC = ground canola. Supplements were provided at 8% of diet DM.

<sup>b</sup>CON vs Canola = hay vs canola supplements; WC vs GC = whole canola vs ground canola supplemented.

## EFFECTS OF MATERNAL UNDERNUTRITION FROM EARLY- TO MID-GESTATION ON VISCERAL ORGANS OF THE EWE AND FETUS

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**ABSTRACT:** From d 28 until d 78 of gestation, 17 multiparous ewes (n = 9 with singletons; n = 8 with twins) were used to determine the influence of maternal nutrient restriction on visceral organ size of the ewe and fetus. Control (**Control**) ewes were fed pelleted beet pulp fortified with vitamins and minerals to meet requirements of early pregnancy, whereas nutrient-restricted ewes (**Restricted**) were fed 50% of Control. Rations were adjusted weekly for BW changes. On d 78 of gestation, ewes were slaughtered, and ewe and fetal digestive tracts were removed, stripped of digesta, and trimmed of fat and measured. Weights of ewe liver, lungs, heart, kidney, and pancreas were collected. Maternal eviscerated BW (EBW) and weights of the liver and kidney were reduced ( $P < 0.01$ ) in Restricted ewes. Total digestive tract weights (g) were greater ( $P = 0.02$ ) for Control ewes, and tended ( $P = 0.12$ ) to be greater as a % of EBW. The stomachs of Restricted ewes were also lighter ( $P < 0.02$ ) than in Control ewes because the rumen of the Restricted ewes made up a lower ( $P = 0.02$ ) percentage of EBW. Small intestine length did not differ ( $P = 0.26$ ) between treatments but small intestine weights were greater ( $P = 0.02$ ) in Control than Restricted ewes. Maternal large intestine weight and length did not differ ( $P \geq 0.38$ ) between treatments. A maternal diet  $\times$  number of offspring interaction ( $P = 0.001$ ) was noted for fetal digestive tract weight (% of EBW) because the magnitude of decreased fetal digestive tract was greater with Restricted ewes gestating singles compared to Restricted ewes gestating twins. Maternal visceral organ weight will decline with BW loss; however, certain organs may be more sensitive to nutrient restriction than others. Likewise, twinning may depress digestive tract development in the fetus, which may be compounded by maternal undernutrition.

**Key Words:** Ewes, Fetal, Nutrient restriction, Visceral organ

### Introduction

Ruminant livestock may experience severe intake restriction when feed resources are severely diminished, such as during blizzards or drought. Unfortunately, these environmental factors can occur during critical times of pregnancy, and maternal nutrient deficiencies during pregnancy can permanently affect development of fetal tissues (Barker, 1995). Such perturbations during fetal tissue development can reduce neonatal viability and create a predisposition to disease later in life (Barker, 1995). The

digestive tract seems to be an area that is highly influenced by undernutrition (Avila et al., 1989; Trahair et al., 1997a; Vonnahme et al., 2003). It has been demonstrated that the small intestine possess the ability to adapt to changing physiologic needs such as pregnancy (Ferraris and Diamond, 1997) or undernutrition (Ferraris et al., 2001). Ferraris et al. (2001) reported that intestinal weights of energy-restricted mice were 12% smaller than in mice fed for ad libitum consumption; however, the absorptive efficiency of the intestine from energy-restricted mice was increased by 60%. Restricting pregnant ewes to 70% of requirements tended to increase fetal gut weight at d 45 of gestation but reduced fetal gut weight on d 135 of gestation (Osgerby et al., 2002). Other researchers (Trahair et al., 1997a; Vonnahme et al., 2003), however, have observed only minor reductions in fetal intestinal weights when ewes were nutrient restricted during gestation.

In addition to the effects of maternal nutrient restriction, number of offspring gestated may influence fetal digestive tract development. Vonnahme et al. (2003) reported higher caruncular vascularity in ewes gestating twins compared to single lambs. Increased caruncular vascularity may be an adaptation mechanism developed to deal with increased nutrient demand of gestating twins. Unfortunately, limited data are available regarding the influence of twinning on digestive tract growth during maternal nutrient restriction. Therefore, our objective was to evaluate the influence of number of developing fetuses and maternal nutrient restriction during early gestation on visceral organs of ewes and their fetuses at d 78 of gestation.

### Materials and Methods

Starting on d 20 of pregnancy, 17 multiparous ewes (initial BW  $90 \pm 3$  kg) were used in a split-plot design experiment in accordance with an approved University of Wyoming Animal Care and Use Committee protocol. Ewes were weighed on two consecutive days to establish initial BW, and diets were formulated based on metabolic BW ( $BW^{0.75}$ ). A control (**Control**) diet consisting of pelleted beet pulp (10.0% CP and 79.7% TDN) was fed to meet 100% of the nutrient requirements for a ewe during the first 15 wk of gestation (NRC, 1985). The Control diet was fortified with a vitamin and mineral mixture that contained 51.4% sodium triphosphate, 47.6% potassium chloride, 0.39% zinc oxide, 0.06% cobalt acetate, and 0.50% ADE vitamin premix [8,000,000 IU vitamin A, 800,000 IU

vitamin D<sub>3</sub>, and 400,000 IU vitamin E/lb; the vitamin premix was fed to meet the vitamin A requirements]). On d 21 of gestation, all ewes were placed into individual pens (1.6 × 2.6 m) and fed the Control ration for 1 wk. On d 28, ewes were randomly allotted to one of two treatments. They either remained on the Control diet or were assigned to a nutrient restricted (**Restricted**) group. Restricted ewes were fed one half the control's daily allowance. Daily rations were delivered at 0700 each day. Pregnancy conformation and number of fetuses were determined via ultrasonography (Ausonics Microimager 1000 sector scanning instrument: Ausonics Pty Ltd, Sydney, Australia) on d 45 of gestation.

On d 78 of gestation, ewes were euthanized with an overdose of sodium pentobarbitol (Abbott Laboratories, Abbott Park, IL) exsanguinated, and the fetus(s) were immediately removed. Ewe and fetal digestive tracts were stripped of digesta, trimmed of fat, and measured. Weights of ewe liver, lungs, heart, kidney, and pancreas were also recorded. Data were analyzed using the GLM procedures of SAS (SAS Inst. Cary, N.C.) using a model for a split-plot design. Maternal dietary treatment was the main plot tested against animal within treatment (error a), and the number of offspring and dietary treatment × number of offspring interaction was the subplot tested against residual error (error b).

## Results and Discussion

### *Maternal undernutrition*

Restricted ewes exhibited reduced rumen, reticulum, and omasal weights ( $P \leq 0.05$ ); therefore, the overall stomach weight was reduced ( $P = 0.01$ ) in ewes receiving the Restricted diet (Table 1). The rumen represented less ( $P = 0.02$ ) percentage of the eviscerated BW (EBW) for Restricted than for Control. This difference was large enough to reduce total stomach weight ( $P = 0.02$ ) with nutrient restriction. A treatment × offspring interaction was noted ( $P = 0.10$ ) for ewe abomasum weight. Control ewes gestating twins had heavier abomasums compared to Control ewes gestating singletons (211.8 vs 228.9 g), whereas Restricted ewes gestating twins had lighter abomasal weights compared to Restricted ewes gestating singletons (163.5 vs 221.2 g). This interaction was more pronounced ( $P = 0.02$ ) when expressed as a percentage of EBW because the abomasums of Control ewes gestating singletons were smaller (0.38%) compared to Restricted ewes gestating a singleton (0.45%), whereas the abomasum weights of Control ewes gestating twins (0.45%) were greater than Restricted ewes gestating twins (0.35%). Using the same dietary treatments as this study, Vonnahme et al. (2003) reported that abomasal weights were not affected by diet. However, Ferraris et al. (2001) demonstrated that the gastrointestinal tract of non-pregnant mice was not greatly reduced, but stomach size increased with energy restriction.

Restricted ewes had less ( $P = 0.02$ ) small intestine weight than Control ewes, which was not attributed to a reduction ( $P = 0.86$ ) in small intestine length. However, the reduction in small intestine weight coincided with a reduction ( $P = 0.01$ ) in EBW; therefore, small intestine

weight did not differ between treatments ( $P = 0.53$ ) when expressed as a percentage of EBW. In a companion study to this trial, Vonnahme et al. (2003) reported a 12% decrease in ewe circulating glucose concentrations. Insulin-like growth factor is highly regulated by nutritional status (Breier et al., 1986) and is positively correlated to serum glucose concentrations (Funston et al., 1995). Because circulating concentrations of IGF can influence the growth of the small intestine (Fohlenhag et al., 1997), a reduction in available glucose in the Restricted ewes and a subsequent theoretical reduction in serum IGF could affect the weight of the small intestine (Trahair et al., 1997b). However, it is important to note that changes in small intestine weight followed a concomitant change in ewe BW. Overall, ewe digestive tracts decreased in weight by 16% ( $P = 0.02$ ) with nutrient restriction; this reduction was not as pronounced when expressed as a percentage of EBW ( $P = 0.12$ ). Nutrient restriction reduced liver and kidney weights when expressed in g ( $P \leq 0.01$ ) or as a percentage of EBW ( $P \leq 0.04$ ) when compared to Control ewes. The liver is a very dynamic organ that has been shown to fluctuate quite readily with alterations in intake (Drouillard et al., 1991; Vonnahme et al., 2003). A low plane of nutrition has been shown to reduce weights of visceral organs in sheep and pigs (Koong et al., 1985). Our data indicate that certain regions of the digestive tract could be less sensitive to plane of nutrition and may compensate for physiological perturbations as suggested by Ferraris and Diamond (1997).

### *Fetal undernutrition*

The weights of individual compartments of the fetal stomach were not influenced ( $P \geq 0.16$ ) by maternal undernutrition on d 78 of gestation (Table 2). However, when expressed as a percentage of EBW the reticulum was smaller ( $P = 0.05$ ) in Restricted fetuses compared to Control fetuses. Intestine length tended ( $P = 0.10$ ) to be shorter in Restricted fetuses, but weight did not differ ( $P = 0.38$ ) between dietary groups. Avila et al. (1989) also reported marked reductions in small intestine length and weight in growth retarded fetal sheep at 140 d of gestation.

Offspring gestated as twins had reduced ( $P = 0.03$ ) intestine weights and tended ( $P = 0.09$ ) to have reduced digestive tract weights. When expressed as a percentage of EBW, the fetal stomach was not influenced by maternal nutrient restriction ( $P \geq 0.27$ ), except for the reticulum ( $P = 0.05$ ). Number of offspring had a more pronounced influence ( $P = 0.06$ ) on fetal intestine weight than dietary treatment ( $P = 0.26$ ). A maternal nutrient restriction × number of offspring interaction was noted ( $P = 0.001$ ) for fetal digestive tract weight (% of EBW). This interaction was due to differences between Control fetuses (2.17%) gestated as singletons and Restricted fetuses gestated as singletons (1.75%) compared to Control fetuses gestated as twins (1.77%) and Restricted fetuses gestated as twins (1.45%).

Vonnahme et al. (2003) reported that twin fetuses gestated by nutrient-restricted ewes had significantly lower blood thyroxine compared to Control fetuses gestated as singletons or twins and Restricted fetuses gestated as singletons. Thyroxine is known to be highly involved in

gut mucosal growth and development (Hodin et al., 1996). Ogueh et al. (2000) demonstrated that women pregnant with twins had lower thyroxine than women with single pregnancies. Therefore, twinning may have a more pronounced influence on the production of thyroxine, which in turn, may explain the stronger relationship of twinning compared to maternal nutrient restriction on fetal digestive tract weight. Overall both dietary treatment and number of offspring influenced fetal digestive tract development.

### Implications

Although the visceral organs in ewes respond to nutrient restriction during early gestation, certain regions of the digestive tract may be less sensitive to plane of nutrition than others. Maternal nutrient restriction may also retard development of the fetal digestive tract, and the number of offspring gestated may compound the reduction in fetal digestive tract growth. However, more research is needed to determine if the reduction in digestive tract growth in response to either maternal nutrient restriction or twinning has any long-term affect on animal performance.

### Literature Cited

- Avila, C. G., R. Harding, S. Rees, and P. M. Robinson. 1989. Small intestine development in growth-retarded fetal sheep. *J. Pediatr. Gastroenterol. Nutr.* 8:507-515.
- Barker, D. J. P. 1995. Fetal origins of coronary heart disease. *Br. Med. J.* 311:171-174.
- Breier, B. H., J. J. Bass, J. H. Butler, and P. D. Gluckman. 1986. The somatotrophic axis in young steers: influence of nutritional status on pulsatile release of growth hormone and circulating concentrations of insulin-like growth factor I. *J. Endocrinol.* 111:209-215.
- Drouillard, J. S., T. J. Klopfenstein, R. A. Britton, M. L. Bauer, S. M. Gramlich, T. J. Wester, and C. L. Ferrell. 1991. Growth, body composition, and visceral organ mass and metabolism in lambs during and after metabolizable protein or net energy restrictions. *J. Anim. Sci.* 69:3357-3375.
- Ferraris, R. P. and J. Diamond. 1997. Regulation of intestinal sugar transport. *Physiol. Rev.* 77:257-302.
- Ferraris, R. P., Q. X. Cao, and S. Prabhakaram. 2001. Chronic but not acute energy restriction increases intestinal nutrient transport in mice. *J. Nutr.* 131:779-786.
- Fohlenhag, K., V. Arrhenius-Nyberg, I. Sjogren, and K. Malmlof. 1997. Effects of insulin-like growth factor I (IGF-I) on the small intestine: a comparison between oral and subcutaneous administration in the weaned rat. *Growth Factors* 14:81-88.
- Funston, R. N., A. J. Roberts, D. L. Hixon, D. M. Hallford, D. W. Sanson, and G. E. Moss. 1995. Effect of acute glucose antagonism on the hypophyseal hormones and concentrations of insulin-like growth factor I (IGF)-I and IGF-binding proteins in serum, anterior pituitary, and hypothalamus of ewes. *Bio. Reprod.* 52:1179-1186.
- Hodin, R. A., A. Shei, M. Morin, and S. Meng. 1996. Thyroid hormone and the gut: selective transcriptional activation of a villus-enterocyte marker. *Surgery.* 120:138-143.
- Koong, L. J., C. L. Ferrell, and J. A. Neinaber. 1985. Assessment of interrelationships among levels of intake and production, organ size and fasting heat production in growing animals. *J. Nutr.* 115:1383-1390.
- NRC, 1985. Nutrient Requirements of Sheep, 6th rev. ed. Natl. Acad. Press, Washington, DC.
- Ogueh, O., A. P. Hawkins, A. Abbas, G. D. Carter, K. H. Nicolaides, and M. R. Johnson. 2000. Maternal thyroid function in multifetal pregnancies before and after fetal reduction. *J. Endocrinol.* 164:7-11.
- Osgerby, J. C., D. C. Wathes, D. Howard, and T. S. Gadd. 2002. The effect of maternal undernutrition on ovine fetal growth. *J. Endocrinology* 173:131-141.
- Trahair, J. F., T. M. DeBarro, J. S. Robinson, and J. A. Owens. 1997a. Restriction of nutrition in utero selectively inhibits gastrointestinal growth in fetal sheep. *J. Nutr.* 127:637-641.
- Trahair, J. F., S. J. Wing, K. J. Quinn, and P. C. Owens. 1997b. Regulation of gastrointestinal growth in fetal sheep by luminally administered insulin-like growth factor I. *J. Endocrinol.* 152:29-38.
- Vonnahme, K. A., B. W. Hess, T. R. Hansen, R. J. McCormick, D. C. Rule, G. E. Moss, W. J. Murdoch, M. J. Nijland, D. C. Skinner, P. W. Nathanielsz, and S. P. Ford. Maternal undernutrition from early- to mid-gestation leads to growth retardation, cardiac ventricular hypertrophy, and increased liver weight in the fetal sheep. *Biol. Reprod.* 69:133-140.

Table 1. Effects of maternal undernutrition from early- to mid-gestation on d-78 of gestation ewe visceral organ measurements

Item	Treatments <sup>a</sup>		SEM	<i>P</i> <sup>b</sup>		
	Control	Restricted		Treatment	Offspring	Treatment × Offspring
Stomach, g <sup>c</sup>	1,577.4	1,219.1	87.9	0.01	0.65	0.64
Rumen	1,020.4	774.3	58.1	0.01	0.94	0.85
Reticulum	189.0	153.5	12.5	0.05	0.55	0.54
Omasum	147.7	99.0	9.4	0.002	0.17	0.60
Abomasum	220.4	192.4	15.8	0.21	0.35	0.10
Small intestine						
Length, cm	2,220.7	2,193.3	116.7	0.86	0.97	0.23
Weight, g	409.3	354.1	16.3	0.02	0.96	0.49
Cecum	65.8	60.4	5.8	0.49	0.59	0.85
Large intestine						
Length, cm	670.7	638.8	26.4	0.38	0.84	0.39
Weight, g	441.8	445.9	23.5	0.90	0.08	0.04
Digestive tract, g <sup>d</sup>	2,494.3	2,079.5	116.2	0.02	0.46	0.38
Lungs	820.5	689.0	65.6	0.16	0.81	0.99
Liver	906.8	691.9	38.9	0.001	0.39	0.22
Pancreas	82.6	84.4	9.8	0.89	0.65	0.52
Heart	368.4	367.9	12.9	0.98	0.27	0.52
Kidney	167.65	127.7	9.3	0.01	0.18	0.81
Eviscerated BW	52,546	47,607	1,914	0.01	0.37	0.82
	----- % of eviscerated BW -----					
Stomach <sup>c</sup>	3.01	2.55	0.12	0.02	0.87	0.32
Rumen	1.95	1.62	0.09	0.02	0.52	0.56
Reticulum	0.36	0.32	0.02	0.15	0.84	0.31
Omasum	0.28	0.21	0.01	0.98	0.32	0.72
Abomasum	0.42	0.40	0.02	0.58	0.43	0.02
Small intestine	0.79	0.75	0.04	0.53	0.49	0.47
Cecum	0.13	0.13	0.01	0.98	0.84	0.97
Large intestine	0.85	0.93	0.04	0.15	0.15	0.01
Digestive tract <sup>d</sup>	4.77	4.36	0.18	0.12	0.94	0.15
Lungs	1.57	1.45	0.12	0.48	0.51	0.83
Liver	1.73	1.45	0.05	0.001	0.83	0.03
Pancreas	0.16	0.18	0.08	0.52	0.43	0.49
Heart	0.70	0.78	0.03	0.08	0.96	0.75
Kidney	0.32	0.27	0.08	0.04	0.35	0.51

<sup>a</sup>Treatments: Control: Pelleted beet pulp fortified with vitamins and minerals fed at 100% of NRC (1985) requirements for ewes in early- to mid-gestation; Restricted: 50% of Control.

<sup>b</sup>Treatment = Control (n = 9) or Restricted (n = 8); Offspring = gestated as a singleton (n = 9) or a twin (n = 8); Treatment × Offspring = interaction between Treatment and number of offspring gestated.

<sup>c</sup>Stomach: Rumen + Reticulum + Omasum + Abomasum.

<sup>d</sup>Digestive tract: Stomach + Small intestine + Cecum + Large intestine.

Table 2 Effects of number of developing fetuses and maternal undernutrition and from early- to mid-gestation on d-78 of gestation on fetal digestive tract measurements

Item	Treatments <sup>a</sup>				SEM	<i>P</i> <sup>b</sup>		
	Single		Twin			Treatment	Offspring	Treatment × Offspring
	Control	Restricted	Control	Restricted				
Stomach, g <sup>c</sup>	2.08	2.00	1.96	1.81	0.23	0.62	0.50	0.87
Rumen	1.30	0.86	0.80	0.81	0.21	0.35	0.24	0.33
Reticulum	0.26	0.22	0.27	0.19	0.04	0.16	0.72	0.66
Omasum	0.41	0.37	0.35	0.33	0.05	0.48	0.33	0.84
Abomasum	0.53	0.56	0.54	0.49	0.09	0.86	0.75	0.63
Intestine								
Weight, g	3.34	3.08	2.57	2.29	3.34	0.38	0.03	0.97
Length, cm	232.7	257.8	231.8	246.5	12.3	0.10	0.59	0.64
Digestive tract, g <sup>d</sup>	5.88	5.08	4.76	4.01	0.43	0.09	0.03	0.95
	-----% eviscerated BW -----							
Stomach <sup>c</sup>	0.75	0.69	0.73	0.67	0.06	0.27	0.63	0.95
Rumen	0.45	0.30	0.30	0.30	0.08	0.28	0.36	0.29
Reticulum	0.10	0.08	0.10	0.07	0.01	0.05	0.83	0.77
Omasum	0.15	0.13	0.13	0.12	0.01	0.22	0.32	0.61
Abomasum	0.19	0.19	0.20	0.18	0.03	0.61	0.93	0.70
Intestine	1.20	1.06	0.95	0.83	0.12	0.26	0.06	0.93
Digestive tract <sup>d</sup>	2.17	1.75	1.77	1.45	0.11	0.01	0.01	0.001

<sup>a</sup>Treatments: Control: Pelleted beet pulp fortified with vitamins and minerals fed at 100% of NRC (1985) requirements for ewes in early- to mid-gestation; Restricted: 50% of Control.

<sup>b</sup>Treatment = Control (n = 9) or Restricted (n = 8); Offspring = gestated as a singleton (n = 9) or a twin (n = 8); Treatment × Offspring = interaction between Treatment and number of offspring gestated.

<sup>c</sup>Stomach: Rumen + Reticulum + Omasum + Abomasum.

<sup>d</sup>Digestive tract: Stomach + Small intestine + Cecum + Large intestine.

**IDENTIFICATION OF THE MECHANISMS BY WHICH OMNIGEN-AF, A NUTRITIONAL SUPPLEMENT, AUGMENTS IMMUNE FUNCTION IN RUMINANT LIVESTOCK**

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**ABSTRACT:** OmniGen-AF, a recently-developed nutritional supplement, has been reported to effectively improve health and performance of lactating dairy cattle. However, its mechanisms of action have not been fully characterized. The hypothesis of this research was that feeding immunocompromised ruminant livestock with OmniGen-AF increases innate immune function. To test this hypothesis, sixty sheep were assigned to five treatments: **1:** control, **2:** immunosuppressed with dexamethasone injection, **3:** immunosuppressed with OmniGen-AF, **4:** immunosuppressed with a pathogen challenge (*Aspergillus fumigatus*) and **5:** immunosuppressed with pathogen challenge and OmniGen-AF. Sheep were given these treatments for 28 days after which two indexes of innate immune function were assessed: **A:** L-selectin and **B:** interleukin-1 $\beta$  (IL-1 $\beta$ ). Dexamethasone injection reduced innate immune function ( $P<0.05$ ; i.e., reduced neutrophil L-selectin and IL-1 $\beta$  concentrations). Administration of OmniGen-AF to non-pathogen-challenged sheep increased ( $P<0.05$ ) L-selectin but did not affect IL-1 $\beta$  ( $P>0.05$ ). Pathogen challenge did not affect ( $P>0.05$ ) L-selectin or IL-1 $\beta$ . However, exposure to pathogen potentiated actions of OmniGen-AF on IL-1 $\beta$ . Specifically, OmniGen-AF restored ( $P<0.05$ ) normal levels of neutrophil L-selectin and boosted levels of IL-1 $\beta$  in pathogen-challenged sheep. OmniGen-AF functions, in part, by augmenting innate immune function in immunosuppressed livestock.

**Key Words:** Immunity, Sheep, OmniGen-AF, L-selectin, Interleukin-1 $\beta$

**Introduction**

Animals are able to resist invasion by pathogens via a combination of the innate immune system and the adaptive (antibody-mediated) immune system (Janeway *et al.*, 2001). The innate immune system represents the first line of defense and thereby provides the adaptive system the time it requires to develop an antibody response. Aspects of the innate system include physical epithelial barriers, gastric acid, digestive enzymes and activities of phagocytic cells which may be recruited to any site of infection (Janeway *et al.*, 2001).

The neutrophil is a phagocytic cell of the innate immune system. It is manufactured in bone and released to circulate in blood where it functions to monitor for sites of an infection (Burton and Erskine, 2003). Neutrophils

monitor sites infection by “rolling” along the endothelial lining of blood vessels where local production of cytokines causes them to firmly attach adjacent to a site of infection, to migrate through the endothelial lining toward pathogens and to subsequently engulf pathogens via phagocytosis (Burton and Erskine, 2003). Following ingestion, neutrophils digest pathogens via one of two mechanisms: an oxidative burst or via engulfment by lysosomes. Of interest, a recent study showed that neutrophils also cast bacteriocidal “nets” extracellularly. These “nets” consist of DNA and a mixture of proteases which digest pathogens (Brinkmann *et al.*, 2004).

Neutrophil rolling is mediated by weak interactions between an extracellular neutrophil adhesion molecule (L-selectin) and other adhesion molecules associated with the endothelial cell surface (Burton and Erskine, 2001). L-selectin is critical to this process and recent studies have documented that glucocorticoid-mediated stress and immunosuppression bring about a reduction in innate immune function by causing release of the extracellular pool of neutrophil L-selectin (Tempelman *et al.*, 2002; Weber *et al.*, 2001). This thereby reduces or eliminates the ability of neutrophils to search for pathogens and enhances the likelihood of an infection. An example of this occurs at parturition in dairy cattle; a time when cows are maximally susceptible to infections such as mastitis (Burton and Erskine, 2003). The glucocorticoid (stress-related) peak which occurs at parturition brings about a marked reduction in neutrophil L-selectin and increases susceptibility to infection (Burton and Erskine, 2003). Similarly, neutrophil function is also supported by a variety of other molecules including the pro-inflammatory cytokine: interleukin-1 $\beta$  (IL-1 $\beta$ ). IL-1 $\beta$  is an inducible cytokine which plays critical roles in the neutrophil’s ability to communicate with other cells and to mediate pathogen sequestration (Janeway *et al.*, 2001).

To support animals’ innate abilities to resist pathogen, producers often provide sub-therapeutic antibiotics in feeds. This is a common practice in North America with about 20-25 million lbs of antibiotics administered to livestock annually (UCS, 2003). However, the practice has fallen into disfavor in the European Union and some Asian countries (e.g., Japan) and there has been interest in developing alternatives to this practice as a strategy to possibly reduce the development of antibiotic-resistant human pathogens.

In this experiment, we tested the ability of a new feed product (OmniGen-AF) to augment innate immune function in ruminant livestock. Early reports have indicated its ability to inhibit fungal growth *in vitro* (Puntenney *et al.*, 2003); however, field reports indicated it was effective in preventing diseases in lactating dairy cattle. To test this hypothesis, a sheep study was completed in which efficacy of OmniGen-AF was evaluated in immunosuppressed sheep and in immunosuppressed sheep challenged with pathogen (*A. fumigatus*).

## Materials and Methods

This study was approved by Oregon State University's Animal Care and Use Committee. Sixty Polypay X Friesian/Suffolk sheep (25 wethers and 35 ewe lambs: ca. 85 lbs each) were assigned to five treatments in January, 2003. The treatments consisted of: **1** control, **2** immunosuppressed, **3** immunosuppressed with OmniGen-AF, **4** immunosuppressed and pathogen-challenged and **5** immunosuppressed, pathogen-challenged and supplemented with OmniGen-AF. Five males and seven females were randomly assigned to each treatment. The duration of the experiment was 28 days. Immunosuppression was induced by twice daily injection of dexamethasone (Azium: 0.1 mg/kg BW, twice/day). This is a model of extreme stress which was reported by Dr. Jeanne Burton at Michigan State University (Weber *et al.*, 2001). Azium was injected sub-cutaneously in the neck region. OmniGen-AF® (Prince-Agri Products, Quincy, IL) was administered to animals in Treatments 3 and 5 by feeding at a dose of 0.5% w/w. Pathogen challenge was provided to Treatments 4 and 5 by daily administration of highly-molded wheat mill run (1 lb/head/day). This sample of wheat mill run had been obtained from a dairy in Washington State with high incidence of hemorrhagic bowel syndrome and abortions. Treatments 1, 2 and 3 were provided additional wheat bran (1 lb/head/day). All animals were fed alfalfa hay free choice and provided 0.75 lbs of ground corn/head/day. Sources of ground corn and wheat bran were baked at 95 °C for 24 hours prior to feeding to reduce the likelihood of introducing viable pathogen to animals on Treatments 1, 2 and 4.

Jugular blood samples (10 ml) were taken from animals on Day 28 of the study using citric acid as an anti-coagulant. Blood samples were immediately placed on ice and transported to the laboratory where neutrophils were isolated by Ficoll-Paque Plus (Amersham Biosciences) using gradient centrifugation according to manufacturer's recommendations.

Protein from neutrophils (20 µg) was applied to SDS-PAGE gels from six randomly-chosen animals on each treatment and electrophoresed. Samples were then transferred to a nitrocellulose membrane via Western blotting (Ausubel *et al.*, 1989) and membranes were blocked with 5% skim milk in TTBS. Primary antibody (L-selectin or IL-1β: VMRD, Pullman, WA) was exposed

to membranes for 1 hour after which membranes were exposed to secondary antibody (goat anti-mouse HRP: BioRad) for 1 hour. Membranes were washed five times with TTBS and then exposed to Xray film for 1 hour (L-selectin) or overnight (IL-1β) and developed. Intensity of exposures was determined by scanning densitometry using a BioRad VersaDoc 1000 imager and PDQuest software.

*Animal Health.* During the study, animal health was closely monitored. Rectal temperatures were taken weekly. When animals appeared ill, a veterinarian from the Oregon State University College of Veterinary Medicine was employed to diagnose illnesses.

Following completion of this study, animals were held in the Oregon State University sheep facility for a 28-day clearance period. This was judged by the OSU College of Veterinary Medicine to provide adequate time for clearance of residual Azium.

*Statistical analyses.* Concentrations of L-selectin and IL-1β were expressed as arbitrary densitometer units. Analysis of a variance was used to determine if differences ( $P < 0.05$ ) existed among the five treatments (Steel and Torrie, 1980) using SAS. A Student-Neuman-Keul multiple range test was then used to test for individual treatment differences. A 5% level of significance was adopted for all comparisons.

## Results and Discussion

Administration of Azium to Treatments 2, 3, 4 and 5 caused marked immunosuppression. Evidence of this is illustrated in both Figures 1 and 2 where Azium injection caused a marked reduction ( $P < 0.05$ ) in L-selectin concentration (Figure 1) and completely abolished ( $P < 0.05$ ) neutrophil IL-1β (Figure 2; Treatment 2 compared to Treatment 1 [control]). These observations indicate clearly that the Burton model (Weber *et al.*, 2001), which was developed as an model of extreme stress in dairy cattle, provides an adequate model for immunosuppression in sheep. Further work in this area may be directed toward titration of an optimal dose for immunosuppression in sheep.

During the 28-day study, few health problems were detected. However, in the fourth week of the study, three sheep on Treatment 4 (immunosuppressed and pathogen-challenged) developed lethargy, ruminal hypomotility and pyrexia (each over 40 °C). Mean body temperatures for all sheep are shown in Figure 3. The attending veterinarian diagnosed the animals as having pneumonia. Two of these three animals were slaughtered in the OSU College of Veterinary Medicine following the trial by a veterinary pathologist. One was devoid of any gastrointestinal pathology. The other demonstrated moderate but locally extensive ulcerative rumenitis with mild, emphysematous lymphadenopathy. Other animals on this treatment were not euthanized. It is possible that the pneumonia and gastrointestinal pathologies noted in one sheep were

related to the immunosuppressive effects of Azium and the presence of food-borne pathogen.

Effects of OmniGen-AF on indexes of innate immune function in non-pathogen-challenged sheep are shown also in Figures 1 and 2 (Treatment 3 versus Treatment 2). Addition of OmniGen-AF to the diet restored normal levels of L-selectin ( $P<0.05$ ) but had no effect ( $P>0.05$ ) on neutrophil IL-1 $\beta$  concentration. These data indicate potential for the feed product to restore ability of neutrophils to monitor the endothelial cell lining for sites of infection but also indicate that IL-1 $\beta$  function remains repressed, even in the presence of OmniGen-AF.

Animals on Treatments 4 and 5 were also challenged with a contaminated feed product (wheat mill run). We tested this feed and determined that it was contaminated with *A. fumigatus* (>1 million spores/g); however, the multitude of colors present in the feed indicated other molds were also present. Addition of OmniGen-AF to pathogen-challenged sheep also increased L-selectin ( $P<0.05$ ) concentration (Figure 1). Of interest, the presence of pathogen potentiated actions of OmniGen-AF on IL-1 $\beta$ . Specifically, OmniGen-AF caused a 2- to 3-fold increase ( $P<0.05$ ) in IL-1 $\beta$  concentration when fed in the presence of a heavily-molded feed sample.

It is difficult to envision how a nutritional product might augment immune function in the manner described here. However, it has been reported that nutrients have profound effects on immune status. Nutrients that have been demonstrated (in either animal or human studies) to be required for the immune system to function efficiently include essential amino acids, the essential fatty acid linoleic acid, vitamin A, folic acid, vitamin B6, vitamin B12, vitamin C, vitamin E, Zn, Cu, Fe and Se (Calder and Kew, 2002). Practically all forms of immunity may be affected by deficiencies in one or more of these nutrients. Undernutrition leading to impairment of immune function can be due to insufficient intake of energy. In this sense, OmniGen-AF, as a nutritional product, may exert its effects on immune function as do other nutrients.

### Implications

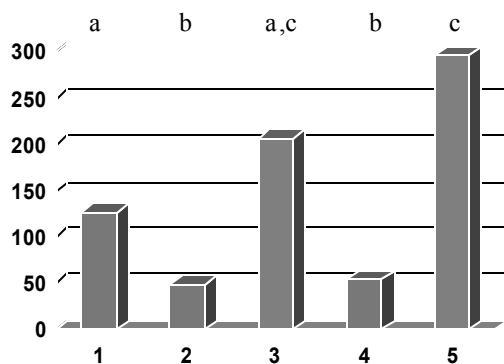
Feed products such as OmniGen-AF have potential to augment innate immune function in ruminant livestock and, possibly, to improve herd health. Further work on abilities of this and similar products to replace a portion of the antibiotics currently fed at sub-therapeutic levels needs further investigation.

### Acknowledgements

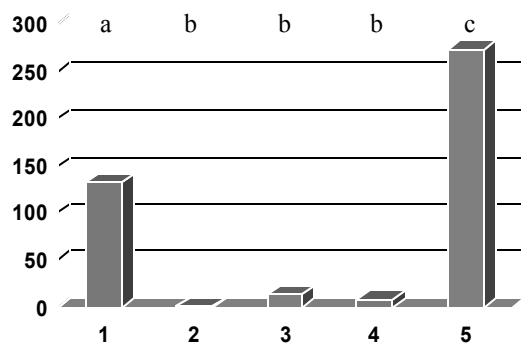
The authors are grateful to Tom Nichols and his crew at the OSU Sheep barns for their support in carrying-out the experiment.

### References

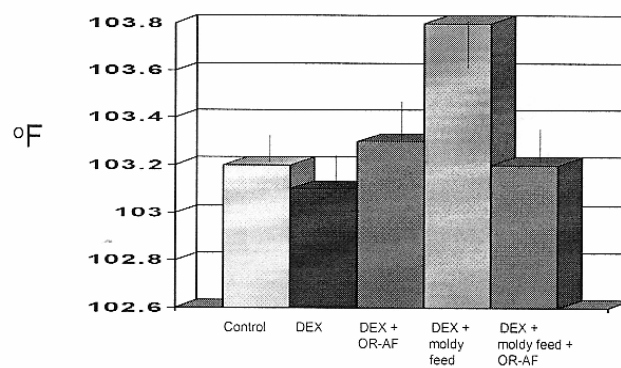
- Ausubel F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Deidman, J.A. Smith, and K. Struhl. Current Protocols in Molecular Biology. Greene Publishing Assoc. and Wiley Interscience. John Wiley and Sons. N.Y. 1989.
- Brinkmann, V., U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann, D.S. Weiss, Y. Weinrauch, and A. Zychlinsky. Neutrophil extracellular traps kill bacteria. *Science* 303, 1532-1525, 2004.
- Burton, J.L., and R.J. Erskine. Immunity and mastitis. Some new ideas for an old disease. *Vet. Clin. Food Anim.* 19:1-45, 2003.
- Calder, P.C., and S. Kew. The immune system: a target for functional foods. *Br. J. Nutr.* 88:165-177, 2002.
- Janeway, C., P. Travers, M. Walport and M. Shlomchik. Immunobiology. Garland Publishing, NY. 2001.
- Puntenney, S., Y.-Q. Wang, N.E. Forsberg. Mycotic infections in livestock: Recent insights and studies on etiology, diagnostics and prevention of Hemorrhagic Bowel Syndrome. *Proc. S.W. Anim. Nutr. Manag. Conf.* 49-62, 2003.
- Steel, R.G.D. and J.H. Torrie. Principles and Procedures of Statistics. McGraw-Hill, NY, 1980.
- Tempelman, R.J., P.M. Saama, A.E. Freeman, S.C. Kelm, A.L. Kuck, M.E. Kehrli, and J.L. Burton. Genetic variation in bovine neutrophil sensitivity to glucocorticoid challenge. *Acta Agric. Scand. Sect. A Animal Sci.* 52:189-202, 2002.
- Weber, P.S.D., S.A. Madsen, G.W. Smith, J.J. Ireland, and J.L. Burton. Pre-translational regulation of neutrophil L-selectin in glucocorticoid-challenged cattle. *Vet. Immunol. Immunopath.* 83:213-240, 2001.
- Union of Concerned Scientists (2003). Food and Environment. Estimates of anti-microbial abuse in livestock. [www.ucsusa.org/food\\_and\\_environment/antibiotic\\_resistance/page.cfm?pageID=264](http://www.ucsusa.org/food_and_environment/antibiotic_resistance/page.cfm?pageID=264)



**Figure 1.** Neutrophil L-selectin concentrations in sheep exposed to the five experimental treatments outlined in text of article. Numbers on the x-axis correspond to Treatment. Numbers on the y-axis correspond to L-selectin in arbitrary densitometry units. Values are means of six animals following 28 days of treatment. If treatments do not share a common letter superscript, they differ significantly ( $P < 0.05$ ).



**Figure 2.** Neutrophil interleukin-1 $\beta$  concentrations in sheep exposed to the five experimental treatments outlined in text of article. Numbers on the x-axis correspond to Treatment. Numbers on the y-axis correspond to IL-1 $\beta$  in arbitrary densitometry units. Values are means of six animals following 28 days of treatment. Statistically-significant differences ( $P < 0.05$ ) are indicated in text. If treatments do not share a common letter superscript, they differ significantly ( $P < 0.05$ ).



**Figure 3.** Effects of the five treatments on body temperatures of the sheep. Values are means of twelve sheep/treatment  $\pm$  SEM. No differences ( $P > 0.05$ ) were detected among treatments.

## INCIDENCE OF FOODBORNE PATHOGENS AND ANTIMICROBIAL SUSCEPTIBILITY OF FECAL COLIFORMS IN STOCKER CALVES FED IONOPHORE

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**ABSTRACT:** Fifty-three crossbred calves ( $232 \pm 3$  kg) were purchased from auction barns to determine: 1) incidence of fecal shedding of *E. coli* O157:H7 and *Salmonella*, 2) influence of feeding an ionophore on shedding of *E. coli* O157:H7 and *Salmonella*, and 3) antimicrobial resistance of putative fecal coliforms isolated from calves receiving ionophore. Calves were blocked by BW and sex, and assigned in replicate, to receive mineral with ionophore (IONOPH; Lasalocid; 1.76 g/kg mineral) or without ionophore (CONTROL) for 60 d. Calves were fed a corn:wheat midds:soybean meal supplement at 1.5% BW/d. Fecal samples were collected and BW recorded on d 0, 33 and 60. Antimicrobial susceptibility of fecal coliforms ( $n = 11$  or 12/treatment) to 14 antibiotics was determined on each collection date. Average daily gain (ADG) was not different ( $P > 0.10$ ) between treatments and averaged  $0.92 \pm 0.07$  kg/d for IONOPH calves and  $0.87 \pm 0.07$  kg/d for CONTROL calves. Sick calves had decreased ( $P < 0.05$ ) ADG compared with healthy calves ( $0.78 \pm 0.10$  vs  $1.01 \pm 0.06$  kg/d). Incidence of *E. coli* O157:H7 was 1.9% and was not different among treatments ( $P > 0.10$ ). No *Salmonella* was isolated from any calf during the experimental period. Patterns of antimicrobial resistance were similar ( $P = 0.14$ ) between IONOPH and CONTROL calves. Isolates demonstrated the most resistance to oxytetracycline, chlorotetracycline, ampicillin and florfenicol (33, 29, 28 and 26% of isolates resistant, respectively). All calves were fed chlorotetracycline in the receiving ration for 10 d and a number of calves in both treatments were administered florfenicol for respiratory illness during the experiment. Feeding ionophore to stocker calves had no affect of fecal shedding of foodborne pathogens or on antimicrobial resistance in fecal coliforms.

**Key Words:** *E. coli* O157:H7, *Salmonella*, ionophores, foodborne pathogens, stocker calves.

### Introduction

It is estimated that nearly 76 million people are infected with a food-borne illness in the United States every year. Recent estimates of these cases attribute 1.5 million to *E. coli* O157:H7 and *Salmonella* (Mead et al., 1999). Furthermore, it has been estimated that bacterial

food-borne illness accounts for \$2.9 to \$6.7 billion in productivity losses and medical costs annually (Buzby et al., 1996). *Escherichia coli* O157:H7 and *Salmonella* have been isolated from cattle at all stages of production (Laegreid et al., 1999; Elder et al., 2000) and are considered reservoirs for these bacteria. Infected animals are typically asymptomatic while shedding these pathogens into the environment (Hancock et al., 1997; Bach et al., 2002).

Since approval by the FDA, ionophores have been used throughout the cattle industry due to their ability to increase production efficiency. Recently, ionophores have gained attention due to the similar timeframe of emergence of *E. coli* O157:H7 cases and the introduction of ionophores into the cattle industry (Griffin and Tauxe, 1991; Rasmussen et al., 1999). It has been hypothesized that ionophores may favor gram negative bacteria, such as *E. coli* by inhibition of gram positive species (Dennis et al., 1981; Henderson et al., 1981; Schelling, 1984).

Previous studies conducted in our laboratory found ionophores had no effect on *E. coli* O157:H7 or *Salmonella* *in vitro* or in short-term studies with experimentally infected animals (Edrington et al., 2003a, 2003b). The effects of long-term feeding of ionophores on naturally colonized animals is less clear. Therefore, we conducted a study with the following objectives: 1) to examine the incidence of fecal shedding of *E. coli* O157:H7 and *Salmonella*, 2) to determine the influence of feeding an ionophore on fecal shedding of these pathogens, and 3) to examine the antimicrobial resistance of putative fecal coliforms isolated from calves receiving ionophore.

### Materials and Methods

All animal procedures used in this study were approved by the committee for animal welfare at the Dale Bumpers Small Farms Research Center, Booneville, AR. Fifty-three crossbred calves (mean body weight =  $232 \pm 3$  kg) were maintained on fescue:bermudagrass pasture with *ad libitum* access to water. Cattle were blocked by BW and sex and assigned in replicate to either receive a mineral supplement containing 1.76 g/kg of lasalocid (IONOPH) or receive the same mineral supplement without ionophore for 60 d. Cattle were fed a commercial

receiving ration containing 1g/kg of chlorotetracycline for the initial 10 d. On day 11, calves were fed a corn:wheat midds:soybean meal supplement at 1.5% BW/d until day 60. Ten calves required medication at least once and received one or a combination of the following: Nuflor® (florfenicol), Baytril® (enrofloxacin), Micotil® (tilmicosin), or LA 200® (oxytetracycline). Calves were gathered on d 0, 33, and 60 and BW was recorded and approximately 15 g of fecal material obtained via rectal palpation from each calf. Fecal samples were placed into Whirlpaks™ (Modesto, CA), packed on ice, and shipped to the laboratory in College Station, TX for isolation of *E. coli* O157:H7 and *Salmonella*.

**Bacterial isolation.** To determine the prevalence of *Salmonella*, 10 g of fecal material was enriched in 90 mL of tetrathionate broth for 24 h at 37°C. Following incubation, 200 µL of the above enrichment were added to 5 mL of Rappaport-Vassilidis R10 broth and incubated an additional 24 h at 42°C, before being spread plated onto brilliant green agar (BGA) supplemented with novobiocin (25 µg/mL). Colonies exhibiting typical *Salmonella* morphology were confirmed by slide agglutination using SM-O antiserum poly A-I and V-I, and group C1 factors. *Escherichia coli* O157:H7 was cultured as previously described (Elder et al., 2000). Ten grams of feces were incubated (6 h, 37°C) in gram negative GN-Hajna broth containing cefixime (1.42 mg/mL), vancomycin (8 mg/mL), and cefsulodin (10 mg/mL). This was followed by immunomagnetic bead separation and enrichment using anti-*E. coli* O157:H7 antibody-labeled paramagnetic beads (Neogen Corporation, Lansing, MI). Fifty microliters of bead suspension were spread plated on CHROMagar™ (DRG International, Mountain Side, NJ) supplemented with potassium tellurite. Plates were incubated (37°C, 18 h) and colonies that exhibited typical *E. coli* O157:H7 morphologies were selected from each plate. Selected colonies were confirmed as *E. coli* O157:H7 using Reveal® microbial screening tests according to manufacturer's instructions (Neogen Corporation, Lansing, MI).

**Determination of Antimicrobial Susceptibility.** Putative fecal coliforms were selected from the chromagar plates on d 0, 33, and 60 and examined for antimicrobial susceptibility using the automated Sensititre antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, OH). Broth microdilution was used according to methods described by the National Committee for Clinical Laboratory Standards (NCCLS, 1999) to determine minimum inhibitory concentrations for the following antimicrobials: ampicillin, apramycin, ceftiofur, chlorotetracycline, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, spectinomycin, sulphachloropyridazine, sulphadimethoxine, sulphathiazole, and trimethoprim/sulphamethoxazole. Interpretive standards of the NCCLS were used to determine resistance breakpoints unless unavailable, in which case the NARMS 2000 Annual Report (FDA, 2000) was used. *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *Enterococcus faecalis* ATCC 29212

were used as quality control strains for broth microdilution susceptibility testing.

**Statistical Analysis.** Effect of ionophore treatment on body weight change and average daily gain were determined by the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). Antimicrobial susceptibility of isolates was determined by Poisson analysis of SAS.

## Results and Discussion

Average daily gain (ADG) was not different ( $P > 0.10$ ) between treatments (Table 1). Improvements in ADG are well documented in cattle fed ionophores (Berger et al., 1981; Potter et al., 1986). Although not statistically significant, the numerical difference we observed in IONOPH calves (+ 0.05 kg) is similar to what has been reported previously (Raun et al., 1976). Mineral intakes between groups were similar and within manufacturer's suggested guidelines. The reason no significant group differences were observed is unclear, but may be related to diet, weather, exposure time, sample size, or other unknown factors.

*Escherichia coli* O157:H7 was isolated from one calf during the experimental period. No *Salmonella* was isolated at any time point. While the incidence of *E. coli* O157:H7 reported in this study is considerably lower than what has been reported for feedlot cattle (Elder et al., 2000; Barkocy et al., 2003) it is within the range reported by others for beef calves on forage based production systems (Laegreid et al., 1999; Looper et al., 2003). Similar to this study, *Salmonella* was not isolated in beef calves prior to entering the feedlot (Corrier et al., 1990) or in grazing cows (Looper et al., 2003).

Antimicrobial susceptibility patterns were similar ( $P = 0.14$ ) among treatments. Overall, CONTROL and IONOPH calves were resistant to an average of 1.7 and 2.5 antibiotics, respectively. The most common antimicrobial resistance was to oxytetracycline, florfenicol, and ampicillin (Table 2).

**Table 1.** Mineral intake, body weight (BW) gain, and average daily gain (ADG) of stocker cattle receiving ionophore for 60 d

Item	Treatment		SEM <sup>a</sup>	P > F
	Control	Ionophore		
Mineral intake, g·hd <sup>-1</sup> ·d <sup>-1</sup>	59.9	60.8	12.7	0.98
BW gain, kg	52.8	56.2	4.31	0.49
ADG, kg·d <sup>-1</sup>	0.87	0.92	0.07	0.49

<sup>a</sup>Standard error of the mean

All isolates were susceptible to apramycin, enrofloxacin, and trimethoprim/sulphamethoxazole. These results are not surprising since all calves received chlorotetracycline for 10 d in their receiving ration. In addition, a number of

calves were treated for respiratory illness with florfenicol. A number of the coliform isolates displayed multiple resistance (data not shown), and while reason for concern, they were to antibiotics commonly used in veterinary medicine and not those used in human medicine. Similar resistance patterns have been observed in *E. coli* O157:H7 (Fitzgerald et al., 2003) and *Salmonella* (Edrington et al., 2004) isolated from dairy cattle.

**Table 2.** Antimicrobial resistance of fecal coliforms isolated from stocker calves.

Antimicrobial	Number of isolates displaying resistance <sup>a</sup>	
	Number	Percent
Oxytetracycline	23	33.8
Chlorotetracycline	20	29.4
Ampicillin	19	27.9
Florfenicol	18	26.5
Sulphathiazole	14	20.6
Sulphadimethazole	13	19.1
Sulphachloropyridazine	12	17.6
Spectinomycin	11	16.2
Ceftiofur	2	2.9
Gentamycin	1	1.5
Neomycin	1	1.5

<sup>a</sup>All isolates collected at each sampling period across treatments (n = 68).

### Implications

Feeding the ionophore lasalocid to stocker calves had no effect on the fecal shedding of *E. coli* O157:H7 or *Salmonella*. Furthermore, ionophore feeding had no effect on antimicrobial susceptibility patterns of fecal coliforms. A thorough understanding of the complexity of pathogen carriage and shedding in the ruminant is difficult but necessary as long as contamination of the food supply is a threat. Incorporation of knowledge from this and other studies, as well as pre- and post-harvest food safety interventions may help to ensure the safety of ruminant derived food products.

### Literature Cited

- Bach, S. J., T. A. McAllister, D. M. Veira, V. P. J. Gannon, and R. A. Holley. 2002. Transmission and control of *Escherichia coli* O157:H7- a review. *Can. J. Anim. Sci.* 82:475-490.
- Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of shiga toxin-producing *Escherichia coli*, including O157:H7 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978-1986.
- Berger, L. L., S. C. Rieke, and G. C. Fahey, Jr. 1981. Comparison of two forms and two levels of lasalocid with monensin on feedlot cattle performance. *J. Anim. Sci.* 53:1440-1445.
- Buzby, J. C., T. Roberts, C. T. Jordan Lin, and J. M. MacDonald. 1996. Bacterial food-borne disease: medical costs and productivity losses. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. Report 741.
- Corrier, D. E., C. W. Purdy, and J. R. DeLoach. 1990. Effects of marketing stress on fecal excretion of *Salmonella spp.* in feeder calves. *Am. J. Vet. Res.* 51:866-869.
- Dennis, S. M., T. G. Nagaraja, and E. E. Bartley. 1981. Effects of lasalocid or monensin on lactate-producing or using rumen bacteria. *J. Anim. Sci.* 52:418-426.
- Edrington, T. S., T. R. Callaway, K. M. Bischoff, K. J. Genovese, R. O. Elder, R. C. Anderson, and D. J. Nisbet. 2003a. Effect of feeding the ionophores monensin and laidlomycin propionate and the antimicrobial bambermycin to sheep experimentally infected with *E. coli* O157:H7 and *Salmonella typhimurium*. *J. Anim. Sci.* 81:553-560.
- Edrington, T. S., T. R. Callaway, P. D. Varey, Y. S. Jung, K. M. Bischoff, R. O. Elder, R. C. Anderson, E. Kutter, A. D. Brabban, and D. J. Nisbet. 2003b. Effects of the antibiotic ionophores monensin, lasalocid, laidlomycin propionate and bambermycin on *Salmonella* and *E. coli* O157:H7 *in vitro*. *J. Appl. Microbiol.* 94: 207-213.
- Edrington, T. S., K. M. Bischoff, T. R. Callaway, M. L. Looper, K. J. Genovese, Y. S. Jung, J. L. McReynolds, R. C. Anderson, and D. J. Nisbet. 2004. Antimicrobial resistance and serotype prevalence of *Salmonella* isolated from dairy cattle in the Southwestern United States. *Microb. Drug. Resist.* (In Press).
- Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohaemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci.* 97:2999-3003.
- FDA. 2000. National Antimicrobial Resistance Monitoring System Annual Report. Available: [http://www.cdc.gov/narms/annual/2000/table\\_2.htm](http://www.cdc.gov/narms/annual/2000/table_2.htm). Accessed January 5, 2002.
- Fitzgerald, A. C., T. S. Edrington, M. L. Looper, T. R. Callaway, K. J. Genovese, K. M. Bischoff, J. L. McReynolds, J. D. Thomas, R. C. Anderson, and D. J. Nisbet. 2003. Antimicrobial susceptibility and factors affecting the shedding of *E. coli* O157:H7 and *Salmonella* in dairy cattle. *Lett. Appl. Microbiol.* 37:392-398.
- Griffin, P. M., and R. V. Tauxe. 1991. The epidemiology of infection caused by *Escherichia coli* O157:H7, other enterohaemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* 13:60-67.
- Hancock, D. D., T. E. Besser, D. H. Rice, D. E. Herriott,

- and P. I. Tarr. 1997. A longitudinal study of *Escherichia coli* O157:H7 in fourteen cattle herds. *Epidemiol. Infect.* 118:193-195.
- Henderson, C., C. S. Stewart, and F. V. Nekrep. 1981. The effect of monensin on pure and mixed cultures of rumen bacteria. *J. Appl. Bacteriol.* 51:159-169.
- Laegreid, W. W., R. O. Elder, and J. E. Keen. 1999. Prevalence of *Escherichia coli* O157:H7 in range beef calves at weaning. *Epidemiol. Infect.* 123:291-298.
- Looper, M. L., C. F. Rosenkrans, Jr., G. E. Aiken, and T. S. Edrington. 2003. *Escherichia coli* and *Salmonella* in beef cattle grazing tall fescue. *Ark. Agr. Exp. Sta. Rep.* 509:58-60.
- Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerging Infectious Disease.* 5(5):1-39.
- NCCLS. 1999. National Committee for Clinical Laboratory Standards. Performance standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard. Natl. Committee for Clin. Lab. Stand., Villanova, PA.
- Potter, E. L., R. D. Muller, M. I. Wray, L. H. Carroll, and R. M. Meyer. 1986. Effect of monensin on the performance of cattle on pasture or fed harvested forages in confinement. *J. Anim. Sci.* 62:583-592.
- Rasmussen, M. A., T. L. Wickman, W. C. Cray Jr., and T. A. Casey. 1999. *Escherichia coli* O157:H7 and the rumen environment. Page 39 in: *E. coli O157:H7 in Farm Animals*. C. S. Stewart and H. J. Flint, eds. CAB Int., New York.
- Raun, A. P., C. O. Cooley, E. L. Potter, R. P. Rathmacher, and L. F. Richardson. 1976. Effect of monensin on feed efficiency of feedlot cattle. *J. Anim. Sci.* 43:670-677.
- Schelling, G. T. 1984. Monensin mode of action in the rumen. *J. Anim. Sci.* 58:1518-1527.

## EFFECTS OF A LONG ACTING TRACE MINERAL RUMEN BOLUS UPON RANGE COW AND CALF TRACE MINERAL PROFILES<sup>1</sup>

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**ABSTRACT:** The objectives were to determine if strategic supplementation of range cows with a long acting (six mo) rumen bolus containing Cu, Se, and Co would alter liver Cu or Zn in cows, or blood Se, Cu, or Zn in cows and calves and exhibit differential response between cow breeds. Treatment and control cows used over three years consisted of 42 and 45 Composite (C) cows (25% Hereford, Angus, Gelbevieh, and Barzona), 35 and 41 Hereford (H) cows, and 44 and 44 Brahman (B) cross cows, respectively. Prior to administering the bolus in January, cows had liver and blood samples obtained. Liver and blood sampling was repeated for cows in May and blood sampling for calves in May and September. Data were analyzed using a restricted maximum likelihood-based mixed effects model appropriate for repeated measures with fixed effects of treatment, cow breed, month, year, and age of dam. Blood data included breed x bolus, bolus x year, and month x year interactions, and for cow Se, bolus x month x year. Liver data included breed x bolus as an interaction. Cow within breed by treatment was included as a random effect. Overall liver Cu was deficient ( $P < 0.0001$ ) in control cows and adequate for treated cows. Liver Cu differed by year ( $P < 0.01$ ). Blood Se was adequate for all cows except in January 2001 and 2002. There was no difference ( $P > 0.05$ ) in Se between treatment groups in January but treated cows always had greater ( $P < 0.10$ ) Se in May. Breed differences for Se existed for both treatment cows and calves ( $P < 0.05$ ). Calves from treatment cows had greater Se than calves from control cows ( $P < 0.0001$ ) and the concentration of calf Se dropped ( $P < 0.0001$ ) from May to September. Cow and calf serum Cu and Zn differed ( $P > 0.05$ ) by breed, month, and year but did not accurately reflect trace mineral status for cow liver. Strategic supplementation of copper and selenium via a long acting trace mineral bolus in late gestation was successful in increasing liver copper in cows and blood selenium in cows and calves, but varied by year for Cu and for Se, year and breed.

**Key Words:** Cattle, Minerals

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### Introduction

Gooneratne and Christensen (1989) demonstrated that the developing fetus draws extensively from maternal liver stores of copper. Similarly, selenium efficiently passes from pregnant cows to the fetus through the placenta (Koller et al., 1984) and this maternal mode of transfer has been shown to more effective in improving selenium status in calves than through the milk of cows supplemented postpartum (Enjalbert et al., 1999).

Breed effects for efficiency in metabolizing copper have been well documented (Littledike et al., 1995); however, breed differences in selenium metabolism are not well understood.

The objective of this study was to determine if strategic supplementation of range cows during late gestation with a long acting (6 mo; Cosecure<sup>®</sup>, Telsol Ltd., Leeds, United Kingdom) rumen bolus containing Cu, Se, and Co would alter liver Cu and Zn in cows, or blood Se, Cu, or Zn in cows and calves and whether the response would vary by breed.

### Materials and Methods

**Range site.** The study site for this experiment was at the V-V Ranch operated by the University of Arizona and located near Camp Verde, Arizona. The ranch ranges in elevation from approximately 975 m to 2195 m. Average yearly precipitation ranges from 40 cm at the lower elevations to 70 cm at the upper elevations. However, annual precipitation during the course of this trial was quite variable, with above average precipitation during the growing season in 2001, below average precipitation during the growing season in 2002, and above average precipitation during March and June 2000 and mostly below average summer precipitation in 2000.

**Forage Sampling.** Forage was sampled by hand clipping four times a year (January, April, June or August, and September) from four different locations on the ranch.

**Forage Analyses.** Prior to mineral analysis, forage samples were dried at 65° C for 48 to 72 h, then ground to pass through an approximately 2 mm screen using a Wiley mill (AOAC, 1995) by the AZ Veterinary Diagnostic Laboratory (AZVDL) in Tucson. For the determination of total Se, samples were first wet digested with a solution of nitric acid and magnesium nitrate followed by dry ashing at 500° C (Shimoishi, 1976). Selenium was selectively extracted from the digests as the 2-nitro piazselenol chelate and

quantified by capillary gas chromatography with electron capture detection (Shimoishi, 1976). Forage samples were analyzed for copper and zinc by a commercial laboratory (Dairy One, Ithaca, NY) using inductively coupled plasma emission spectroscopy as described by Sirois et al. (1991).

**Animals.** The trial commenced in January 2000 and concluded in September 2002. Treatment and control cattle were randomly allocated at the onset and each yr cattle were chosen from within these groups for a total of 42 and 45 Composite (**C**) cows (25% Hereford, Angus, Gelbevieh, and Barzona or Senepol), 35 and 41 Hereford (**H**) cows, and 44 and 44 Brahman (**B**) cross cows, respectively. Cows ranged in age from 3 to 8 yr for C, 3 to 11 yr for H, and 5 to 15 yr for B.

Cows in the treatment group were orally dosed each January with two 100 gram Cosecure<sup>®</sup> boluses consisting of 0.30% (wt/wt) selenium as sodium selenate, 13.4% (wt/wt) copper, and 0.5% (wt/wt) cobalt. According to company literature validated with rumen fistulated cattle on a silage and concentrate ration, boluses dissolved in 175 d and released 156, 5.9, and 3.4 mg/d of Cu, Co, and Se, respectively.

Cattle remained in a common herd without any type of oral trace mineral supplement for the three years of the trial except for free choice white iodized salt blocks. In the winter of 2002, from early February to late April 2002, cows were provided free pasture access to protein blocks (27% crude protein; Eagle Milling Co., Inc., Casa Grande, AZ) containing 17 ppm Cu, 149 ppm Zn, and 0.301 ppm Se. At an average daily intake of 0.88 kg of protein supplement, it was estimated that cattle received 15 mg/d Cu, 131 mg/d Zn, and 0.26 mg/d Se from the protein supplement.

**Data Sampling for Cattle.** During the three-yr study, liver and blood samples were obtained from cows in January and May (except that liver samples were not collected in January 2000), and blood samples were collected from calves in May and September. Blood samples were collected via tail venapuncture for cows and by jugular venapuncture for calves using trace mineral free Vacutainers (Becton-Dickinson, Inc., Franklin Lakes, NJ) and stored on ice during transport to the laboratory. Whole blood samples were used for Se (except for May 2000 calves, for which serum was used) and blood serum for Cu and Zn. Blood serum for calves was used for Se analysis in May 2000 due to whole blood samples accidentally getting frozen. Blood samples were centrifuged at 2,400 x g for 20 min and serum collected and stored at

-20°C. Whole blood samples were stored at 5°C. Liver samples were collected using a Schackelford-Courtney Liver Biopsy Instrument (Sontec Instruments, Englewood, CO) according to the technique described by Rogers et al. (2001) and stored at -20°C until analyses.

**Mineral Analyses.** Selenium analyses of whole blood were determined at the AZVDL using the same methods as previously described for forage samples. Serum samples were shipped overnight to the Animal Nutrition and Growth Lab, Department of Animal Science, Texas A & M University, College Station, TX and analyzed for Cu and Zn by flame atomic absorption spectroscopy (Model S11, Thermal Jarrel Ash Corp.). Serum samples were diluted 1:1 with double-distilled, deionized water, and standards prepared in a 15% (vol/vol) glycerol solution. Liver samples

(approximately 0.1 g wet tissue) were freeze dried for 24 h and then predigested with 5 ml of nitric acid for three d. Following addition of 1 ml of hydrogen peroxide, samples were digested in a MSP 1000 microwave sample preparation unit (CEM Corp.) for 2 h at 100°C. Digested liver samples were diluted with double distilled water, and Cu and Zn analyses conducted as described above. Atomic absorption standards were prepared in 2% nitric acid solution.

**Missing Data.** Due to the extensive nature of the V-V Ranch (31,161 ha), we were not able to gather all the cattle for the May sampling period in 2002 (78 total cows gathered). In May 2001, 15 cows failed to calve by the time the liver biopsy was obtained (4 B, 5 C, and 6 H), so these cows were not included in data analyses. There were 77 calves sampled in May 2000, 78 in September 2000, 69 in May 2001, 81 in September 2001, 73 in May 2002, and 67 in September 2002. There were 34 calf serum samples which froze in September 2002 and had to be eliminated from analyses due to excessive hemolysis.

**Statistical Analyses.** Data were analyzed using a restricted maximum likelihood-based mixed effects model appropriate for repeated measures (SAS Inst., Inc., Cary, NC) with fixed effects of treatment, cow breed, month, year, and Beef Improvement Federation (BIF, 1990) age of dam. An unstructured correlation structure was used to model within subject error. Blood data included breed x bolus, bolus x year, and month x year interactions, and for cow Se, bolus x month x year. Liver data included breed x bolus as an interaction. Cow within breed by bolus was included as a random effect. Yearly concentrations of forage were analyzed using a restricted maximum likelihood-based mixed effects model appropriate for repeated measures with fixed effects of plant species, year, month, and plant species x year. Pasture was included as a random effect. An unstructured correlation structure was used. Treatment means for blood and liver data and yearly forage concentrations of Cu, Se, and Zn were separated using all pairwise tests using unprotected least squares difference tests.

## Results and Discussion

**Overall Forage Trace Mineral Concentrations.** Concentrations of Cu in forage were nearly adequate (10 ppm; NRC, 1996) in 2000 ( $9.2 \pm .46$  ppm), marginally deficient in 2002 ( $4.9 \pm .45$  ppm), and severely deficient in 2001 ( $3.9 \pm .46$  ppm), a year with more favorable precipitation during the growing season. The concentrations of Se in acted in an opposite fashion to that of Cu, being  $0.042 \pm .006$  ppm in 2000,  $0.079 \pm .006$  ppm in 2001, and  $0.045 \pm .006$  ppm in 2002), though the concentrations of Se were always deficient in forage ( $< 0.1$  ppm; NRC, 1996). Concentrations of Zn in forage were severely deficient in 2001 ( $16.0 \pm 1.04$  ppm) and 2002 ( $15.1 \pm 1.03$  ppm) and marginally deficient ( $20.2 \pm 1.04$  ppm) in 2000.

Ganskopp and Bohnert (2003) found that the concentrations of Cu in forage decreased in wetter years and they related this phenomenon to a dilution effect with increased biomass in favorable years. Conversely, from the data we have presented, it appears that increased moisture on semi-arid rangelands may in fact increase Se translocation in forage.

*Trace Mineral Values in Cattle.* Tables 1 and 2 present the least squares means for cow and calves, respectively. Liver Cu for control cows was deficient (Corah and Dargatz, 1996) at  $73 \pm 4.8$  ppm while liver Cu was adequate ( $121 \pm 4.8$  ppm) for cows receiving the Cosecure® boluses. There were no significant ( $P = 0.17$ ) breed effects detected for liver Cu, but concentrations varied by year ( $P < 0.0001$ ), being  $122 \pm 7.6$  ppm in 2000,  $78 \pm 3.3$  ppm in 2001, and  $91 \pm 5.3$  ppm in 2002 when pooled over all cows. The levels of liver Cu corresponded to forage concentrations of Cu, but did not correlate well to cow serum Cu levels. There was no difference between treated and control cows ( $P = 0.42$ ) for serum Cu (Table 1) and these data add to a growing body of evidence (Radostits et al., 1994) that serum Cu is not a good indication of trace mineral status until levels approach deficient levels.

Concentrations of liver Zn never fell below adequate levels (80 to 90 ppm; Corah and Dargatz, 1996), though they declined ( $P < 0.0001$ ) from January ( $133 \pm 3.0$  ppm) to May ( $116 \pm 2.1$  ppm). These data indicate that dietary levels recommended for grazing beef cattle by NRC (1996) may not accurately match dietary needs for range cattle in central AZ. Liver Zn and serum Zn did not differ ( $P = 0.79$ ; Table 1) between treatment groups and was to be expected since the cattle in this study did not receive any supplemental Zn.

Serum Cu and Zn did not differ ( $P > 0.0585$ ) by treatment for calves (Table 2), though serum Cu concentrations for both treated and control calves fell below what is considered adequate (0.60 ppm; Puls, 1994) in 2000. As noted for serum Cu data for cows, serum Cu data for calves in this study are questionable.

For cows, whole blood Se differed between treatment groups, by month, year, month within year, and by age of dam ( $P < 0.0001$ ), as well as by breed ( $P = 0.0058$ ). In spite of the low concentrations of Se in forage, cow blood Se levels were adequate for both treatment groups ( $> 0.1$  ppm; Radostits et al., 1994) for all time periods except in January 2001 and 2002 when they were marginally (0.05 to 0.1 ppm; Radostits et al., 1994) deficient (Table 1). There were no differences in blood Se for control vs treated cows in January ( $P > 0.05$ ; Table 1), but bolused cows had greater Se in May 2000 and 2002 ( $P < 0.0001$ ) and tended to have greater blood Se in May 2001 ( $P = 0.0611$ ).

Calves nursing bolused cows had greater ( $P = 0.0002$ ) whole blood Se than did calves nursing control cows (Table 2). The concentration of Se for calves dropped from May ( $0.137 \pm .003$  ppm) to September ( $0.103 \pm .003$  ppm) and was to be expected as stores of Se acquired prenatally declined. The much lower Se observed in 2000 (Table 2) was an artifact related to the necessity of using serum Se instead of whole blood Se in May.

Figures 1 and 2 illustrate breed effects we observed for whole blood Se for cows and calves, respectively. Among treatment cows, Se was greater for B than for C ( $P = 0.0133$ ) cattle and tended to be greater ( $P = 0.0532$ ) than that observed for H cattle. Control calves from H cows had less ( $P = 0.0100$ ) Se than did C cattle and tended ( $P = 0.0737$ ) to have less Se than did B cattle. In reviewing data for both

cows and calves, it is apparent that H cattle benefit from supplemental Se when grazing Se deficient rangelands.

## Implications

Strategic supplementation of copper and selenium via a long acting trace mineral bolus in late gestation was successful in increasing liver copper in cows and blood selenium in cows and calves, but varied by year for Cu and for Se, year and breed. When favorable growing season moisture occurs, it is critical to evaluate Cu status in forage and supplement accordingly. Brahman cross cattle in this study appeared to be more efficient at metabolizing supplemental Se. Hereford cattle appeared to have a greater need for supplemental Se than did Brahman cross and Composite cattle when grazing Se deficient rangelands. Serum Cu was not a good indicator of trace mineral status and should be avoided when assessing Cu status of range cows except when cattle are known to be severely deficient in Cu. Suggested dietary requirements for zinc for range cows may need to be reevaluated.

## Literature Cited

- AOAC. 1995. Official Methods of Analysis. 16<sup>th</sup> Ed. Association of Official Analytical Chemists, Arlington, VA.
- BIF, 1990. BIF Guidelines for Uniform Beef Improvement Programs. 6<sup>th</sup> Ed. Beef Improvement Federation, University of GA, Athens, GA.
- Corah, L.R., and D. Dargatz. 1996. Forage analyses from cow/calf herds in 18 states. Report: Beef cow/calf health and productivity audit. U.S. Dept. of Agri. [http://www.aphis.usda.gov/vs/ceah/cahm/Beef\\_Cow-Calf/chapa/chapfora.pdf](http://www.aphis.usda.gov/vs/ceah/cahm/Beef_Cow-Calf/chapa/chapfora.pdf) Accessed March 30, 2004.
- Enjalbert, F., P. Lebreton, O. Salat, and F. Schelcher. 1999. Effects of pre- or postpartum selenium supplementation on selenium status in beef cows and their calves. J. Anim. Sci. 77:223-229.
- Ganskopp, D., and D. Bohnert. 2003. Mineral concentration dynamics among 7 northern Great Plain grasses. J. Range Manag. 56:174-184.
- Gooneratne, S.R., and D.A. Christensen. 1989. A review of maternal copper status and fetal tissue copper concentrations in Saskatchewan bovine. Can. J. Anim. Sci. 69:141-150.
- Koller, L. D., G. A. Whitbeck, and P. J. South. 1984. Transplacental transfer and colostral concentrations of selenium in beef cattle. Am. J. Vet. Res. 45:2507-2510.
- Littledike, E. T., T. E. Wittum, and T. G. Jenkins. 1995. Effect of breed, intake, and carcass composition on the status of several macro and trace minerals of adult beef cattle. 73:2113-2119.
- NRC, 1996. Pages 54 to 74 in Nutrient Requirements of Beef Cattle. (7<sup>th</sup> Ed.). National Academy Press, Washington, DC.
- Puls, R. 1994. Mineral Levels in Animal Health. Diagnostic Data. 2nd Edition. Sherpa International, Clearbrook, BC, Canada.

- Radostits, O.M., D.C. Blood, and C.C. Gay. 1994. Diseases caused by nutritional deficiencies. In: O.M. Radostits, D.C. Blood, and C.C. Gay (Ed.) Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses. 8<sup>th</sup> Edition. pp 1368-1453. Bailliere Tindall, London.
- Rogers, G. M., D. J. Capucille, M. H. Poore, J. Maas, and J. E. Smallwood. 2001. Growth performance of cattle following percutaneous liver biopsy utilizing a Schackelford-Courtney biopsy instrument. Bov. Pract. 35:177-184.
- Shimoishi, Y. 1976. The gas-chromatographic determination of selenium (VI) in milk with 1,2-diamino-4-nitrobenzene. Analyst 101:298-305.
- Sirois, P. K., M. J. Reuter, C. M. Laughlin, and P. J. Lockwood. 1991. A method for determining macro and micro elements in forages and feeds by inductively coupled plasma atomic emission spectrometry. Spectroscopist 3:6-9.

Table 1. Effects of a long acting trace mineral bolus upon cow liver copper, liver zinc, whole blood selenium, serum copper, and serum zinc<sup>a</sup>

Item	No Bolus	Bolus	SE	P-value
Liver Cu, DM ppm				
Pooled over all months and all years	73	121	4.8	0.0001
Liver Zn, DM ppm				
Pooled over all months and all years	124 ± 2.4	125 ± 2.5		0.7873
Serum Cu, DM ppm				
Pooled over all months and all years	0.78	0.79	0.015	0.4216
2000, pooled over mo	0.95	0.97	0.023	0.6568
2001, pooled over mo	0.78	0.79	0.017	0.4527
2002, pooled over mo	0.60 ± .022	0.61 ± .020		0.6063
Serum Zn, DM ppm				
Pooled over all months and all years	0.92	0.92	0.015	0.8986
2000, pooled over mo	1.00 ± .022	1.00 ± .023		0.9354
2001, pooled over mo	1.03	1.04	0.022	0.7576
2002, pooled over mo	0.73 ± .022	0.72 ± .020		0.6279
Whole blood Se, DM ppm				
Pooled over all months and all years	0.124	0.141	0.003	0.0001
2000, pooled over mo	0.144	0.161	0.004	0.0014
2001, pooled over mo	0.123	0.135	0.005	0.0793
2002, pooled over mo	0.106	0.129	0.004	0.0001
	January	May	January	May
2000, mo within yr	0.130 ± .004 <sup>b</sup>	0.157 ± .005 <sup>c</sup>	0.127 ± .004 <sup>b</sup>	0.194 ± .005 <sup>d</sup>
2001, mo within yr	0.088 ± .004 <sup>b</sup>	0.158 ± .008 <sup>c</sup>	0.091 ± .004 <sup>b</sup>	0.178 ± .008 <sup>c</sup>
2002, mo within yr	0.066 ± .004 <sup>b</sup>	0.146 ± .005 <sup>c</sup>	0.069 ± .003 <sup>b</sup>	0.189 ± .005 <sup>d</sup>

<sup>a</sup>Cosecure<sup>®</sup> trace mineral boluses had an expected life of approximately 175 d and provided approximately 156 mg/d Cu, 5.9 mg/d Co, and 3.4 mg/d Se.

<sup>b,c,d</sup> Within a row, means lacking a common superscript letter differ ( $P < 0.05$ ).

Table 2. Whole blood selenium, serum copper, and serum zinc for calves nursing cows administered a long acting trace mineral bolus<sup>a</sup>

Item	No Bolus	Bolus	SE	P-value
Serum Cu, DM ppm				
Pooled over all months and all years	0.64	0.63	0.012	0.4025
2000, pooled over mo	0.41	0.45	0.014	0.0905
2001, pooled over mo	0.81	0.79	0.017	0.2838
2002, pooled over mo	0.69 ± .019	0.65 ± .017		0.0585
Serum Zn, DM ppm				
Pooled over all months and all years	1.05 ± .016	1.06 ± .018		0.4493
2000, pooled over mo	0.96 ± .033	0.97 ± .034		0.8196
2001, pooled over mo	1.20 ± .026	1.20 ± .027		0.8938
2002, pooled over mo	0.98 ± .025	1.01 ± .024		0.3635
Whole blood Se, DM ppm <sup>b</sup>				
Pooled over all months and all years	0.113	0.128	0.003	0.0002
2000, pooled over mo	0.058 ± .002	0.065 ± .003		0.0366
2001, pooled over mo	0.138	0.156	0.006	0.0478
2002, pooled over mo	0.142	0.163	0.005	0.0030

<sup>a</sup>Cosecure<sup>®</sup> trace mineral boluses had an expected life of approximately 175 d and provided approximately 156 mg/d Cu, 5.9 mg/d Co, and 3.4 mg/d Se.

<sup>b</sup>Selenium concentrations for May 2000 were determined with blood serum instead of whole blood.

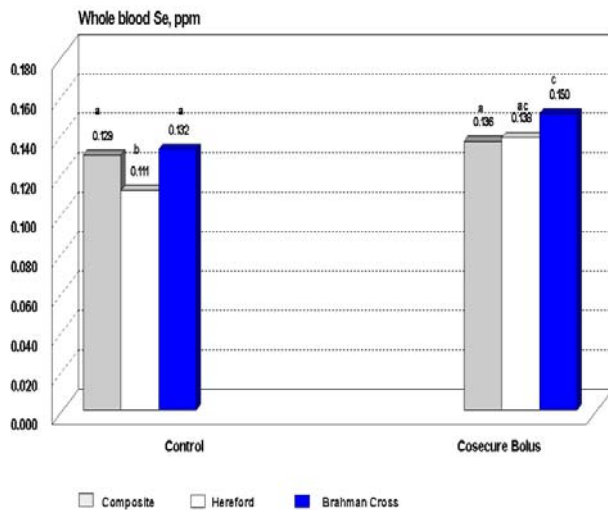


Figure 1. Whole blood selenium concentrations pooled over all years and months for cow beef breeds administered a long acting (6 mo) trace mineral bolus. The SE was .004 ppm for Hereford cattle and Composite control cattle, and .005 ppm for bolused Composite cattle and Brahman cattle.

<sup>abc</sup> Means lacking a common superscript differ ( $P < 0.05$ ).

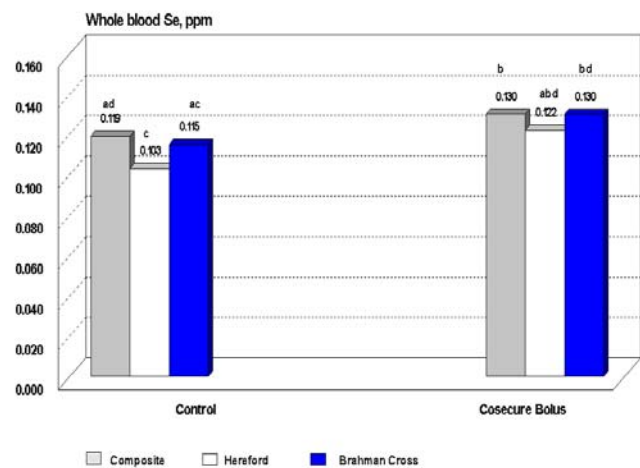


Figure 2. Whole blood selenium concentrations pooled over all years and months for calves nursing cow beef breeds administered a long acting (6 mo) trace mineral bolus. The SE was .004 ppm for calves from bolused Hereford cattle and control Composite control cattle, and .005 ppm for calves from all the other cattle. <sup>abcd</sup> Means lacking a common superscript differ ( $P < 0.05$ ).

## EFFECTS OF A LONG ACTING TRACE MINERAL RUMEN BOLUS UPON RANGE COW PRODUCTIVITY <sup>1</sup>

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**ABSTRACT:** The objectives were to determine if strategic supplementation of range cows in Arizona during the last trimester of gestation with a long acting (six mo) rumen bolus containing Cu, Se, and Co would (1) increase cow body condition and body weights, and calf birth weights, weaning weights, post weaning weights, or weight per day of age (WDA); and (2) to see if any of the above traits varied by breed. There were 192 control and 144 treated Composite cows (25% Hereford, Angus, Gelbevieh, and Senepol or Barzona), 236 control and 158 Hereford treated cows, and 208 control and 149 treated Brahman cross cows used over three years. Cows were weighed and scored for body condition (1 to 9, 9 = fattest) in January, May, and September. Data were analyzed using a restricted maximum likelihood-based, mixed effects model appropriate for repeated measures. The statistical models included the fixed effects of treatment, breed of cow, and year, and all two and three way interactions between those, and age of dam and calf sex and their interaction. Cow within breed by bolus was included as a random effect. The continuous fixed effect of weaning age was included in the model. Control cows lost less weight ( $P < 0.05$ ) than treated cows from January to May ( $-10 \pm 3.4$  vs  $-21 \pm 4.0$  kg), but there was a significant breed by year by treatment interaction ( $P < 0.001$ ). Calf WDA, weaning, and post weaning weights did not differ ( $P > 0.05$ ) between treated and control cows, however there was a significant ( $P < 0.05$ ) breed by year by treatment interaction for birth weight. Strategic supplementation via a long acting trace mineral bolus increased or decreased body weight in early lactation cows, depending upon the breed and year and may indicate the need for breed and year specific supplementation programs.

**Key Words:** Cattle, Minerals, Copper, Selenium

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### Introduction

A long acting (6 mo) rumen trace mineral bolus containing Cu, Se, and Co has been developed in the United Kingdom (Cosecure<sup>®</sup>, Telsol Ltd., Leeds, United Kingdom) and has shown promise for helping alleviate trace mineral deficiencies (Buckley et al., 1987; Givens et al., 1988) though calf growth did not differ for the one study in which it was measured (Givens et al., 1988). If the long acting rumen bolus can be shown to be successful on Arizona rangelands, then cattle dosed with the boluses could pack their own trace mineral supplement for up to six mo instead of having to deliver an oral trace mineral supplement to the cattle by pack horse for rugged topography Arizona rangelands.

A companion study in this proceedings (Sprinkle et al., 2004) examines the effect of the Cosecure<sup>®</sup> bolus upon cow and calf trace mineral profiles. In this paper, we will examine the effect of the trace mineral bolus upon cow and calf productivity. Specifically, we wished to determine if strategic supplementation of range cows in Arizona during the last trimester of gestation with a long acting (six mo) rumen bolus containing Cu, Se, and Co would (1) increase cow body condition and body weights, and calf birth weights, weaning weights, post weaning weights, or weight per day of age (WDA) at weaning; and (2) to see if any of the above traits varied by breed within treatment.

### Materials and Methods

**Range site.** The study site for this experiment was at the 32,161 ha V-V Ranch operated by the University of Arizona and located near Camp Verde, Arizona. The ranch ranges in elevation from approximately 975 m to 2195 m. Average yearly precipitation ranges from 40 cm at the lower elevations to 70 cm at the upper elevations. However, annual precipitation during the course of this trial was quite variable, with above average precipitation during the growing season in 2001, below average precipitation during the growing season in 2002, and above average precipitation during March and June 2000 and mostly below average summer precipitation in 2000.

**Forage Sampling.** Forage was sampled by hand clipping four times a year (January, April, June or August, and September) from four different locations on the ranch and analyzed for Cu and Se according to the methods described in this proceedings (Sprinkle et al., 2004).

**Animals.** The trial commenced in January 2000 and concluded in September 2002. Treatment and control cattle were randomly allocated at the onset and remained in each treatment group throughout the three-yr trial. Control and treatment cattle over the three-yr trial included 192 and 144

Composite (C) cows (25% Hereford, Angus, Gelbevieh, and Barzona or Senepol), 236 and 158 Hereford (H) cows, and 208 and 149 Brahman (B) cross cows, respectively. Cows ranged in age from 2 to 8, 2 to 18, and 2 to 15 yr for C, H, and B, respectively.

In January of each yr, cows in the treatment group were orally dosed with two 100 gram Cosecure® boluses consisting of 0.30% (wt/wt) selenium as sodium selenate, 13.4% (wt/wt) copper, and 0.5% (wt/wt) cobalt. According to company literature validated with rumen fistulated cattle on a silage and concentrate ration, boluses dissolved in 175 days and released 156, 5.9, and 3.4 mg/d of Cu, Co, and Se, respectively.

Cattle remained in a common herd and rotated through 32 upland pastures without any type of oral trace mineral supplement for the three years of the trial except for free choice white iodized salt blocks. In the winter of 2002, from early February to late April 2002, cows were provided free pasture access to protein blocks (27% crude protein; Eagle Milling Co., Inc., Casa Grande, AZ) containing 17 ppm Cu and 0.301 ppm Se. At an average daily intake of 0.88 kg of protein supplement, it was estimated that cattle received 15 mg/d Cu and 0.26 mg/d Se from the protein supplement.

The majority (98.2%) of calves born in this trial were sired by Hereford bulls via artificial insemination or pasture exposure. The breeding seasons extended from May 20 to November 15 in 2000, from May 16 to October 31 in 2001, and June 29 to October 26 in 2002. For a portion of the cow herd (56, 43, and 67% for C, H, and B over the three-yr trial, respectively), the natural exposure breeding season was preceded by estrus synchronization and artificial insemination using both OV-SYNCH and Select SYNCH in 2000 and 2001 and Easi-Breed CIDRs in 2002. In September and January, cows were checked for pregnancy by rectal palpation. Cattle were weighed and scored for BCS (1 to 9; 9 = fattest) in January, May, and September. Birth and weaning weights were collected on all calves. The majority of the calves were weaned in September at approximately 184 d and weaning weights were adjusted to 205 days of age and for age of dam according to BIF (1990) guidelines. In 2000, all calves were shipped to the University of Arizona Feedlot (UAF) 3 days after weaning. In 2001 and 2002 ten days after weaning, larger steers were shipped to UAF while smaller steers and all the heifers were shipped to the Maricopa Agricultural Center where they were placed on Sudan pasture. Calves too young to wean in September were weaned in late November 2000, early December in 2001 and mid-November in 2002. The WDA for each calf at weaning was calculated using the actual weaning weights and dividing by age at weaning.

**Statistical Analyses.** Data were analyzed using a restricted maximum likelihood-based mixed effects model appropriate for repeated measures (SAS Inst., Inc., Cary, NC) with fixed effects of treatment, cow breed, year, and all two and three way interactions between those, and Beef Improvement Federation (BIF, 1990) age of dam and calf sex and their interaction. The continuous fixed effect of weaning age was included in the model and cow within breed by bolus was included as a random effect. An unstructured correlation structure was used to model within subject error. Treatment

means for all statistical models were separated using all pairwise tests using unprotected least squares difference tests.

## Results

**Overall Forage Trace Mineral Concentrations.** Concentrations of Cu in forage were nearly adequate (10 ppm; NRC, 1996) in 2000 ( $9.2 \pm .46$  ppm), marginally deficient in 2002 ( $4.9 \pm .45$  ppm), and severely deficient in 2001 ( $3.9 \pm .46$  ppm), a year with more favorable precipitation during the growing season. The concentrations of Se acted in an opposite fashion to that of Cu, being  $0.042 \pm .006$  ppm in 2000,  $0.079 \pm .006$  ppm in 2001, and  $0.045 \pm .006$  ppm in 2002), though the concentrations of Se were always deficient in forage ( $< 0.1$  ppm; NRC, 1996).

**Cow Performance Data.** Over the course of the trial, BCS for treatment cows tended ( $P = 0.07$ ) to be greater for treatment cows in January but cow weight in January did not differ ( $P = 0.3655$ ; Table 1). The BCS in May and September did not differ ( $P > 0.32$ ), though bolused H cattle had less BCS ( $P = 0.0006$ ) than did control H in May 2000 ( $4.5 \pm .13$  vs  $5.0 \pm .10$ ).

There were no significant overall differences by treatment in cow weights (Table 1) but the year x breed x treatment interaction was important ( $P < 0.0001$ ) for cow weight in May. Most of this interaction was due to control H cattle having greater body weight in May than did bolused H cattle ( $P < 0.0001$ ;  $448 \pm 7.2$  vs  $396 \pm 9.6$  kg). By September, the interaction of cow breed with year and treatment had diminished ( $P = 0.1071$ ) and the only significant difference ( $P = 0.0268$ ) occurred in September 2001, when control C cattle had greater weights than did treated cows ( $465 \pm 6.7$  vs  $444 \pm 2.7$  kg).

Cattle treated with Cosecure® boluses lost more weight from January to May ( $P = 0.0200$ ; Table 1). A highly significant ( $P = 0.0004$ ) year x breed x treatment interaction was present and is presented in Figure 1. By far ( $P < 0.0001$ ), H cattle were most profoundly affected by bolus administration in 2000, a better Cu yr. Treated B cattle also tended ( $P = 0.0543$ ) to lose more weight than control in 2002.

There are at least two assumptions that can be made as to why bolused cattle lost more weight in early lactation. First, it can be assumed that bolused cattle had greater milk production than did control cattle. Alternately, it can be assumed that increased supplemental Cu (especially when forage Cu levels were close to dietary requirements in 2000) could have had an antagonistic effect upon cow productivity by interacting with other trace minerals in the forage base or by decreasing forage digestibility (Arthington et al., 2003).

Limited experimental research has examined the influence of added Cu and Se in the diet upon milk production. Lacetera et al. (1996) reported that milk production ( $P = 0.06$ ) and total milk solids ( $P = 0.02$ ) were greater for dairy cows provided supplemental Se. Engle et al. (2001) failed to show any increase in milk production with added Cu in the diets of dairy cattle.

Although some research has shown a decrease in forage digestibility with added Cu (Arthington et al., 2003), other research (Lopez-Guisa and Satter, 1992) failed to demonstrate the same effect.

Since we were unable to obtain milk production for these cattle, it is uncertain whether the decline in BCS for bolused cattle resulted from increased milk production or antagonisms with other trace minerals in the diet and/or reduced fiber digestibility.

**Calf Performance Data.** In this study, we failed to demonstrate any added growth for adjusted weaning weights or WDA for calves suckling cows bolused with a long acting trace mineral bolus (Table 2). Other research has reported variable results for added Cu, increasing ADG during finishing trials (Ward and Spears, 1997) and decreasing gain for growing dairy heifers (Lopez-Guisa and Satter, 1992). Awadeh et al. (1998) and Gunter et al. (2003) failed to demonstrate any added growth performance for calves nursing Se supplemented cows while Nelson and Miller (1987) reported that weaning weights for calves nursing Se supplemented cows increased by 20 kg.

It appears that any added weight gains for calves nursing cows supplemented with either Cu or Se are dependent upon several factors, chief of which are the dietary Cu or Se concentrations for cows in the study and the presence or absence of any antagonistic trace minerals in the diet such as Mo, Fe, and S. Villar et al. (2002) reported that positive growth responses appear to occur when dietary Se in the forage base is less than 0.05 ppm DM and when plasma Se levels are less than 0.030 ppm. The pasture forage Se reported by Gunter et al. (2003) was 0.11 ppm and 0.07 ppm by Awadeh et al. (1998).

There was a trend ( $P = 0.0675$ ; Table 2) for increased post weaning ADG for H calves from supplemented cows. Most of this response occurred ( $P = 0.0892$ ) in 2001 ( $179 \pm 8.1$  vs  $200 \pm 9.5$  kg) when Cu concentrations in forage were severely deficient and Se concentrations slightly elevated (though still below adequate levels).

We detected a year x breed x treatment interaction ( $P = 0.022$ ) for birth weight, due mostly to B cattle in 2001 ( $P = 0.0170$ ;  $37.2 \pm .63$  and  $35.4 \pm .68$  for control vs bolused cows) and C cattle in 2000 ( $P = 0.0735$ ;  $37.2 \pm .54$  vs  $35.8 \pm .63$  for control vs bolused cows). We are uncertain for the nature of this effect unless it was related to forage digestibility factors discussed earlier.

### Implications

Cow and calf performance responses to added Cu and Se varied with yr and by breed, necessitating careful monitoring of levels of these trace minerals in the forage during different growing conditions and altering trace mineral supplementation programs accordingly.

### Literature Cited

- Arthington, J. D., F. M. Pate, and J. W. Spears. 2003. Effect of copper source and level on performance and copper status of cattle consuming molasses-based supplements. *J. Anim. Sci.* 81:1357-1362.
- Awadeh, F. T., R. L. Kincaid, and K. A. Johnson. 1998. Effect of level and source of dietary selenium on concentrations of thyroid hormones and immunoglobulins in beef cows and calves. *J. Anim. Sci.* 76:1204-1215.
- Buckley, W. T., G. Strachan, and R. Puls. 1987. Copper and selenium supplementation to calves by means of a soluble glass bolus. *Can. J. Anim. Sci.* 67:877-881.
- BIF, 1990. BIF Guidelines for Uniform Beef Improvement Programs. 6<sup>th</sup> Ed. Beef Improvement Federation, University of GA, Athens, GA.
- Engle, T. E., V. Fellner, and J. W. Spears. 2001. Copper status, serum cholesterol, and milk fatty acid profile in Holstein cows fed varying concentrations of copper. *J. Dairy Sci.* 84:2308-2313.
- Givens, D. I., G. Zervas, V. R. Simpson, and S. B. Telfer. 1988. Use of soluble glass rumen boluses to provide a supplement of copper for suckled calves. *J. Agric. Sci., Camb.* 110:199-204.
- Gunter, S. A., P. A. Beck, and J. M. Phillips. 2003. Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves. *J. Anim. Sci.* 81:856-864.
- Lacetera, N. U. Bernabuci, b. Ronchi, and A. Nardone. 1996. Effects of selenium and vitamin E administration during a late stage of pregnancy on colostrum and milk production in dairy cows, and on passive immunity and growth of their offspring. *Am. J. Vet. Res.* 57:1776-1780.
- Lopez-Guisa, J. M., and L. D. Satter. 1992. Effect of copper and cobalt addition on digestion and growth in heifers fed diets containing alfalfa silage or corn crop residue. *J. Dairy Sci.* 75:247-256.
- NRC, 1996. Pages 54 to 74 in Nutrient Requirements of Beef Cattle. (7th Ed.). National Academy Press, Washington, DC.
- Nelson, A. O., and R. F. Miller. 1987. Responses to selenium in a range beef herd. *California Agriculture*. March-April:4-5.
- Sprinkle, J. E., S. P. Cuneo, H. M. Frederick, R. M. Enns, D. W. Schafer, G. E. Carstens, L. J. Slay, S. R. Daugherty, T. H. Noon, B. M. Rickert, and C. Reggiardo. 2004. Effects of a long acting trace mineral bolus upon range cow and calf trace mineral profiles. *Proceedings Western Section American Society of Animal Science*. (In press) 54:
- Villar, D., J. R. Arthur, J. M. Gonzalez, F. J. Pallares, and T. L. Carson. 2002. Selenium status in cattle: Interpretation of laboratory results. *Bov. Pract.* 36:73-80.
- Ward, J. D., and J. W. Spears. 1997. Long-term effects of consumption of low-copper diets with or without supplemental molybdenum on copper status, performance, and carcass characteristics of cattle. *J. Anim. Sci.* 73:3057-3065.

Table 1. Effects of a long acting trace mineral bolus upon range cow weight and body condition score<sup>a</sup>

Item	No Bolus	Bolus	P-value
January BCS <sup>b</sup>			
All cows and all yr	4.8 ± .05	4.9 ± .06	0.0738
C cows, over all yr <sup>c</sup>	4.6 ± .08	4.7 ± .09	0.1783
H cows, over all yr <sup>c</sup>	4.8 ± .08	4.9 ± .08	0.0694
B cows, over all yr <sup>c</sup>	5.0 ± .09	5.1 ± .10	0.0600
May BCS <sup>b</sup>			
All cows and all yr	4.5 ± .11	4.4 ± .12	0.3283
C cows, over all yr <sup>c</sup>	4.6 ± .18	4.3 ± .20	0.1559
H cows, over all yr <sup>c</sup>	4.5 ± .21	4.4 ± .21	0.8642
B cows, over all yr <sup>c</sup>	4.5 ± .19	4.5 ± .22	0.8616
September BCS <sup>b</sup>			
All cows and all yr	4.6 ± .05	4.7 ± .06	0.5162
C cows, over all yr <sup>c</sup>	4.5 ± .08	4.5 ± .09	0.8543
H cows, over all yr <sup>c</sup>	4.5 ± .07	4.6 ± .09	0.2051
B cows, over all yr <sup>c</sup>	4.9 ± .09	5.0 ± .10	0.9388
January wt, kg			
All cows and all yr	445 ± 3.3	449 ± 3.8	0.3655
C cows, over all yr <sup>c</sup>	432 ± 5.5	426 ± 6.4	0.3811
H cows, over all yr <sup>c</sup>	435 ± 5.2	445 ± 5.8	0.1737
B cows, over all yr <sup>c</sup>	467 ± 6.0	475 ± 6.7	0.2740
May wt, kg			
All cows and all yr	436 ± 3.8	426 ± 4.4	0.1293
C cows, over all yr <sup>c</sup>	421 ± 6.7	407 ± 7.5	0.1481
H cows, over all yr <sup>c</sup>	442 ± 5.6	433 ± 6.6	0.2922
B cows, over all yr <sup>c</sup>	438 ± 6.9	437 ± 7.7	0.8993
September wt, kg			
All cows and all yr	435 ± 3.3	434 ± 3.7	0.8823
C cows, over all yr <sup>c</sup>	427 ± 5.8	413 ± 6.6	0.1021
H cows, over all yr <sup>c</sup>	431 ± 4.9	436 ± 5.8	0.4954
B cows, over all yr <sup>c</sup>	447 ± 6.0	453 ± 6.6	0.4100
Change in wt January to May, kg			
All cows and all yr	- 10 ± 3.4	- 21 ± 4.0	0.0200
C cows, over all yr <sup>c</sup>	- 9 ± 5.6	- 17 ± 6.6	0.2999
H cows, over all yr <sup>c</sup>	2 ± 5.6	- 13 ± 6.4	0.0713
B cows, over all yr <sup>c</sup>	- 23 ± 6.0	- 32 ± 6.7	0.2353

<sup>a</sup>Cosecure<sup>®</sup> trace mineral boluses had an expected life of approximately 175 d and provided approximately 156 mg/d Cu, 5.9 mg/d Co, and 3.4 mg/d Se.

<sup>b</sup>(1 to 9, 9 = fattest)

<sup>c</sup>Breeds: C = Composite (25% Hereford, Angus, Gelbevieh, and Barzona or Senepol); H = Hereford; B = Brahman cross

Table 2. Weight per day of age, birth, adjusted weaning, and post-weaning wt for calves nursing cows administered a long acting trace mineral bolus<sup>a</sup>

Item	No Bolus	Bolus	P-value
Adjusted weaning wt, kg <sup>b</sup>			
From all cows and all yr	201 ± 1.9	199 ± 2.1	0.5201
From C cows, over all yr <sup>c</sup>	210 ± 3.1	211 ± 3.6	0.8148
From H cows, over all yr <sup>c</sup>	177 ± 2.8	177 ± 3.3	0.9739
From B cows, over all yr <sup>c</sup>	215 ± 3.4	210 ± 3.7	0.1872
November post-weaning wt, kg			
From all cows and all yr	181 ± 3.4	184 ± 3.5	0.4016
From C cows, over all yr <sup>c</sup>	193 ± 5.4	194 ± 5.3	0.9370
From H cows, over all yr <sup>c</sup>	155 ± 5.2	169 ± 6.3	0.0675
From B cows, over all yr <sup>c</sup>	194 ± 5.3	190 ± 5.4	0.4424
Wt/d of age at weaning, kg			
From all cows and all yr	0.95 ± .01	0.94 ± .01	0.5959
From C cows, over all yr <sup>c</sup>	0.99 ± .01	1.00 ± .02	0.7866
From H cows, over all yr <sup>c</sup>	0.84 ± .01	0.84 ± .02	0.9599
From B cows, over all yr <sup>c</sup>	1.01 ± .02	0.99 ± .02	0.2154
Birth wt, kg			
From all cows and all yr	35.4 ± .27	34.9 ± .27	0.5167
From C cows, over all yr <sup>c</sup>	35.4 ± .41	35.4 ± .45	0.5223
From H cows, over all yr <sup>c</sup>	34.9 ± .41	34.9 ± .45	0.6972
From B cows, over all yr <sup>c</sup>	35.8 ± .45	34.9 ± .50	0.3700

<sup>a</sup>Cosecure<sup>®</sup> trace mineral boluses had an expected life of approximately 175 d and provided approximately 156 mg/d Cu, 5.9 mg/d Co, and 3.4 mg/d Se.

<sup>b</sup>Weaning weights adjusted according to Beef Improvement Federation guidelines (BIF, 1990).

<sup>c</sup>Breeds: C = Composite (25% Hereford, Angus, Gelbevieh, and Barzona or Senepol); H = Hereford; B = Brahman cross

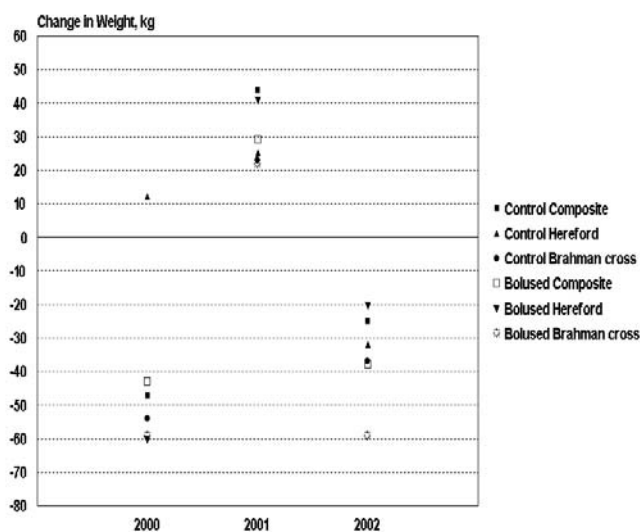


Figure 1. Change in wt from January to May over three yr for beef cowbreeds administered a long acting (6 mo) trace mineral bolus. By breed and yr, Hereford cattle differed by treatment in 2000 ( $P < 0.0001$ ) and Brahman cross cattle tend ( $P = 0.0543$ ) to differ in 2002.

# EFFECT OF LEVEL AND SOURCE OF SELENIUM ON MATERNAL AND FETAL METABOLIC HORMONES IN PREGNANT YEARLING EWES

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**Abstract:** To examine effects of source (organic vs. inorganic) and level (0.1, 3, and 15 ppm) of dietary Se on maternal and fetal insulin like growth factor-1 (IGF-1), and thyroid hormones (T3 and T4), 32 pregnant Targhee ewe lambs (45.6 ± 10.5 kg, 330 ± 30 d of age) were randomly allotted to one of four treatments in a completely randomized design. Treatments were: control (CON; 0.1 ppm Se), Se-wheat (SW; 3 ppm Se), 3 ppm selenate (S3), and 15 ppm selenate (S15). The SW diet was formulated using 32% high Se wheat. Diets were similar in CP (15.5%) and energy (2.68 Mcal of ME), and fed to meet or exceed requirements. Diets were initiated at 50 ± 5 d of gestation. The SW and S3 diets (supranutritional Se levels) provided 75 µg/kg BW of Se, while the S15 treatment provided 375 µg/kg BW of Se. Jugular blood samples were taken from ewes at 50, 64, 78, 92, 106, 120, and 134 d of gestation. Fetal blood samples were taken at d 134 (slaughter). Maternal IGF-1, T3, and T4 were not affected ( $P > 0.1$ ) by treatment, but were altered ( $P < 0.05$ ) by stage of gestation. Maternal IGF-1 increased as gestation progressed, whereas T3 decreased, and T4 remained unchanged. In the fetus, correlations were observed between fetal BW and serum IGF-1. In CON and SW fed ewes,  $r$  values were 0.53 and 0.66, respectively. Conversely this correlation did not exist in ewes fed 3 ppm or 15 ppm as selenate. The S15 treated ewes had the greatest ( $P < 0.001$ ) plasma Se levels (1.3 ppm), the S3 and SW had similar plasma Se levels and CON had the lowest plasma Se concentration. Fetal serum selenium concentrations for SW and S15 treatments were greater ( $P < 0.001$ ) than S3 and CON (0.327 and 0.366, vs. 0.177 and 0.114 ± 0.015 ppm, respectively). Selenium source may influence fetal IGF, but has little impact on T3 and T4. Fetal plasma was higher in Se when the dietary source was high Se wheat compared with Se salt.

Key Words: Selenium, Hormones, Pregnancy

## Introduction

Selenium is a mineral that has diverse biological functions (Sunde, 1997; McDowell, 2003). Selenium has been shown to be required for the conversion of thyroxine (T4) into triiodothyronine (T3), the more active form of the thyroid hormones (Beckett et al., 1987). This relationship is apparent when increasing levels of Se in the diet result in higher levels of serum T3 (Awadeh et al.,

1997). Serum insulin like growth factor (IGF-1) levels were found to be lower in children from Se deficient regions versus Se adequate regions (Aydin et al., 2002). Likewise elevated levels of selenite provided to rats resulted in lower serum IGF-1 versus control (Gronbaek et al., 1995). Currently, relationships between dietary selenium source and serum IGF-1 remain unclear and need defining. Organically bound selenium has been shown to be metabolized differently than inorganic salt forms (Thompson, et al, 1982). This variation in metabolism may explain some of the differences in responses of these metabolic hormones. The objectives of this study were to determine how source and level of selenium affect maternal and fetal serum levels of IGF-1, T3, T4, and Se.

## Materials and Methods

Thirty-two pregnant Targhee ewe lambs (45.6 ± 10.5 kg, 330 ± 30 d of age) were randomly allotted to one of four treatments in a completely randomized design. Treatments were: control (CON; 0.1 ppm Se), Se-wheat (SW; 3 ppm Se), 3 ppm Se from selenate (S3), and 15 ppm Se from selenate (S15). Both diets contained 32% wheat; however, the SW diet was formulated using a high Se (8 ppm) wheat source. Diets were similar in CP (15.5%) and energy (2.68 Mcal ME), and fed to meet or exceed NRC requirements (NRC, 1985). Diets were initiated at 50 ± 5 d of gestation. The SW and S3 diets (supranutritional Se levels) provided 75 µg/kg BW daily of Se, while the S15 treatment provided 375 µg/kg BW daily of Se. Jugular blood samples were taken from ewes at 50, 64, 78, 92, 106, 120, and 134 d of gestation. Fetal blood samples were taken at d 134 (slaughter) via cardiac puncture. All blood samples were centrifuged at 1,500 X g for 30 minutes. Serum was pipetted into 2-ml screw cap vials and stored frozen. Serum IGF-1 was analyzed in a single assay according to procedures of Berrie et al. (1995). Thyroxine and T3 samples were quantified utilizing components of DPC kits (Diagnostic Products Corp., Los Angeles, CA) with modifications in procedures described by Richards et al. (1999), and Wells et al (2003). Serum selenium samples were digested in trace mineral-grade nitric acid under heat. The digests were then diluted with ultra-pure water to a final nitric acid content of 5%, which provided a matrix match for the analytical standards. The prepared samples were

analyzed by ICP-MC and assessed against concentration curves of known standards. Standard curves and quality control samples were analyzed every 5 samples.

Hormone and selenium data were analyzed using the PROC MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). Sources of variation included treatment, period, and treatment by period.

## Results and Discussion

Serum IGF-1 concentrations in maternal serum increased ( $P = 0.001$ ) with advancing gestation (Figure 1). There were no differences ( $P = 0.78$ ), in IGF-1 concentrations among treatments. Conversely, serum T3 decreased ( $P = 0.001$ ) with advancing gestation (Figure 2). Serum T4 concentrations were not altered ( $P = 0.90$ ) by advancing gestation. Treatment did not affect T3 or T4 ( $P = 0.38$  and  $0.42$ ) in pregnant ewes (Table 1). These data disagree with those of Awadeh et al, (1997), who reported a reduction in serum T3 in pregnant cows fed various levels (1.0, 3.3, and 7.3 mg/d) of selenite versus cows fed a selenized yeast source (3.4 mg/d).

Maternal dietary Se did not affect fetal serum concentrations of IGF-1, T3 or T4. However, across dietary treatment, there was an overall correlation ( $P = 0.04$ ;  $r = 0.36$ ) between fetal carcass weight (Fetcw) and serum IGF-1. When correlations were evaluated for individual treatments, both CON and SW had positive  $r$  values of 0.53 and 0.66, respectively. Dietary treatments containing Na selenate showed no correlations of Fetcw and serum IGF-1. These data indicate that the relationship of fetal Fetcw to IGF-1 can be influenced by source of dietary Se. Interestingly, fetal carcass weight was positively correlated with fetal serum T3 concentrations (Table 2) in CON and S15 ( $r = 0.68$  and  $0.57$ , respectively). However, when ewes were fed 3 ppm of Se from wheat (SW) or selenate (S3), there was no relationship between fetal carcass weight and fetal serum T3 concentrations. Fetal carcass weight also was correlated with serum T4 in the S3 ( $r = 0.76$ ) but not in other treatments (Table 2). Reasons for these differences are unclear and require further investigation.

As day of gestation progressed, serum Se increased in the supranutritional treatments (Tables 3 and 4). Maternal serum Se concentrations were greatest in S15, intermediate in S3 and SW, and lowest in CON fed ewes (Table 3;  $P < 0.01$ ) at all times after d 50 of gestation. Because S15 treatment resulted in 3 to 4 times the serum Se concentrations, compared with S3 and SW, serum Se concentrations were reanalyzed without the S15 treatment. Results indicate that serum Se concentrations were lowest in CON, intermediate in S3 and greatest in SW treated ewes ( $P < 0.01$ ) from day 78 to slaughter.

Fetal serum selenium concentrations for SW and S15 treatments were greater ( $P < 0.001$ ) compared with S3 or CON (Figure 4). These data indicate that maternal dietary source of Se influences fetal serum Se concentrations.

## Implications

In the ewe IGF-1, T3, and T4 were not influenced by source or level of selenium. Selenium source and level had a significant impact on serum Se concentrations in both the ewe and the fetus. Fetal serum Se is responsive to maternal dietary Se source.

## Literature Cited

- Awadeh, F. T., R. L. Kincaid, and K. A. Johnson. 1997. Effect of level and source of dietary selenium on concentrations of thyroid hormones and immunoglobins in beef cows and calves. *J. Anim. Sci.* 76:1204-1215.
- Aydin, K., A. Bideci, M. Kendirci, P. Cinaz, and S. Kurtoglu. 2002. Insulin-like growth factor-1 and insulin like growth factor binding protein-3 levels in children living in iodine and selenium deficient endemic goiter areas. *Bio. Trace Elem. Res.* 90:25-30.
- Beckett, G. J., S. W. Beddows, P. C. Morrice., F. Nicol, and J. R. Arthur. 1993. Effects of combined iodine and selenium deficiency on thyroid hormone metabolism in rats. *Am. J. Clin. Nutr.* 57:2405-2435.
- Berrie, R. A., D. M. Hallford, and M. L. Galyean. 1995. Effects of zinc source and level on performance and metabolic hormone concentrations of growing and finishing lambs. *Prof. Anim. Sci.* 11:149-155.
- Gronbaek H., J. Grastyk, H. Orskov, and A. Flyubjerg. 1995. Effect of sodium selenite on growth, insulin like growth factor binding proteins, and insulin like growth factor-1 in rats. *J. Endocrinology* 145:105-112.
- McDowell, L. R. 2003. Minerals in Animal and Human Nutrition (2<sup>nd</sup> ed). Elsevier press. Amsterdam.
- NRC. 1985. Nutrient Requirements of Sheep. 6<sup>th</sup> Ed. National Academy Press, Washington, DC.
- Richards, J. B., D. M. Hallford, and G. C. Duff. 1999. Serum luteinizing hormone, testosterone and thyroxine and growth responses of ram lambs fed locoweed (*Oxytropis sericea*) and treated with vitamin E/selenium. *Theriogenology* 52:1055-1066.
- Sunde, R. A. 1997. Selenium. In: Handbook of Nutritionally Essential Mineral Elements. O'Dell and Sunde, Ed. Marcel Dekker, Inc. New York

Thompson C. D., M. F. Robinson, D. R. Campbell, and

H. M. Rea. 1982. Effect of prolonged supplementation with daily supplements of selenoethionine and sodium selenite on glutathione peroxidase activity in blood of New Zealand residents. *Amer. J. Clin. Nutr.* 36:21-31.

Wells N. H., D. M. Hallford, and J. A. Hernandez. 2003. Serum thyroid hormone and reproductive characteristics of Ramboulet ewe lambs treated with propylthiouracil before puberty. *Theriogenology* 59:1403-1413.

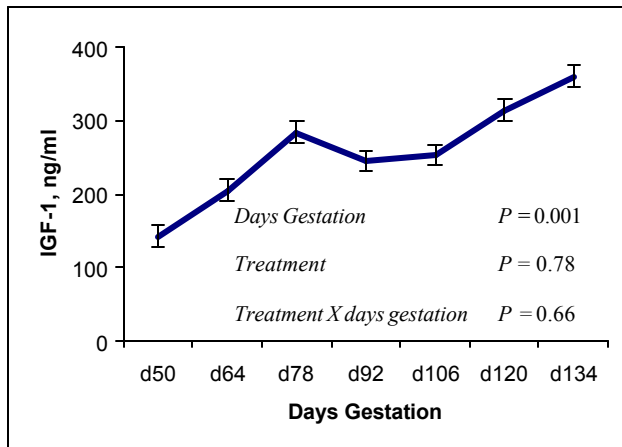


Figure 1. Serum IGF-1 concentrations in pregnant ewes fed different levels and sources of selenium.

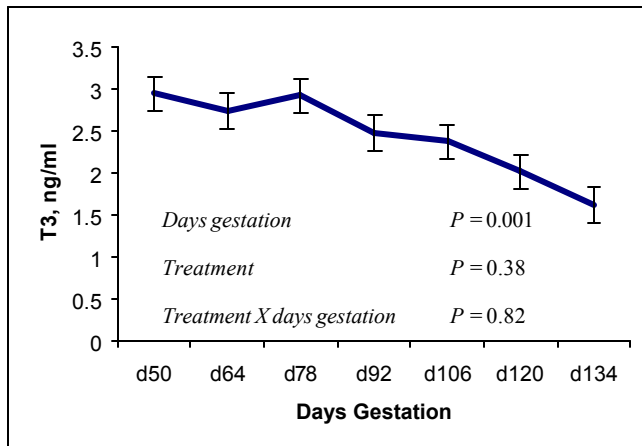


Figure 2. Serum T3 concentrations, in pregnant ewes fed different levels and sources of selenium.

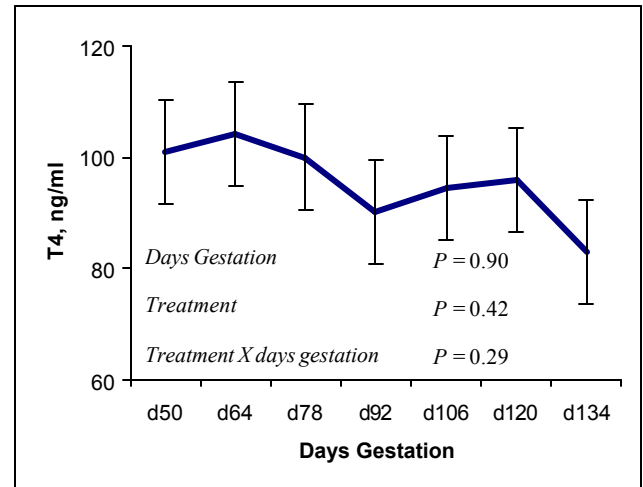


Figure 3. Serum T4 concentrations in pregnant ewes fed different levels and sources of selenium.

Table 1. Serum concentrations of insulin like growth factor-1 (IGF-1), triiodothyronine (T3), and thyroxine (T4) in pregnant ewes fed different levels and sources of selenium.

Item	Treatment <sup>a</sup>				SE	P-value
	CON	SW	S3	S15		
IGF-1	272.8	264.5	235.7	257.1	21.5	0.66
T3	2.57	2.26	2.46	2.49	0.13	0.38
T4	105.1	88.1	95.7	103.9	8.0	0.42

<sup>a</sup> Control (CON), selenium wheat (SW; 3 ppm), selenate (S3; 3ppm), and (S15; 15 ppm).

Table 2. Correlations of fetal carcass weight with serum Insulin like growth factor-1 (IGF-1), triiodothyronine (T3), and thyroxine (T4) concentrations (ng/ml).

Treatment <sup>a</sup>	IGF-1		T3		T4	
	r	P- value	r	P - value	r	P - value
CON	0.53	0.11	0.69	0.03	0.32	0.37
SW	0.66	0.08	0.10	0.84	0.57	0.14
S3	0.10	0.32	0.64	0.17	0.76	0.08
S15	0.04	0.90	0.56	0.08	0.14	0.69

<sup>a</sup> Control (CON), selenium wheat (SW; 3 ppm), selenate (S3; 3ppm), and (S15; 15 ppm).

Table 3. Serum selenium (ppm) in pregnant ewe lambs fed different levels and sources of selenium during gestation.

Days <sup>a</sup>	Treatment <sup>b</sup>				SE	P-value
	CON	SW	S3	S15		
d50	0.24	0.23	0.23	0.26 <sup>a</sup>	0.01	0.15
d64	0.24 <sup>c</sup>	0.34 <sup>d</sup>	0.32 <sup>d</sup>	1.33 <sup>d</sup>	0.08	0.01
d78	0.22 <sup>c</sup>	0.39 <sup>d</sup>	0.34 <sup>d</sup>	1.14 <sup>c</sup>	0.04	0.01
d92	0.23 <sup>c</sup>	0.44 <sup>d</sup>	0.37 <sup>cd</sup>	1.46 <sup>c</sup>	0.07	0.01
d106	0.23 <sup>c</sup>	0.47 <sup>d</sup>	0.38 <sup>cd</sup>	1.34 <sup>c</sup>	0.09	0.01
d120	0.24 <sup>c</sup>	0.43 <sup>d</sup>	0.38 <sup>cd</sup>	1.28 <sup>e</sup>	0.09	0.01
d134	0.27 <sup>c</sup>	0.55 <sup>d</sup>	0.47 <sup>cd</sup>	1.73 <sup>e</sup>	0.06	0.01

<sup>a</sup> Days of gestation.

<sup>b</sup> Control (CON), selenium wheat (SW; 3 ppm), selenate (S3; 3ppm), and (S15; 15 ppm).

<sup>cde</sup> Means within row having differing superscripts differ ( $P < 0.10$ ).

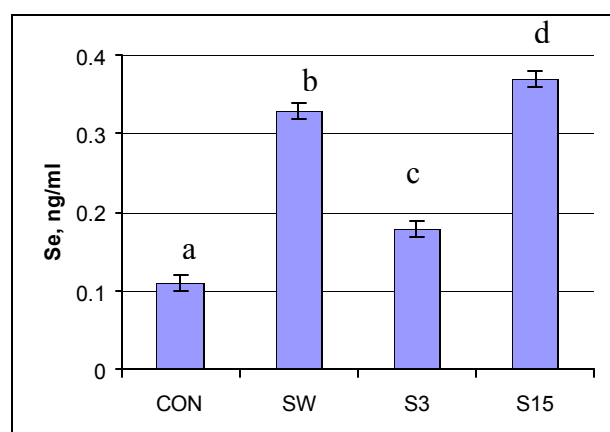


Figure 4. Fetal serum selenium concentrations from ewes fed different levels and sources of selenium.

<sup>abcd</sup> Means within differing superscripts differ ( $P < 0.06$ ).

# EFFECTS OF VITAMIN E LEVELS IN CREEP FEEDS FOR NURSING CALVES ON PERFORMANCE AND SUBSEQUENT FEEDLOT PERFORMANCE<sup>1</sup>

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**ABSTRACT:** To determine the benefits of supplementing nursing calves with vitamin E before weaning, 120 spring-calving cow/calf pairs were stratified by cow age, calving date, and calf gender than randomly assigned to one of 12 5.1-ha bermudagrass/tall fescue pastures from July 1 until weaning (23 October 2002). After weaning calves were received in the SWREC feedlot for 28 d in 12 pens with fence-line feed bunks and started on a 70% concentrate diet (soybean hull based). One of four treatments was randomly assigned to three pastures for 43 d beginning 10 September 2002: 1) no creep feed, or a cooked molasses supplement with 2) no added vitamin E, 3) 1,103 IU of vitamin E/kg, or 4) 2,205 IU of vitamin E/kg (target intake = 227 g/d). Liver biopsies were collected from four calves in each pasture on d 0 and 43 of creep feeding for vitamin E analysis. Data were analyzed by ANOVA with pasture/pen serving as the experimental unit. Least-square means were separated using the following contrast: treatment 1 vs. the average of 2, 3, and 4, and linear and quadratic test for treatments 2, 3, and 4. Creep feed intake averaged 81 g/d and did not differ ( $P \geq 0.42$ ). On pasture, calf BW on d 0 and 43, ADG, and vitamin E concentration in wet liver tissue on d 0 did not differ ( $P \geq 0.10$ ). On d 43, calves fed creep feed had greater ( $P < 0.01$ ) vitamin E concentration in liver tissue (42 g/kg) than calves fed no creep feed (35 g/kg). Increasing vitamin E levels in the creep feed linearly increased ( $P = 0.01$ ) its concentration in liver tissue. After weaning, BW on d 0 and 28, ADG, and feed DMI in the feedlot did not differ ( $P \geq 0.07$ ) between creep fed and not creep fed. A linear decrease ( $P = 0.04$ ) in feed DMI was noted with the increasing levels of vitamin E levels in the creep feed. Calves not creep fed had a superior ( $P < 0.01$ ) G:F (0.23) compared to creep fed calves (0.19). However, a linear increase ( $P < 0.01$ ) was noted in G:F with the increasing levels of vitamin E levels of the creep feed. Adding vitamin E to creep feed had some beneficial effects on subsequent feedlot performance.

Key Words: Vitamin E, Cattle, Beef

## Introduction

The incidence and recovery from morbidity of newly received calves in the feedlot is an important concern for most cattle feeders (Rivera et al., 2002). Typically, vaccination and antibiotic treatments are techniques used by producers to control bovine respiratory disease complex and are effective (Galyean et al., 1995); however,

preconditioning of calves before weaning would be a more effective and easier method of controlling bovine respiratory disease complex. The following trial was designed to evaluation of the effects of feeding various levels of vitamin E in creep feeds on calf creep performance and subsequent 28-d feedlot performance during receiving.

## Materials and Methods

One hundred twenty cows (predominantly Angus with  $\leq 25\%$  Brahman breeding) nursing Angus sired spring-born calves from the SWREC cow herd were stratified by cow age, calving date, and calf gender then randomly assigned to one of 12 bermudagrass/dallisgrass pastures (5.1 ha each) on 1 July 2002 after the completion of the 60-d breeding season. After 71 d of grazing the pastures (10 September 2002), treatments were randomly assigned to pastures as follows: 1) no creep feed, 2) a cooked molasses tub supplying no supplemental vitamin E, 3) a cooked molasses tub containing 1,103 IU vitamin E/kg, or 4) a cooked molasses tub containing 2,205 IU vitamin E/kg. Targeted intakes for the creep feeds were 227 g/d.

Calves were weighed on 10 September 2002 at the beginning of creep feeding. After calves had been weighed, four calves (2 steers and 2 heifers) per pasture had liver biopsies taken as described Corah and Arthington (1994) to measure initial liver vitamin E concentration. In short, liver

Table 1. Composition of the feedlot receiving diets

Feedstuff	Concentrate (DM basis)			
	70%	75%	80%	85%
Chopped grass hay	30.0	25.0	20.0	15.0
Soybean hulls	29.0	34.0	39.0	44.0
Rice bran	10.0	10.0	10.0	10.0
Corn, cracked	15.0	15.0	15.0	15.0
Cottonseed meals	8.0	8.0	8.0	8.0
Mineral 1822 <sup>a</sup>	0.5	0.5	0.5	0.5
Limestone	1.5	1.5	1.5	1.5
QLF Pasture Plus <sup>b</sup>	5.0	5.0	5.0	5.0
Salt	1.0	1.0	1.0	1.0

<sup>a</sup>Contained (% as-fed): 14.0% Salt, 15.0% Ca, 7.0% P, 5.0% Mg, 1.0% S, 1,000 g/kg Mn, 2,355 g/kg Fe, 1,250 g/kg Cu, 3,000 g/kg Zn, 20 g/kg Co, 25 g/kg I, 662 KIU of vit. A/kg, 66 KIU of vit. D<sub>3</sub>/kg, and 221 IU of vit. E/kg.

<sup>b</sup>QLF Pasture Plus molasses based supplement contained the following: 30% CP, 1.7% P, 4.8% K, 30.0% total inverted sugars, 81.7% TDN (DM basis; Quality Liquid Feeds, Inc., Dodgeville, WI.).

tissue was obtained through a puncture incision between the 11<sup>th</sup> and 12<sup>th</sup> rib along the perceptual line from the tuber coxae to the point of the scapula. Using a biopsy needle (Tru-Cut biopsy needle, 14 gauge, 15 cm, 20-mm specimen notch, VWR Scientific Products, Batavia, IL), 400 to 500 mg of tissue was obtained. Four to five livers samples were collected on the anterior end of a sterile glove, physically separated from contaminating blood, and placed in a labeled borosilicate glass (12 x 75 mm) culture tube, capped, and frozen (-20° C) until overnight shipment. Samples were analyzed for vitamin E in a manner similar to that described by Bottje et al. (1997).

To determine creep-feeding intake, containers of cooked molasses were weighed on 10 September 2002 to determine initial weight and were re-weighed at 7 d intervals. Cows were restricted from the creep feeds by surrounding them with four livestock panels; one of the panels being a creep gate. Calves were trained to the creep gates between 1 July and 10 September 2002 by supplementing the cows in front of the creep gate 3 d/wk; cows with non-creep fed calves were also be supplemented. Creep feeders were located in areas where the cows loafed and feeders were observed daily to insure that calves were feeding. On 3 October 2002, calves were re-weighed and injected with a killed 4-way vaccine for IBR, PI<sub>3</sub>, BRSV, and BVD (Triangle 4 + Type II BVD; Fort Dodge Animal Health; Overland Park, KS) and with a seven-way *Clostridial* (Vision 7 Somnus; Bayer Corp., Shawnee Mission, KS). On 23 October 2002, calves were weaned, weighed, and re-vaccinated with a killed 4-way vaccine for IBR, PI<sub>3</sub>, BRSV, and BVD (Triangle 4 + Type II BVD), with a seven-way *Clostridial* (Vision 7 Somnus), treated for internal and external parasites (Cydectin; Fort Dodge Animal Health), and implanted (Ralgro; Schering-Plough Animal Health; Union, NJ). After processing, calves that were initially liver biopsied were re-biopsied.

On the day calves were weaned, they were placed in the SWREC feedlot (23 October 2002). Calves from each pasture were kept together as groups and placed into 12

individual pens. Calves were weighed without fasting on d 0, 14, and 28. The calves were started on a 70% concentrate growing diet (Table 1). For the first seven days of feeding, calves were offered a maximum of 2.3 kg daily of medium-quality bermudagrass hay (12.5% CP, 57.5% TDN). Hay intake was limited in order to encourage consumption of the milled feedlot diet. Additionally, a step-up feeding process was used to deliver 70, 75, 80, and 85% concentrate of the dietary DM on a weekly basis throughout the 28-d receiving period (Table 1). Dietary DMI was limited to a maximum prescribed feeding rate to program 1.1 kg/d of gain as described by Galyean (1999). Samples of feed were collected weekly for analysis of DM, ash, CP (AOAC, 1990), ADF (Goering and Van Soest, 1970), Ca, and P (Sirois, 1991). Feed DMI was monitored and orts were weighed back and discarded on days calves were weighed.

During the 28-d feeding period, calves were observed daily for signs of morbidity. Any calves deemed to have symptoms of bovine respiratory disease complex were to be removed from the pen, rectal temperature was recorded, and calves with rectal temperatures greater than 39.7° C were treated by antibiotic injection. Treated calves were returned to the home pen and all pulls and treatments will be recorded for all calves.

The experiment was analyzed as a completely randomized block design; the main effects were treatment, calf gender, cow age, calf birth date, and pasture/pen(treatment). Pasture or pen within treatment were used as the random experimental error. Least-square means were separated using contrast statements for no creep feed versus the average of pastures receiving creep feed, and linear and quadratic effect of vitamin E level in the creep feed.

## Results and Discussion

*Calf performance on pasture.* Creep feed intake averaged 82 g/d across the three treatments where creep

Table 2. Creep feed intake, BW, and ADG of nursing calves and subsequent feedlot performance during receiving as affected creep feeds with three different levels of vitamin E

Item	No creep	Creep feed			SE <sup>b</sup>	Contrast <sup>a</sup>		
		No added vitamin E	1,103 IU vitamin E/kg	2,205 IU vitamin E/kg		No creep vs. creep	Linear creep	Quadratic creep
Creep feed intake, g/d	---	68	91	86	15.0	---	0.42	0.46
BW, kg								
d 0 (9/10) <sup>c</sup>	164	178	170	157	6.1	---	---	---
d 43 (10/23) <sup>c</sup>	181	186	186	202	2.7	0.26	0.74	0.81
ADG (d 0 to 43), kg	0.32	0.45	0.45	0.45	0.06	0.10	0.73	0.84
Liver vitamin E, g/kg								
d 0 (9/10)	35	32	36	40	6.8	0.95	0.44	0.997
d 43 (10/23) <sup>d</sup>	35	38	44	45	1.4	< 0.01	0.01	0.18

<sup>a</sup>n = 3.

<sup>b</sup>Contrast: no creep feed vs. creep feed = no creep feed vs. the average of creep feed with no vitamin E, 1,103 IU vitamin E/kg, and 2,205 IU vitamin E/kg; linear creep = the linear effect of no vitamin E, 1,103 IU vitamin E/kg, and 2,205 IU vitamin E/kg; quadratic creep = the quadratic effect of no vitamin E, 1,103 IU vitamin E/kg, and 2,205 IU vitamin E/kg.

<sup>c</sup>Body weight measured on 10 September 2002 used as a covariate.

<sup>d</sup>Initial liver vitamin E concentration (10 September 2002) used as a covariate in the analysis.

feed was fed (Table 2) and did not differ among treatments ( $P \geq 0.42$ ). The targeted creep feed intake was 227 g/d. The lower intake estimates than targeted did not result from calves not entering the creep feeders; calves were regularly observed in the creep feeding pens. This lower intake probably resulted from one of two things or a combination of the two: 1) only a portion of the calves consumed creep feed or 2) all calves consumed smaller amounts of feed than targeted. Body weight on d 0 did not differ among treatments or on d 43 ( $P \geq 0.26$ ); BW of creep fed calves was only slightly greater (5 kg) than non-creep fed calves. As a result, ADG did not differ among treatments during the creep feeding period ( $P \geq 0.10$ ).

The vitamin E concentration in wet liver tissue at the start of creep feeding averaged 36 g/kg and did not differ among treatments ( $P \geq 0.44$ ; Table 1). After 43 d of creep feeding, the vitamin E concentration in wet liver tissue was greater ( $P < 0.01$ ) in calves from pastures where creep feed was offered compared to pastures where no creep feed was offered; also, there was a linear increase ( $P = 0.01$ ) with no added vitamin E to 1,000 IU Vitamin E in the creep feed. Based on these data, the lower than targeted creep feed intake was not sufficient enough to stimulate an increase in grow rate (if it would), but it was sufficient to increase the vitamin E status of these calves as evidenced by the liver tissue analysis.

*Calf performance in feedlot.* Body weight on d 0, 14, and 28 did not differ among treatments ( $P \geq 0.26$ ; Table 3). Subsequently, no differences were detected in ADG in any period or the overall ADG ( $P \geq 0.07$ ). These results are

similar to those reported by Rivera et al. (2002) and Hill (1987) who fed supplemental vitamin E during receiving. No calves were treated for bovine respiratory disease during the trial; subsequently, no health data are shown.

Hay was fed to these calves during the first 5 d of receiving to augment adaptation to the high-concentrate milled feed offered in the feedlot and there were no differences ( $P \geq 0.18$ ; Table 2) among treatments in the amount of hay consumed. During the first 14 d in the feedlot, calves consumed an average of 3.3 kg of DM from the feedlot diet; however, there were no differences ( $P \geq 0.16$ ) as a result of treatments. Feed DMI from d 15 to 28 tended ( $P = 0.07$ ) to be greater in for calves that had been creep fed compare to calves that had not be creep fed; also, there was a linear decrease ( $P = 0.04$ ) as the concentration of vitamin E was increased in the creep feed. Similar to that noted for feed DMI between d 15 to 28, feed DMI on d 0 to 28 showed a linear decrease ( $P = 0.04$ ) as the concentration of vitamin E was increased in the creep feed. Interestingly, Rivera et al. (2002) showed that when supplemental vitamin E was included in the receiving, intake was not affected. Feed efficiency, G:F, for d 0 to 14, 15 to 28, and 0 to 28 showed that calves that had not been creep fed had superior ( $P \leq 0.04$ ) feed efficiency compare to calves that had been creep fed. During d 15 to 28, calves offered creep feed had a quadratic response ( $P = 0.05$ ) to the vitamin E concentration in the creep feed with feed efficiency improving at a diminishing rate as vitamin E concentration was add to the creep feed. Also interesting to note is that calves that were creep feed displayed a linear increase ( $P < 0.01$ ) in the G:F ratio as the vitamin E concentration of the

Table 3. Body weight, DMI, and G:F during receiving (weaning) of calves as affected by creep feeding before weaning with three different levels of vitamin E

Item	No creep	Creep feed			SE <sup>b</sup>	Contrast <sup>a</sup>		
		No added vitamin E	1,103 IU vitamin E/kg	2,205 IU vitamin E/kg		No creep vs. creep	Linear creep	Quadratic creep
BW, kg								
d 0 (10/23)	181	186	186	188	2.7	---	---	---
d 14 (11/6) <sup>c</sup>	195	198	198	198	2.7	0.40	0.99	0.91
d 28 (11/20) <sup>c</sup>	211	212	212	215	3.7	0.68	0.57	0.80
ADG, kg								
d 0 to 14 <sup>c</sup>	1.0	0.8	0.8	0.7	0.12	0.17	0.59	0.88
d 15 to 28 <sup>c</sup>	1.1	1.0	1.0	1.2	0.19	0.75	0.42	0.81
d 0 to 28 <sup>c</sup>	1.1	0.9	0.9	1.0	0.06	0.07	0.47	0.82
Daily DMI, kg/animal								
Hay, d 0 to 5 <sup>c</sup>	1.3	1.0	1.1	1.1	0.11	0.18	0.43	0.95
Receiving diet								
d 0 to 14 <sup>c</sup>	3.2	3.4	3.4	3.2	0.14	0.58	0.16	0.77
d 15 to 28 <sup>c</sup>	6.0	6.5	6.3	6.0	0.13	0.07	0.04	0.70
d 0 to 28 <sup>c</sup>	4.6	5.0	4.9	4.6	0.11	0.16	0.04	0.69
G:F, kg:kg								
d 0 to 14 <sup>c</sup>	0.31	0.24	0.24	0.23	0.015	0.04	0.57	0.35
d 15 to 28 <sup>c</sup>	0.19	0.15	0.16	0.20	0.012	< 0.01	< 0.01	0.05
d 0 to 28 <sup>c</sup>	0.24	0.18	0.19	0.21	0.011	< 0.01	< 0.01	0.22

<sup>a</sup>n = 3.

<sup>b</sup>Contrast: no creep feed vs. creep feed = no creep feed vs. the average of creep feed with no vitamin E, 1,103 IU vitamin E/kg, and 2,205 IU vitamin E/kg; linear creep = the linear effect of no vitamin E, 1,103 IU vitamin E/kg, and 2,205 IU vitamin E/kg; quadratic creep = the quadratic effect of no vitamin E, 1,103 IU vitamin E/kg, and 2,205 IU vitamin E/kg.

<sup>c</sup>Body weight measured on 10 September 2002 used as a covariate.

creep feed was increased between d 0 to 28. To the contrary, Rivera et al. (2002) reported that increase the supplemental vitamin E concentrations linearly reduced feed efficiency. The differences noted in G:F ratios suggest the adding vitamin E to the creep feed compensated for the majority of perceived (Drioullard and Kuhl, 1998) deleterious effects normally associated with creep feeding in that it reduces feed efficiency in the feedlot and that it is a result of creep feeding before weaning.

### **Implications**

Results from this study indicate that vitamin E supplementation of nursing calves via the creep feeding with self-fed cooked molasses supplements can be beneficial to the performance during receiving in the feedlot. Vitamin E supplementation during creep feeding seems to be able to alleviate some of the deleterious effects normally associate with the increased energy intake caused by creep feeding that result in decreased feed efficiency in the feedlot.

### **Literature Cited**

- AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.
- Bottje, W. G., G. F. Erf, K. Bersi, S. Wang, D. Barnes, K. W. Beers. 1997. Effect of dietary d,l- $\alpha$ -tocopherol on tissue  $\alpha$ - and  $\gamma$ -tocopherol and pulmonary hypertension syndrome (ascites) in broilers. *Poultry Sci.* 76:1506-1512.
- Corah, L. R., and J. D. Arthington. 1994. Determining the trace mineral status in cattle. Publication and video distribution. Kansas State Univ. Coop. Ext. Ser., Manhattan.
- Drioullard, J. S., and G. L. Kuhl. 1998. Effect of previous grazing nutrition and management on feedlot performance of cattle. *J. Anim. Sci.* 77(Suppl. 2):136-146.
- Galyean, M. L. 1999. Review: restricted and programmed feeding of beef cattle definitions, application, and research results. *Prof. Anim. Sci.* 15:1-6.
- Galyean, M. L., S. A. Gunter, and K. J. Malcolm-Callis. 1995. Effects of arrival medication with tilmosin phosphate on health and performance of newly received cattle. *J. Anim. Sci.* 73:1219-1226.
- Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). *Agric. Handbook No. 379.* ARS, USDA, Washington, DC.
- Hill, G. A. 1987. Vitamin E and selenium supplementation of cattle. Pages 57-65 in *Proc. 1987 Georgia Nutr. Conf. For the Feed Industry.* Agric. Exp. Sta., Univ. of Georgia, Athens.
- Rivera, J. D., G. C. Duff, M. L. Galyean, D. A. Walker, and G. A. Nunnery. 2002. Effects of supplemental vitamin E on performance, health, and humeral immune response of beef cattle. *J. Anim. Sci.* 80:933-941.
- Sirois, P. K., M. J. Reuter, C. M. Laughlin, and P. J. Lockwood. 1991. A method for determining macro and micro elements in forages and feeds by inductively coupled plasma atomic emission spectrometry. *The Spectroscopist* 3:6-9.

## EFFECTS OF THIAMIN SUPPLEMENTATION ON PERFORMANCE AND HEALTH OF GROWING STEERS CONSUMING HIGH SULFATE WATER

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**ABSTRACT:** Thiamin injections are often used to treat sulfate induced polioencephalomalacia (PEM) in beef cattle. It is unclear whether supplemental thiamin will reduce the incidence of PEM and improve performance in steers consuming water with elevated sulfate levels. This study was conducted to determine the effects of thiamin supplementation on performance and health of growing steers consuming water with high sulfate levels. Sixty-three steers (335 kg) were stratified by weight and randomly allotted to one of nine pens. Pens were assigned to one of three treatments (3 pens/treatment) based on water sulfates and thiamin supplementation. Treatments were: 1) low sulfate water (average = 393 mg/L sulfates) with no supplemental thiamin (LS); 2) high sulfate water (average = 3786 mg/L sulfates) with no supplemental thiamin (HS); and 3) high sulfate water (average = 3790 mg/L sulfates) with supplemental thiamin at 1 g/hd/d (HST). The study was conducted from June 16 to August 22, 2003. Water was obtained from a rural water system and sodium sulfate was mixed in the water to create desired sulfate levels in the HS and HST treatments. Steers were fed a diet containing grass hay, wheat middlings, and supplement. The supplement was identical for all treatments except for the addition of thiamin to HST. Water intake did not differ between treatments ( $P = 0.24$ ). Steers on HST had a higher ( $P = 0.05$ ) ADG than those on HS, and steers on LS had a higher ( $P = 0.01$ ) ADG than HS or HST (0.81, 0.49, 0.63 kg/d for LS, HS, and HST, respectively). Steers on LS had higher ( $P = 0.01$ ) DMI than steers in HS or HST. Steers on LS and HST had a higher ( $P < 0.10$ ) gain/feed than steers on HS (0.087, 0.064, and 0.078 for LS, HS, and HST, respectively). The incidence of PEM was 4.8 and 14.3% for HST and HS, respectively, compared to no cases of PEM in the LS treatment ( $P < 0.10$ ). There were no differences in the incidence of PEM between HST and HS ( $P = 0.29$ ). Thiamin supplementation (1 g/hd/d) improved ADG and gain/feed in steers receiving high sulfate water.

**Key Words:** Steers, Water, Sulfate, Thiamin, Polioencephalomalacia

### Introduction

Water in South Dakota is often high in sulfates (APHIS, 2000). Gould et al. (2002), accounting for the sulfur contribution of both water and feed, found diets of cattle in South Dakota and the surrounding region. To commonly have sulfur levels above those recommended by the NRC (1996). High dietary sulfur levels have been associated with polioencephalomalacia (PEM; McAllister et al. 1997), a neurological disorder that causes incoordination, blindness,

anorexia, depression, seizures, and possibly death. Data from our laboratory showed that increased levels of sulfates in the water supplied to growing steers decreased performance and increased the incidence of PEM (Patterson et al. 2002; 2003).

Thiamin injections are often recommended as therapeutic treatment for diagnosed cases of PEM. A thiamin deficiency has been associated with PEM (McDowell, 1989) due to an antagonism between the ingested sulfur and thiamin in the rumen (Brent and Bartley, 1984). However, recent evidence has shown PEM to be associated with hydrogen sulfide production in the rumen, and not with blood thiamin levels (McAllister et al. 1997; Loneragan et al. 1998). Data are lacking to show the effects of supplemental thiamin to cattle receiving water with elevated sulfate levels. Therefore, the objective of this study was to evaluate the effects of supplemental thiamin on the performance and health of growing steers consuming water with high sulfate levels during the hot summer months.

### Materials and Methods

The study was conducted from June 16 to August 22, 2003, at South Dakota State University's Cottonwood Range and Livestock Research Station, near Phillip, SD. Sixty-three crossbred steers (avg. wt = 335 kg) were stratified by weight and randomly assigned to one of nine pens (7 steers/pen). The pens were randomly assigned to one of three treatments based on water sulfates and thiamin supplementation. Treatments were 1) low sulfate water with no supplemental thiamin (LS), 2) high sulfate water with no supplemental thiamin (HS), and high sulfate water with supplemental thiamin at 1 g/hd/d (HST).

The LS water was from a rural water system, and the HS and HST treatments were created by adding sodium sulfate to the LS water to a targeted 4000 mg/L sulfates (Table 1). Water samples were taken daily and composited by week. Weekly composites were analyzed for sulfates by the SDSU Water Resource Institute, Brookings, SD (Table 1).

Steers were housed in dry-lot pens and fed grass hay and wheat middlings (diet consisted of 55% hay and 45% wheat middlings; 13.16% CP, .41 Mcal/lb NEg, 0.95% Ca, 0.58% P; DM basis). Supplements (Table 2) were top dressed at a rate of .45 kg/head/day. The supplement fed to the steers in HST had thiamin mononitrate included at 2.2 gr/kg (1 g of supplemental thiamin/d). Rations were fed in concrete bunks at 0800 daily. Bunks were managed to be clean just prior to feeding, and any orts were weighed and recorded. Free choice salt was fed ad-libitum to all

treatments in covered mineral feeders. Water was supplied in oval aluminum tanks. Water consumption was measured by the daily change in water depth and adjusted for evaporation and precipitation (measurements of evaporation and precipitation taken from a weather station located adjacent to the research feedlot). Animal health was monitored daily. Cattle were diagnosed with PEM when showing multiple clinical symptoms characterizing the disease, including depression, blindness, incoordination, star gazing, anorexia, and seizures. Necropsies were performed on all mortalities, and tissue samples were submitted to the South Dakota State University Diagnostic Laboratory for disease confirmation.

Steer weights were taken in the morning on two consecutive days at the beginning and end of the experiment. Access to water was denied 12-h prior to weight measurements. At the end of the experiment all pens were placed on rural water (LS treatment) and limit fed for 5 d (approximately 2% of BW, DM Basis) prior to final weight measurements. Steer ADG was calculated for each experimental unit (pen) with data from dead cattle removed. Feed efficiency was calculated as ADG divided by average daily DMI.

The effects of treatment on steer weight, ADG, DMI, gain/feed, and water intake were analyzed by ANOVA using PROC GLM of SAS. Morbidity and mortality were analyzed with Chi-Square analysis in Proc Freq of SAS. (SAS Inst. Inc., Cary, NC)

## Results and Discussion

The daily high temperature recorded during the study period was 44°C, with an average high of 33°C. Steer weights at the end of the experiment (Table 3) were different between all treatments ( $P < 0.10$ ), with the LS steers the heaviest and HS steers the lightest. Thiamin supplementation (HST) improved ADG by 29% over the HS treatment ( $P < 0.05$ ), but the HST treatment had lower ADG ( $P < 0.05$ ) than the LS treatment (Table 3). Dry matter intake (Table 3) in the HST and HS treatments was 13.2% and 18.4% less, respectively, than LS treatment ( $P < 0.05$ ). Dry matter intake did not differ ( $P = 0.22$ ) between HST and HS. Gain/feed (Table 3) was higher in the LS and HST treatments compared to the HS treatment ( $P < 0.10$ ), and LS was not different from HST ( $P > 0.10$ ). Water intake did not differ between treatments ( $P = 0.24$ ).

Patterson et al. (2002) reported a 27% reduction in ADG of steers receiving water with 3100-3900 mg/L sulfates over steers on a 400 mg/L sulfate treatment, compared to a 45% and 23% reduction for HS and HST in this experiment, respectively. Patterson et al. (2002; 2003) also showed a decrease in water intake, DMI, and gain/feed when comparing treatments above 3000 mg/L to the 400 mg/L sulfate treatment. Unlike Weeth and Hunter (1971) and Patterson et al. (2002; 2003), this study showed no difference in water intake between sulfate levels.

There was no incidence of morbidity or mortality in the LS treatment, but calves on the HS treatment had a 14.3% (3/21) and 9.5% (2/21) morbidity and mortality rate, respectively (morbidity difference between LS and HS,  $P < 0.10$ ). The steers on HST treatment had a 4.8% (1/21)

incidence of morbidity and mortality, which was not different than either the HS or LS treatments ( $P > 0.50$ ). All calves that were morbid were pulled out of their home pen for showing the symptoms of PEM. There were no differences in mortality between treatments ( $P > 0.50$ ). Out of the two calves that died in the HS treatment, one was confirmed PEM by diagnostic analysis of brain tissue. The remaining animal showed signs of PEM prior to death, but PEM was not confirmed due to autolysis of the tissue. The single mortality in the HST treatment resulted in an inconclusive diagnosis as well. This steer was not observed showing signs of PEM prior to death. We have observed cases in the past where sudden death occurred and diagnostics did confirm PEM, but a diagnosis was not made in this mortality. Patterson et al. (2002; 2003) showed a difference in the incidence of PEM and mortality when comparing steers on water with low sulfate levels versus those receiving water with high sulfate levels.

High daily sulfur intakes were likely associated with the onset of PEM. The HS and HST steers had dietary sulfur levels of 0.85% and 0.88%, respectively, compared to a dietary sulfur level of 0.25% in the LS treatment. Loneragan et al. (1998) found that a dietary sulfur level of 0.90% from feed was associated with PEM. Patterson et al. (2002) found that 0.70% dietary sulfur was associated with PEM. The NRC (1996) gives a maximum tolerable dietary sulfur level of 0.40%, and a requirement of 0.15%. Diets with sulfur levels above 0.20% have shown to impair digestion in the rumen of finishing steers (Zinn et al., 1997).

Thiamin deficiency is a potential cause of PEM (McDowell, 1992). McAllister et al. (1997) and Loneragan et al. (1998) found that PEM was associated with hydrogen production in the rumen, however, and not with blood thiamin levels. The active form of thiamin may not be impacted by hydrogen sulfide production (Loneragan et al., 1997). Hydrogen sulfide is a toxic gas that can be inhaled following eructation from rumen (Kandylis, 1984). The inhaled hydrogen sulfide disrupts energy metabolism in brain cells and causes necrotic lesions which characterize PEM. We evaluated our data with all cattle diagnosed with PEM removed (data not shown), and found the same statistical differences between treatments in ADG and feed efficiency. The fact that thiamin supplementation improved performance independent of clinical PEM could be due to thiamin having an effect on sub-clinical PEM, or supplemental thiamin could have voided a deficiency.

Thiamin supplementation to cattle on high sulfate water improved ADG and feed efficiency.

## Implications

Water high in sulfates decreased weight gains and feed efficiency in steers fed a growing ration. Sulfates in the water were associated with polioencephalomalacia. Thiamin added to the daily diet improved gain in steers on high sulfate water. More research is needed to examine the effects of thiamin levels in the diet on performance and health of cattle on high sulfate water.

## Literature Cited

- APHIS. 2000. Water quality in U.S. feedlots. December Info Sheet #N341.12000. United States Department of Agriculture, Animal and Plant Health Inspection Service.
- Brent, B. E. and E. E. Bentley. 1984. Thiamin and niacin in the rumen. *J. Anim. Sci.* 59:813-822.
- Gould, D. H., D. A. Dargatz, F. B. Garry, D. W. Hamar, and P. F. Ross. 2002. Potentially hazardous sulfur conditions on beef cattle ranches in the United States. *J. Am. Vet. Med. Assoc.* 221:673-677.
- Kandyliis, K. 1984. Toxicology of sulfur in ruminants: Review. *J. Dairy Sci.* 67:2179-2187.
- Loneragan, G. H., D. H. Gould, J. J. Wagner, F. B. Garry, and M. A. Thoren. 1997. The effect of varying water sulfate content on hydrogen sulfide generation and health of feedlot cattle. *J. Anim. Sci.* 75 (Suppl. 1):272 (Abstr.)
- Loneragan, G. H., D. H. Gould, R. J. Callan, C. J. Sigurdson, and D. W. Hamar. 1998. Association of excess sulfur intake and an increase in hydrogen sulfide concentrations in the ruminal cap of recently weaned beef calves with polioencephalomalacia. *J. Am. Vet. Med. Assoc.* 213. 1599-1604.
- McAllister, M. M., D. H. Gould, M. F. Raisbeck, B. A. Cummings, and G. H. Loneragan. 1997. Evaluation of ruminal sulfide concentrations and seasonal outbreaks of polioencephalomalacia in beef cattle in the feedlot. *J. Am. Vet. Med. Assoc.* 211:1275-1279.
- McDowell, L. R. 1989. *Vitamins in Animal Nutrition* Academic Press, Inc. San Diego, CA.
- McDowell, L. R. 1992. *Minerals in Animal and Human Nutrition*. Academic Press, Inc. San Diego, CA.
- NRC. 1996. *Nutrient Requirements of Beef Cattle*. 7<sup>th</sup> ed. National Academy Press, Washington, DC.
- Patterson, H. H., P. S. Johnson, T. R. Patterson, D. B. Young, and R. Haigh. 2002. Effects of water quality on performance and health of growing steers. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 53:217-220.
- Patterson, H. H., P. S. Johnson, and W. B. Epperson. 2003. Effect of total dissolved solids and sulfates in drinking water for growing steers. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 54:*In Press*.
- Weeth, H. J. and J. E. Hunter. 1971. Drinking of sulfate-water by cattle. *J. Anim. Sci.* 32:277-281.
- Zinn, R.A., E. Alvarez, M. Mendez, M. Montano, E. Ramirez, and Y. Shen. 1997. Influence of dietary sulfur level on growth performance and digestive function in feedlot cattle. *J. Anim. Sci.* 75:1723-1728.

**Table 1.** Sulfate levels in water supply for growing steers in western South Dakota in 2003.

Water Analysis	Low Sulfate (LS)	High Sulfate (HS)	High Sulfate with Thiamin (HST)
Projected (mg/L)	400	4000	4000
Average (mg/L)	393	3786	3790
Range (mg/L)	372 to 416	2188 to 4081	2188 to 4081

**Table 2.** The nutrient content of supplements feed to growing steers receiving various sulfate levels in the water supply.

Item	As Fed Specifications	
	LS and HS Supplement	HST Supplement
Ca, %	11	11
Mn, mg/kg	50.0	50.0
Zn, mg/kg	100.0	100.0
Cu, mg/kg	150.0	150.0
Co, mg/kg	1.5	1.5
Se, mg/kg	0.5	0.5
I, mg/kg	5.0	5.0
Vit. A (added), IU/kg	20,408	20,408
Vit. D (added), IU/kg	2,040	2,040
Vit. E (added), IU/kg	27	27
Thiamin (active), g/kg	NA	2.2

**Table 3.** Intake and performance of growing steers supplied water with various sulfate levels and thiamin supplement in 2003.

Item	Low Sulfate (LS)	High Sulfate(HS)	High Sulfate with Thiamin (HST)	SEM
Observations	3	3	3	
Initial wt, kg	334	332	339	1
Final wt, kg	389 <sup>a</sup>	366 <sup>b</sup>	380 <sup>c</sup>	3
ADG, kg/d	0.81 <sup>d</sup>	0.49 <sup>e</sup>	0.63 <sup>f</sup>	0.042
Dry matter intake, kg/d	9.31 <sup>d</sup>	7.60 <sup>e</sup>	8.08 <sup>e</sup>	0.25
Gain/Feed	0.087 <sup>a</sup>	0.065 <sup>b</sup>	0.078 <sup>a</sup>	0.004
Water intake, Liters/d	45.05	40.81	43.34	1.59

<sup>a,b,c</sup>Within a row, means without a common superscript letter differ ( $P < 0.10$ ).

<sup>d,e,f</sup>Within a row, means without a common superscript letter differ ( $P \leq 0.05$ ).

# COMPARATIVE FEEDING VALUE OF SEASONAL KIKUYUGRASS WITH SUMMER AND WINTER GRASSES IN HOLSTEIN STEERS FED FORAGE BASED DIETS<sup>12</sup>

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**ABSTRACT:**Four Holstein steers (167 kg) with cannulas in the rumen and proximal duodenum were used in a 4 x 4 Latin square experiment to evaluate the comparative feeding value of seasonal Kikuyugrass hay (Pennisetum clandestinum var. Whittet; winter and summer) vs summer (Sudangrass; Sorghum sudanense) and winter (Annual Ryegrass; Lolium multiflorum var. Oregon) grass hays in 70% forage-based diets (88.4% OM, 35.5% NDF, and 11.8% CP). Chromic oxide (0.20% DM basis) was added as digesta marker. There were no treatment effects ( $P > .05$ ) on ruminal NDF and N digestion, ruminal microbial efficiency (grams MN/ gk OM fermented), and ruminal N efficiency (non-ammonia N entering the small intestine/ N intake). There were no seasonal effects (summer versus winter harvest;  $P > 0.10$ ) on ruminal and total tract digestion of OM, N, or NDF of kikuyugrass-based diets. Ruminal and total tract digestion of OM, NDF and N were similar ( $P > .10$ ) for sudangrass- and kikuyugrass-based diets. However, ruminal digestion of OM and total tract digestion of OM and N was lower (19, 12, and 9%, respectively;  $P < .05$ ) for the kikuyugrass-based diets than for the annual ryegrass-based diet. Kikuyugrass-based diets had higher ( $P < 0.05$ ) ruminal molar proportions of acetate, and acetate:propionate ratio than ryegrass- and sudangrass-based diets. There were not ( $P > .05$ ) treatment effects on acetate:propionate ratio. We conclude that kikuyugrass has a feeding value similar to that of sudangrass hay. But, its feeding value is much lower (17% with respect to OM digestion) than that of annual ryegrass hay.

Keywords: Kikuyu grass, Cattle, Digestion

## Introduction

Kikuyu grass (*Pennisetum clandestinum*, variety Whittet) has been established in the Mexicali Valley of Baja California, as a perennial sward. Its agronomic characteristics and resistance to extreme climate conditions are considered advantageous (Fribourg, 1995; Krestschmer and Pitman, 1995). Shultz (2000) compared kikuyu grass with Piper sudan and alfalfa hays using ruminal in situ techniques. Following a 48-h incubation, kikuyu grass hay was 34 and 9% more digested than sudan or alfalfa. There do not appear to be any published studies that evaluate site of digestion of kikuyu in vivo. The objective of this study was to evaluate the comparative feeding value of seasonal kikuyu grass hay (winter and summer) vs summer (Sudangrass) and winter (annual ryegrass) grass hays in 70%-forage diets fed to Holstein steers.

## Materials and Methods

Four Holstein steers (167 kg) with cannulas in the rumen and

proximal duodenum were used in a 4 x 4 Latin square experiment to evaluate the comparative feeding value of seasonal kikuyu grass hay (winter- and summer-harvest) vs summer (Sudangrass) and winter (annual ryegrass) grass hays in 70% forage-based diets (88.4% OM, 35.5% NDF, and 11.8% CP). Chromic oxide (0.20% DM basis) was added as digesta marker. Experimental diets are shown in Table 1. Experimental periods consisted of a 10-d diet adjustment period followed by a 4-d collection period. During the collection period duodenal and fecal samples were taken from all steers, twice daily as follows: d 1, 0750 and 1350; d 2, 0900 and 1500; d 3, 1050 and 1650; and d 4, 1200 and 1800. Individual samples consist of approximately 700 mL duodenal chyme and 200 g (wet basis) fecal material. Samples from each steer and within each collection period were composited for analysis. During the final day of each collection period, ruminal samples were obtained from each steer at 4 h after feeding via the ruminal cannula. Upon completion of the trial, ruminal fluid was obtained from all steers and composited for isolation of ruminal bacteria via differential centrifugation (Bergen et al., 1968). Samples were subjected to all or part of the following analysis: DM (oven drying at 105 C until no further weight loss); ash, Kjeldahl N, ammonia N (AOAC, 1975), purines (Zinn and Owens, 1986), NDF (Chai and Uden, 1998), and chromic oxide (Hill and Anderson, 1958). Microbial organic matter (MOM) and N (MN) leaving the abomasum is calculated using purines as a microbial marker (Zinn and Owens, 1986). OM fermented in the rumen (OMF) is considered equal to OM intake minus the difference between the amount of total OM reaching the duodenum and MOM reaching the duodenum. Feed N escape to the small intestine is considered equal to total N leaving the abomasum minus ammonia-N and MN and, thus, includes any endogenous contributions. The trial was analyzed as a 4 x 4 Latin square. Treatment effects were tested for the following orthogonal contrasts: summer-harvest kikuyu grass vs sudrangrass, winter-harvest kikuyu grass vs ryegrass, and summer- vs winter-harvest kikuyu grass (Hicks, 1973).

## Results and Discussion

Treatment effects on characteristics of digestion are shown in Table 2. There were no treatment effects ( $P > 0.10$ ) on ruminal NDF and N digestion, ruminal microbial efficiency (grams MN/ gk OM fermented), and ruminal N efficiency (non-ammonia N entering the small intestine/ N intake). Ruminal and total tract digestion of OM was greater ( $P < .05$ ) for ryegrass hay than the other hays.

There were no seasonal effects (summer versus winter harvest;  $P > 0.10$ ) on ruminal and total tract digestion of OM, N, or NDF of kikuyu grass hay-based diets. Fulkerson et al.

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(1998) reported that kikuyu grass hay was least affected by harvest season on its nutritive value when compared with annual or perennial ryegrass. Treatment effects on molar proportions of VFA and ruminal NDF kinetics are shown in Table 3. Kikuyu grass-based diets had greater ( $P < 0.05$ ) ruminal molar proportions of acetate, and acetate:propionate ratio than ryegrass- and sudangrass-based diets. Ryegrass based diet had the lowest ( $P < 0.01$ ) ruminal pH, and the highest ( $P < 0.01$ ) Kd and Kp for NDF in this experiment.

### **Implications**

The nutritive value of kikuyu grass is not affected by harvest date, provided that the forage is harvested at similar stage of growth. Kikuyugrass has a feeding value similar to that of sudangrass hay. However, its feeding value is lower (17% with respect to OM digestion) than that of annual ryegrass hay.

### **Literature Cited**

- AOAC. 1975. Official methods of analysis (12th ed). Association of Official Analytical Chemists, Washington, DC.
- Bergen, W. G., D. B. Purcer, and J. H. Cline. 1968. Effect of ration on the nutritive quality of rumen microbial protein. *J. Anim. Sci.* 27:1497-1501.
- Chai W., and P. Udén. 1998. An alternative oven method combined with different detergent strengths in the analysis of neutral detergent fiber. *Anim. Feed Sci. Technol.* 74:281-288.
- Fribourg, A. H. 1995. Summer Annual Grasses. In: *Forages, an Introduction to Grassland Agriculture*. Fifth Edition. Iowa State Univ. Press, Ames, Iowa. p 283-304.
- Fulkerson, W. J., K. Slack, D. W. Hennessy, and G. M. Hough. 1998. Nutrients in ryegrass (*Lolium* spp.), white clover (*Trifolium repens*) and kikuyu (*Pennisetum clandestinum*) pastures in relation to season and stage of regrowth in a subtropical environment. *Aust. J. Exp. Agric.* 38:227-240.
- Hicks, C. R. 1973. *Fundamental concepts in the design of experiments*. Holt, Rinehart and Winston, New York.
- Hill, F. N., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. *J. Nutr.* 64:587-603.
- Krestschmer, A. E. and Pitman W. 1995. Tropical and Subtropical Forages In: *Forages, an Introduction to Grassland Agriculture*. Fifth Edition. Iowa State Univ. Press, Ames, Iowa. p 283-304.
- Zinn, R. A., and F. N. Owens. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. *Can. J. Anim. Sci.* 66:157-166.

Table 1. Composition of experimental diets fed to Holstein steers<sup>a</sup>.

Item	Treatments <sup>a</sup>			
	RG	SD	KS	KW
Ingredient composition, % (DM basis)				
Ryegrass hay	69.97			
Sudangrass hay		66.77		
Kikuyu grass			66.77	66.77
Cane molasses	4.16	4.26	4.26	4.26
Steam-flaked wheat	19.63	19.59	19.59	19.59
Chromic oxide	0.28	0.29	0.29	0.29
Yellow grease	1.78	2.74	2.74	2.74
Limestone	0.88	0.9	0.9	0.9
Fishmeal	1.96	3.01	3.01	3.01
Urea	0.89	0.46	0.46	0.46
Sal	0.44	0.45	0.45	0.45
Soybean meal		1.52	1.52	1.52

<sup>a</sup>Treatments: RG: Ryegrass hay; SG: Sudangrass; KS: Summer Kikuyugrass; KW: Winter Kikuyu grass.

Table 2. Effect of seasonal kikuyu grass vs summer and winter grasses on digestive function in Holstein steers fed forage based diets.

Holstein steers fed storage-based diets.					
Item	Treatments <sup>a</sup>				S D
	RG	SG	KS	KW	
Intake, g/d					
DM	4045	4088	4105	4055	
OM	3604	3712	3615	3485	
NDF	770	1494	1450	1318	
N	88	68	76	85	
Flow to the duodenum, g/d					
OM	1436	1607	1695	1752	163
NDF <sup>b</sup>	324	657	623	625	63
N <sup>c</sup>	74.9	53.2	58.3	66.1	7.4
Non-Ammonia N <sup>c</sup>	72.5	51.1	55.2	62.8	7.1
MN <sup>c</sup>	48.4	31.5	25.6	27.9	9.1
Feed N	24.2	19.6	24.5	29.3	6.9
Ruminal digestion, % of intake					
OM <sup>c</sup>	73.9	65.2	61.8	59.6	4.8
NDF	57.8	49.4	53.7	52	12.5
Feed N	73.5	71.1	67.2	65.7	8
MN efficiency <sup>d</sup>	18.3	13	14	16.5	2.6
N efficiency <sup>e</sup>	0.82	0.77	0.74	0.74	0.07
Postruminal digestion, % of flow to duodenum					
OM <sup>bc</sup>	64	42	49.4	51.6	3.8
NDF	41.2	19.5	27.5	34.4	7.2
N <sup>c</sup>	75.1	67.5	69.1	63.9	4.2
Total tract digestion, %					
DM <sup>c</sup>	83.9	73.8	74.7	72.3	3.3
OM <sup>c</sup>	85.7	74.9	76.6	75.7	3.6
NDF	75.2	58.5	66.4	68.8	10.9
N <sup>c</sup>	79	74.3	76.4	71.8	2.9
Digestible Energy, % <sup>c</sup>	83.9	75.2	75	74	3.8

<sup>a</sup>Treatments: RG: Ryegrass hay; SG: Sudangrass; KS: Summer Kikuyugrass; KW: Winter Kikuyugrass.

<sup>b</sup>SG vs KS, P < .05.

<sup>c</sup>RG vs KW, P < .05.

<sup>d</sup>Microbial N, g/kg of OM truly fermented.

<sup>e</sup>Non-ammonia N/feed N

Table 3. Effect of seasonal kikuyu grass vs summer and winter grasses on VFA molar proportions and ruminal kinetics characteristics of the NDF in Holstein steers fed forage based diets.

Item	Treatments <sup>a</sup>				SD
	RG	SD	KS	KW	
pH <sup>c</sup>	5.46	6.2	6.26	6.08	0.18
Ruminal VFA, mol/ 100 mol					
Acetate <sup>bc</sup>	61.6	67.4	76.2	73.4	4.1
Propionate	21.2	18.1	14.1	15.5	3.8
Butirate <sup>c</sup>	17.24	14.46	9.72	11.03	3.35
Acetate:propionate	2.98	4.03	5.78	4.84	1.44
Kinetics of NDF digestion					
kp	9.77	4.74	4.7	5.86	0.02
kd <sup>c</sup>	13.95	5.95	5.45	6.5	3.95

<sup>a</sup>Treatments: RG: Ryegrass hay; SG: Sudangrass; KS: Summer Kikuyugrass; KW: Winter Kikuyugrass.

<sup>b</sup>SG vs KS, P <.05.

<sup>c</sup>RG vs KW, P <.05.

# EFFECTS OF DIETARY UREA CONCENTRATION ON ACID-BASE BALANCE OF FEEDLOT STEERS FED A HIGH CONCENTRATE STEAM-FLAKED CORN BASED DIET.

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**ABSTRACT:** Urea is commonly used in feedlot diets to supply ruminally degradable N. Additionally, it has a transient rumen buffering effect and recent speculation has suggested urea could possibly combat systemic acidosis. Thus, this experiment was conducted to determine the effects of dietary urea on acid-base balance in feedlot steers. A 4 x 4 Latin square was used to determine the effects of isonitrogenous steam-flaked corn based diets containing 0, 0.5, 1.0 or 1.5% urea (DM basis). Steers (306 ± 11 kg) were allowed a 21 d adjustment period to each diet followed by a 1 d sampling period. Immediately prior to, and 1, 2, 4, and 8 h after feeding, arterial (auricular) and jugular blood, as well as urine samples were collected from each steer. Arterial blood samples were immediately analyzed for pH, pCO<sub>2</sub>, and pO<sub>2</sub> and urine samples were immediately analyzed for pH. Serum was harvested from venous blood, stored (-20 °C), and later analyzed for serum urea N (SUN) concentration (mg/dL) evaluation. A treatment x time interaction ( $P < 0.001$ ) was observed for urine pH, however, the biological significance of this interaction is doubtful; no other treatment x time interactions ( $P > 0.26$ ) were observed. No treatment effects were observed for arterial pH ( $P > 0.52$ ; 7.51, 7.53, 7.51, and 7.55 for 0, 0.5, 1.0, and 1.5% urea, respectively), SUN ( $P > 0.40$ ; 14.67, 12.42, 12.08, and 12.66 mg/dL for 0, 0.5, 1.0, and 1.5% urea, respectively), pCO<sub>2</sub> ( $P > 0.62$ ), pO<sub>2</sub> ( $P > 0.32$ ), or urine pH ( $P > 0.89$ ). These results indicate that increasing urea concentrations in high concentrate feedlot diets has no effect on arterial pH, blood gas profile, SUN, or urine pH. Therefore, dietary urea is unlikely to combat systemic acidosis.

source from 0 to 100% increased carcass adjusted ADG, G:F, carcass adjusted G:F, hot carcass weight, and LM area. Additionally, urea has been shown to be an effective ruminal-alkalizing agent in the first hour post feeding (Zinn et al., 2003). This is likely due to its rapid hydrolyzation to CO<sub>2</sub> and NH<sub>3</sub>, and the H<sup>+</sup> scavenging capacity of NH<sub>3</sub> as it is rapidly converted to NH<sub>4</sub><sup>+</sup> in the rumen. The concurrent increase in rumen pH and NH<sub>3</sub> concentration leads to more rapid absorption of NH<sub>3</sub> (Owens and Zinn, 1988). Galyean (1996) speculated that buffering effects, via NH<sub>3</sub>, could also moderate systemic acid loads. Guyton and Hall (2000) explain that NH<sub>3</sub> is utilized in the kidney by diffusing into the tubular lumen and reacting with secreted H<sup>+</sup> ions to form NH<sub>4</sub><sup>+</sup> ions. Each time a NH<sub>4</sub><sup>+</sup> ion is formed and secreted into the urine a complimentary HCO<sub>3</sub><sup>-</sup> ion is also formed and reabsorbed (Guyton and Hall, 2000). Therefore, feeding increasing amounts of urea should provide a buffer to guard against systemic acidosis. Consequently, this has brought about speculation by researchers that feeding urea should, hypothetically, provide a buffer against systemic acidosis. Nonetheless, Cole et al. (2003) indicated that acid-base balance was not significantly affected by dietary CP based on blood gas concentrations. However, no research has been reported on the ability of urea to decrease systemic acid stress. Consequently, the focus of this study was the efficacy of incremental increases of urea in the diet to combat the increased systemic acid load associated with feeding high concentrate feedlot diets.

## Materials and Methods

A 4 x 4 Latin square design was used to determine the effects of feeding four isonitrogenous (12.17% CP) steam-flaked corn based diets containing 0, 0.5, 1.0 or 1.5% urea (Table 1; DM basis). Steers (306 ± 11 kg) were allowed a 21 d adjustment period to each diet followed by a 1 d sampling period. Immediately prior to, and 1, 2, 4, and 8 h after feeding, urine and blood samples were collected. Blood was sampled via arterial (auricular) and jugular puncture. Venous blood was allowed to clot at room temperature and serum was harvested via centrifugation at 1,000 x g for 20 min and stored (-20 °C) for later serum urea N (SUN) concentration (mg/dL) evaluation. Serum urea N was determined using a direct colorimetric determination method (TECO Diagnostics, Anaheim, CA 92807). Arterial blood was sampled using heparinized syringes (3 mL) and placed on ice before being analyzed for

Key Words: Acid-Base Balance, Beef cattle, Urea

## Introduction

The capacity to convert non-protein N (NPN), such as urea, into true protein is a capability inimitable to the ruminant digestive tract. Rumen bacterial enzymes hydrolyze urea to CO<sub>2</sub> and ammonia (NH<sub>3</sub>; Owens and Zinn, 1988). This ammonia is then either incorporated into microbial protein, absorbed across the rumen wall, or flushed to the omasum, with absorption from the rumen being greater as ruminal concentrations increase (Owens and Zinn, 1988). Urea is added to feedlot rations at concentrations ranging from 0.5 to 1.5% (DM basis; Galyean, 1996). Gleghorn et al. (2003) indicated that increasing the proportion of urea as a supplemental protein

pCO<sub>2</sub>, pO<sub>2</sub>, and pH using an IRMA SL Blood Analysis System (Diametrics Medical, Inc. St. Paul, MN 55113). Feed samples were taken weekly and subjected to all of the following analysis: DM (oven drying at 55°C until no further weight loss), CP (% N x 6.25; LECO Corporation, St. Joseph, MI 49085), and ash (combusted 6-h in a muffle furnace at 500°C). Urine Samples were immediately analyzed for pH and then discarded. All response variables including blood pH, pCO<sub>2</sub>, pO<sub>2</sub>, SUN, and urine pH were analyzed using the MIXED procedure, with repeated measures, of SAS (SAS Inst. Inc., Cary, NC). All treatment x time interactions were tested and LS means were reported for each variable with significance considered to be  $P < 0.10$ .

## Results and Discussion

A treatment x time interaction (Figure 1;  $P < 0.001$ ) was observed for urine pH. Treatment differences were only observed at 8 h post feeding with urine pH being greater ( $P < 0.06$ ) for the 0, 0.5, and 1.0% diets than for the 1.5 % diet. However, the biological significance of this difference is doubtful. No other treatment x time interactions ( $P > 0.26$ ) were observed. No treatment effects were observed for arterial pH, pCO<sub>2</sub>, or pO<sub>2</sub> (Table 2;  $P > 0.33$ ). This is in accordance with Cole et al. (2003) as they found similar effects on pH, pCO<sub>2</sub>, and pO<sub>2</sub> when feeding varying or oscillating amounts of CP to finishing steers. No treatment effects were observed for SUN (Table 2;  $P > 0.39$ ); however, concentrations of urea N in the serum indicate that the diet was not limiting in protein as Johnson and Preston, (1995) suggested that plasma urea N values in excess of 5 to 8 mg/dL are indicative of excessive N intake and N wastage.

## Implications

These results indicate that increasing urea concentrations in the diet of finishing steers has no effect on pH, blood gas profile, SUN, or urine pH. Consequently, dietary urea is unlikely to combat systemic acidosis.

## Literature Cited

- Cole, N.A., L.W. Greene, F.T. McCollum, T. Montgomery, and K. McBride. 2003. Influence of oscillating dietary crude protein concentration on performance, acid-base balance, and nitrogen excretion of steers. *J. Anim. Sci.* 81:2660-2668.
- Galyean, M.L. 1996. Protein levels in beef cattle finishing diet: Industry application, university research, and systems results. *J. Anim. Sci.* 74:2860-2870.
- Gleghorn, J.F., N.A. Elam, M.L. Galyean, G.C. Duff, and N.A. Cole. 2003. Effects of dietary crude protein level and degradability on performance and carcass

characteristics of growing – finishing beef steers. *J. Anim. Sci.* 81(Suppl. 1):109(Abst.).

- Guyton A.C. and J.E. Hall. 2000. Regulation of acid-base balance. Page 356 in *Textbook of Medical Physiology*. W. B. Saunders Company. Philadelphia, PA 19106.
- Johnson, J.W., and R.L. Preston. 1995. Minimizing N waste by measuring plasma urea-N levels in steers fed different dietary crude protein levels. Texas Tech University Tech. Rept. T-5-356, 62-63.
- Owens, F.N. and R.A. Zinn. 1988. Protein metabolism of ruminant animals. Pages 229-231 in *The Ruminant Animal: Digestive Physiology and Nutrition*. D.C. Church ed. Prentice-Hall, Inc. Englewood Cliffs, NJ 07632.
- Zinn, R.A., R. Barajas, M. Montano, and R. A. Ware. 2003. Influence of dietary urea level on digestive function and growth performance of cattle fed steam-flaked barely-based diets. *J. Anim. Sci.* 81:2383-2389.

Table 1. Ingredient (DM basis) and nutrient composition of diets

Item	Dietary Urea, %, DM basis			
	0	0.5	1.0	1.5
Sudangrass hay	10.00	10.00	10.00	10.00
Steam-flaked corn	68.20	71.20	74.20	77.20
Soybean meal	11.30	7.80	4.30	0.80
Urea	-	0.50	1.00	1.50
Tallow	3.00	3.00	3.00	3.00
Cane molasses	5.00	5.00	5.00	5.00
Mineral Supplement <sup>a</sup>	2.50	2.50	2.50	2.50
Dry matter	90.13	89.81	89.69	89.56
Ash	4.60	4.60	4.30	3.90
CP	12.39	11.93	12.07	12.29

<sup>a</sup>mineral supplement composition (DM basis): limestone, 47.059%; dicalcium phosphate, 1.036%; potassium chloride, 8.000%; magnesium oxide, 3.448%; ammonium sulfate, 6.667%; salt, 12.000%; cobalt carbonate, 0.002%; copper sulfate, 0.157%; iron sulfate, 0.133%; calcium iodate, 0.003%; manganese sulfate, 0.500%; selenium premix (0.16%), 0.125%; zinc sulfate, 0.845%; vitamin A (30,000 IU/g), 0.264%; vitamin E (500 IU/g) 0.540%; Rumensin-80, 0.675%; Tylan 40, 0.450%; ground corn, 18.096%.

Figure 1: Treatment x time interaction on urine pH ( $P < 0.001$ )

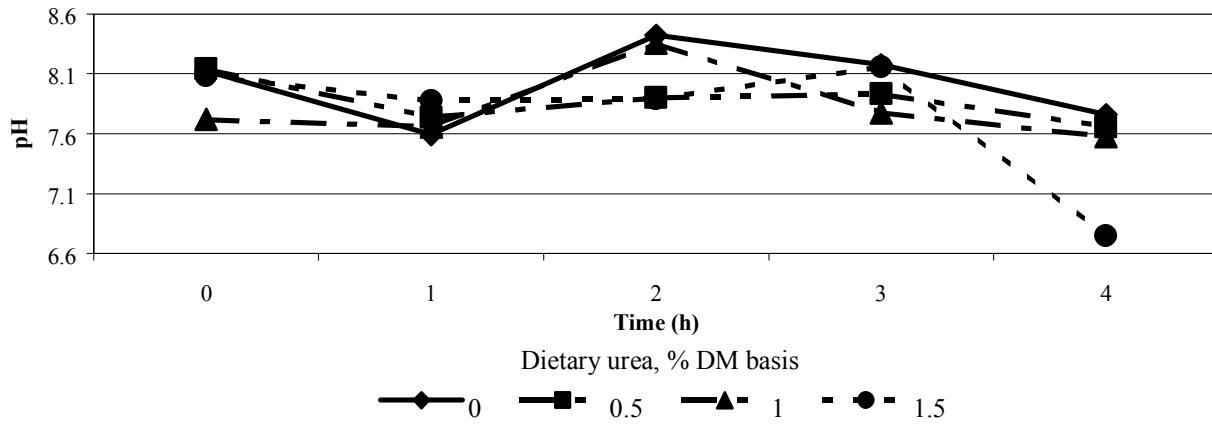


Table 2: Arterial blood acid-base profile and venous serum urea N (SUN) of steers fed four different isonitrogenous diets containing 0, 0.5, 1.0, or 1.5% urea (DM basis)

Item	Dietary Urea, %, DM Basis				SEM <sup>a</sup>	P value
	0	0.5	1.0	1.5		
Arterial pH	7.51	7.53	7.51	7.55	0.02	0.52
pCO <sub>2</sub> , mm Hg	31.01	32.74	33.52	29.20	2.66	0.63
PO <sub>2</sub> , mm Hg	102.45	102.62	94.68	110.78	8.33	0.33
SUN, mg/dL	14.67	12.42	12.08	12.66	1.75	0.40

<sup>a</sup> Standard error of the mean (Most conservative estimate; n = 3)

## EFFECTS OF BARLEY PROCESSING FOR BACKGROUNDING DIETS ON PERFORMANCE IN BEEF STEERS

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**ABSTRACT:** One hundred forty-three crossbred beef steers ( $277 \pm 19$  kg initial BW) were used in a randomized complete block design, to evaluate the optimum level of particle size processing of barley in backgrounding diets. Steers were blocked by weight, sorted, and allotted randomly to one of four dietary treatments (6 pens/treatment). Treatments were coarse rolled barley ( $2569 \pm 1.32$   $\mu$ m), medium rolled barley ( $1980 \pm 1.43$   $\mu$ m), fine rolled barley ( $1324 \pm 1.54$   $\mu$ m), and a mixture of fine and coarse rolled barley ( $1762 \pm 1.73$   $\mu$ m) to approximate medium barley. Diets contained (DM basis) 41.82% barley, 35% pressed sugar beet pulp, 15% grass hay, 5% desugared molasses, and 3.18% supplement. Diets were formulated to contain a minimum of 12.5% CP, 0.6% Ca, 0.3% P, 0.6% K, and 27.5 mg/kg monensin. Initial and final weights were taken with an average 2 d weights used. Steers were implanted with Synovex-S on d 66 and fed for 93 d. Final weight and average daily gain were not affected ( $P \geq 0.43$ ) by degree of processing. Dry matter intake was lower for the fine treatment as compared with coarse and mixed treatments ( $P = 0.008$ ). Gain efficiency was greater for the fine treatment compared with the coarse and mixed treatments ( $P = 0.002$ ) and gain efficiency was greater in the medium than coarse rolled barley ( $P = 0.002$ ). There was a 13% increase in efficiency from the coarse to the fine, and a 9% increase from coarse to medium treatments. Apparent dietary NEm and NEg were higher for the fine and medium treatments than the coarse and mixed treatments ( $P < 0.001$ ). In conclusion, performance of backgrounding steers was improved by medium and fine processing. Further research is needed to determine optimum particle size and how it interacts with roughage level and CP level.

Key Words: Barley, Beef, Particle Size Processing

### Introduction

Backgrounding cattle is another tool that allows producers and feedlot operators to adjust the time cattle will eventually spend in feedlots. Backgrounding involves feeding for moderate growth, allowing for maturation of muscle and bone, while restricting fat deposition (Block et al., 2001). Corn is the predominant feed grain fed in North America; however, barley is used in cooler climates of northern United States and Canada (Hunt, 1996). Not only is the digestibility of whole barley lower than processed barley, but whole barley also increases the incidence of bloat (Mathison, 1996). Little research has focused on the degree of processing in backgrounding or growing diets and resulting effects on growth, feed efficiency, and dry matter

intake. Recent research in our laboratory (Reed et al., 2002; Bengochea et al., 2004) indicated that finer processing of sprout damaged barley and sound (non-sprout damaged) barley in growing diets increased steer performance. Finely grinding corn for dairy cows fed 55% concentrate diets resulted in lower ruminal ammonia values, indicating a possible increase of fermentation in the rumen (San Emeterio et al., 2000). In cows fed the same diets twice daily, finely grinding high-moisture ensiled corn resulted in increased milk production and enhanced starch utilization (San Emeterio et al., 2000). Callison et al. (2001) reported increased ruminal digestion of non-structural carbohydrate with increasing level of corn processing. Mixtures of high moisture corn and dry whole corn improved steer performance (Stock et al., 1987) and combinations of processed grains alters mixing characteristics that may influence steer preference of grain (Pritchard and Stateler, 1997). The objectives of this study were to evaluate; 1) the effect of increased degree of barley processing and; 2) mixing barley particle sizes on the performance of beef steers fed a 50% roughage diet.

### Materials and Methods

One hundred forty-three crossbred beef steers ( $277 \pm 19$  kg initial BW) were used in a randomized complete block design to evaluate the degree of processing (particle size) barley in backgrounding diets. Steers originated from south-central North Dakota and were shipped approximately 300 km where they were housed at the North Dakota State University Animal Research Center in concrete pens with access to a barn. Steers were fed in concrete fence-line bunks and had ad libitum access to water. During the receiving period, all steers received common transition diets of hay, silage, supplement, and desugared molasses. A booster vaccination against bovine rhinotracheitis, virus, diarrhea, parainfluenza<sub>3</sub>, respiratory syncytial virus and *Haemophilus somnus* was given; and steers were treated with doramectin (Pfizer, Exton, PA) for control of internal and external parasites, dehorned if needed, and ear tagged. Steers were blocked by weight and allotted randomly to dietary treatment (6 pens/treatment). Particle sizes were targeted at 2700  $\mu$ m for coarse rolled barley, 2000  $\mu$ m for medium rolled barley, 1300  $\mu$ m for fine rolled barley, and 2000  $\mu$ m mixing fine and coarse rolled barley to assimilate medium barley. Diets contained (DM basis) 41.82% barley, 35% pressed beet pulp, 15% grass/alfalfa hay, 5% desugared molasses, and 3.18% supplement. Diets were formulated to contain a minimum of 12.5% CP, 0.6% Ca, 0.3% P, 0.6% K, and 27.5 mg/kg monensin. Steers were

implanted with 200 mg progesterone-20 mg estradiol (Synovex S; Fort Dodge Animal Health; Fort Dodge, IA) on d 66. Initial and final weights were taken with an average 2 d weights used. Steers were fed for 93 d. Feed offered was adjusted daily based on bunk assessment made prior to feeding. Orts were weighed weekly and dietary ingredient samples were composited and subsampled for analysis of particle size and lab analysis. Barley was sampled and composited weekly for density and particle size analysis. Particle size was analyzed following the procedure of ASAE (1993) using a sieve shaker (Ro-Tap W. S. Tyler, Mentor, OH). Diets were analyzed for DM, OM, N, starch (AOAC, 2000), ADF, and NDF (ANKOM, Fairport, NY). Apparent net energy for each diet was calculated from estimates of energy gain (EG, Mcal/d) for a large frame steer calf, based on growth performance [(NRC, 1984; EG = (0.0493 BW<sup>0.75</sup>) ADG<sup>1.097</sup>, where BW is the mean shrunk body weight (full weight x 0.96)], and maintenance energy expended (NRC, 1984; EM, Mcal/d; EM = 0.077 BW<sup>0.75</sup>) using the quadratic equation

$$NE_m = \frac{-b \pm \sqrt{b^2 - 4ac}}{2c},$$

where a = 0.41EM, b = 0.877EM + 0.41DMI + EG, and c = -0.877 DMI; dietary NE<sub>g</sub> = 0.877NE<sub>m</sub> - 0.41 (Zinn and Shen, 1998).

Data were analyzed using a mixed model (SAS Inst., Cary, NC) with pen being the experimental unit. The model included treatment for fixed effect and block as random effect. Treatment least square means were separated using LSD if the F test was significant ( $P < 0.05$ ).

## Results and Discussion

Laboratory analysis of the diet (DM basis) was 93.9% OM, 13.1% CP, 43.9% NDF, 18.1% ADF, and 22.0% starch. The test weight of the barley was 0.596 kg/L (46.2 lb/bu). Particle size analysis of the treatments resulted in 2569 ± 1.32 µm for coarse rolled barley, 1980 ± 1.43 µm for medium rolled barley, 1324 ± 1.54 µm for finely rolled barley, and 1762 ± 1.73 µm for the mixed barley.

There were no difference across treatments for final weight ( $P = 0.51$ ) or average daily gain ( $P = 0.43$ ); Table 1. This is similar results from Bengochea et al. (2004) that had a 1385 µm fine particle size and a 2132 µm coarse particle size; and in contrast to Reed et al. (2002) who reported an increase in ADG with increased degree of processing. Reed et al. (2002) fed barley with a particle size of 2628 µm coarse roll, and 1998 µm fine roll.

Dry matter intake was greatest ( $P < 0.01$ ) for steers fed the coarse and mixed barley, and least for the fine barley with the medium barley not different than the fine, coarse, or mixed barleys. Callison et al. (2001) reported no differences for DMI among fine, medium, and coarse ground corn treatments in lactating dairy diets. Research in our laboratory (Reed et al., 2002; Bengochea et al., 2004) indicated similar intakes among treatments.

The fine and medium treatments had the best ( $P < 0.01$ ) feed efficiency (ADG/DMI), the mixed treatment was intermediate, and the coarse had the poorest feed efficiency. Reed et al. (2002) and Bengochea et al. (2004) reported an

increase in feed efficiency with a decrease in particle size. In the present experiment, the fine treatment had a 13% improvement in feed efficiency above the coarse treatment and a 9% improvement in feed efficiency from the medium to the coarse treatment. Apparent dietary NE<sub>m</sub> and NE<sub>g</sub> were higher for the fine and medium treatments compared with the coarse rolled and mixed barley. Bengochea et al. (2004) reported an increase in NE<sub>m</sub> and NE<sub>g</sub> with increased degree of processing.

Increasing the degree of processing to medium and fine levels indicated an increase in feed efficiency above the coarse treatments with no benefit to mixing of barley of different particle sizes, which is similar to findings of Callison et al. (2001) who compared corn particle size mixtures for lactating dairy cattle.

## Implications

Performance of backgrounding steers was improved by medium and fine processing for feed efficiency. Further research is needed to determine optimum particle size and how it interacts with roughage level and CP level in backgrounding.

## Literature Cited

- AOAC. 1997. Official Method of Analysis. (16<sup>th</sup> ed.) Association of Official Analytical Chemists, Gaithersburg, MD.
- ASAE. 1993. Method of determining and expressing fineness of feed materials by sieving. ASAE Standard ASAE S319.2.
- Bengochea, W. L., M. L. Bauer, G. P. Lardy, T. C. Gilbery, and S. A. Soto-Navarro. 2004. Effects of grain processing for backgrounding diets on performance of beef steers. Presented at Midwest Section ASAS and Midwest Branch ADSA 2004 Meeting, Des Moines, IA. Abs. #269.
- Block, H. C., J. J. McKinnon, A. F. Mustafa, and D. A. Christensen. 2001. Manipulation of cattle growth to target carcass quality. *J. Anim. Sci.* 79:133-140.
- Callison, S. L., J. L. Firkins, M. L. Eastridge, and B. L. Hull. 2001. Site of nutrient digestion by dairy cows fed corn of different particle sizes or steam rolled. *J. Dairy. Sci.* 84:1458-1467.
- Hunt, C. W. 1996. Factors affecting the feeding quality of barley for ruminants. *Anim. Feed Sci. Tech.* 62:37-48.
- Mathison, G. W. 1996. Effects of processing on the utilization of grain by cattle. *Anim Feed Sci. Tech.* 58:113-125.
- Prichard, R. H., and D. A. Stateler. 1997. Grain Processing: Effects on mixing, prengension, and other characteristics of feeds. *J. Anim. Sci.* 1997. 75:880-884.
- Reed, J. J., E. R. Loe, M. L. Bauer, G. P. Lardy, and J. S. Caton. 2002. Inclusion of sprouted grain in steer grower diets and effect on performance. *J. Anim. Sci. (Suppl.2)* 80:89.
- San Emeterio, F., R. B. Reis, W. E. Campos, and L. D. Satter. 2000. Effect of coarse or fine grinding on

- utilization of dry or ensiled corn by lactating dairy cows. J. Dairy Sci. 83:2839-2848.
- Stock, R. A., D. R. Brink, R. T. Brandt, J. K. Merrill, and K. K. Smith. 1987. Feeding combination of high moisture corn and dry corn to finishing cattle. J. Anim. Sci. 65:282-289.
- Zinn, R. A., and Y. Shen. 1997. An evaluation of ruminally degradable intake protein and metabolizable amino acid requirements of feedlot calves. J. Anim. Sci. 76:1280-1289.

Table 1. Effect of particle size processing barley for backgrounding diets of beef steers

Item	Treatments				SEM <sup>a</sup>	P-value
	Coarse	Medium	Fine	Mixed		
Initial weight, kg	277	277	277	278	19	0.99
Final weight, kg	392	398	399	396	19	0.51
DMI, kg/d	10.21 <sup>y</sup>	9.85 <sup>xy</sup>	9.55 <sup>x</sup>	10.20 <sup>y</sup>	0.35	0.009
ADG, kg	1.22	1.29	1.30	1.26	0.03	0.43
ADG/DMI, g/kg	120 <sup>x</sup>	131 <sup>yz</sup>	136 <sup>z</sup>	124 <sup>xy</sup>	5	0.002
Dietary NEm, Mcal/kg	1.48 <sup>x</sup>	1.57 <sup>y</sup>	1.61 <sup>y</sup>	1.51 <sup>x</sup>	0.02	<0.001
Dietary NEg, Mcal/kg	0.88 <sup>x</sup>	0.97 <sup>y</sup>	1.01 <sup>y</sup>	0.91 <sup>x</sup>	0.02	<0.001

<sup>a</sup>n = 6.

<sup>x,y,z</sup>Within a row, means lacking a common superscript letter differ ( $P < 0.05$ ).

# EFFECT OF CORN VITREOUSNESS AND GRAIN PROCESSING ON STARCH DIGESTION

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**ABSTRACT:** Eight Holstein steer (251 ± 12 kg) were used in a split plot design experiment to evaluate four varieties of yellow corn with different proportions of vitreous endosperm (vitreousness): **A** = 55.3, **B** = 60.6, **C** = 63.2 and **D** = 64.9 % and two processing methods: dry rolled (**DRC**) and steam-flaked (**SFC**; flake density = 0.35 kg/L). Corn vitreousness was determined by manual dissection of 50 kernels from each variety. Diets consisted of 73.2% corn, 4% alfalfa hay, 8% sudangrass hay, 7.5% cane molasses, 3.5% yellow grass, 1.4% limestone, 0.20% magnesium oxide, 1% urea, and 0.5% TM salt. Treatments were evaluated for in vitro enzymatic starch digestion based on changes in starch solubility and amylase reactive insoluble starch. Predicted ruminal starch digestion (**PRSD** = 1.32Soluble starch + 0.93Insoluble reactive starch) for hybrids A, B, C and D, respectively, were 68.5, 67.9, 67, 66.7 with dry rolling, and 84.3, 82.7, 82.4 and 82.3 with steam flaking. There was a close relationship between corn vitreousness and PRSD for DRC ( $r^2 = 0.95$ ,  $P < .01$ ) and SFC ( $r^2 = 0.88$ ,  $P < 0.06$ ). For DRC, corn vitreousness explained 64% of the variation in percentage fecal starch. However, with SFC, percentage fecal starch was low and not affected by corn vitreousness ( $r^2 = 0.04$ ,  $P > .79$ ). We conclude that differences in corn vitreousness have an appreciable impact on the feeding value of dry processed corn for feedlot cattle. This effect is minimized by steam flaking.

**Key Words:** Corn, Starch Digestion, Vitreousness, Grain Processing, Cattle.

## Introduction

The structure and composition of cereal starches and their interaction with proteins play a major role in the digestibility and feeding value of grain for livestock (Rooney and Pflugfelder, 1986; Zinn et al., 2000). Digestibility of starch from corn grain is limited by the protein matrix that encapsulates starch granules, and by the compact nature of the starch itself, particularly in the hard endosperm portion of kernels where penetration by amylolytic enzymes is restricted (McAllister et al., 1990). The disruption of the protein matrix is the first limiting step toward optimizing starch digestion. Zinn et al. (1995), observed that steam flaking increased starch digestion by 10% (99 vs 90%) and digestibility of nonstarch OM was increased by flaking to the same degree (10%) that starch digestibility was increased. Increased kernel vitreousness has been associated with decreased ruminal starch

degradation (Philippeau and Michalet, 1997, Correa et al., 2002). The feeding value of corn grain is influenced by variety or endosperm type. The objective of this study was to evaluate the interaction of corn vitreousness and grain processing method on in vivo and in situ starch digestion characteristics of four varieties of yellow dent corn.

## Materials and Methods

Eight Holstein steers (251 ± 12 kg) with cannulas in the rumen and proximal duodenum were used in a split-plot design, consisting of two 4 x 4 Latin Square to evaluate the influence of corn processing and variety on characteristics of starch digestion. Whole plots (Latin square replicates) consisted of two types of corn processing (dry rolled and steam flaked corn). Subplot consisted of four corn varieties (pioneer hybrids). Composition of experimental diets is shown in Table 1. Steers were individually maintained in concrete slatted-floor pens (3.9 M<sup>2</sup>) with access to water at all times. Dry matter intake was restricted to 2.2% of BW. Diets were fed in equal proportions at 0800 and 2000 daily. Experimental periods consisted of a 10-d diet adjustment period followed by a 4-d collection period. During the collection period, fecal samples were taken from all steers twice daily as follows: d 1, 0750 and 1350; d 2, 0900 and 1500; d 3, 1050 and 1650; and d 4, 1200 and 1800. Individual samples consisted of approximately 200 g (wet basis) of fecal material. Samples from each steer and within each collection period were composited for analysis. Samples were subjected to the following analysis: DM (oven drying at 105°C until no further weight loss) and starch (Zinn, 1990a).

Corn vitreousness was determined by manual dissection of the kernels (50 kernels from each variety were randomly selected for each sample). Kernels were soaked in distilled water for 2 minutes, dried with a paper towel, the pericarp, tipcap and germ were removed with a scalpel, and the total endosperm was weighed. The floury endosperm was then removed using a grinder mill, and the weight of the remaining vitreous endosperm was expressed as a percentage of the total endosperm.

The thickness of the SFC was determined by breaking the flake approximately in half and measuring the thickness in millimeters (using a micrometer) of the flattest spot near the center of the flake. Estimates of thickness represent an average value for 10 whole flakes selected randomly.

Four varieties of yellow corn were each processed as

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DRC and SFC (eight samples) and then evaluated for chemical composition and in vitro enzymatic starch digestion. All samples were ground through a Wiley Mill (1mm screen). Amyloglucosidase reactive starch (**AGR**) was determined according to Zinn (1990a), except that the incubation time was increased to 4 h. Enzymatic reactivity of insoluble starch (**RS**) was determined according to Rodriguez et al. (2001). Insoluble reactive starch (**ISR**, %) was determined as:  $ISR = (RS - AGR)/6$ , and insoluble starch digestible (**ISD**, %) was calculated as  $ISD = (100 - AGR)(ISR/(ISR + .05))$ , where .05 represents passage rate of grain from the rumen. Predicted ruminal starch digestion (**PRSD**, %) was calculated as:  $PRSD = 1.32 AGR + .93 ISD$ . Statistical relationships (vitreousness vs predicted ruminal starch digestion, vitreousness vs fecal starch excretion) were determined by regression.

## Results and Discussion

The characteristics of the corn hybrids used are presented in Table 2. Vitreousness of the four yellow corn hybrids averaged 61% (A = 55.3, B = 60.6, C = 63.2 and D = 64.9 %). Philippeau and Mitchalet-Doreau (1997) studying flint and dent cultivars at different stages of growth, found that when corn grain reaches maturity, vitreousness averaged 48.1%. Philippeau et al (1999) reported that vitreous endosperm averaged 51.7% of grain DM for dent hybrids. Correa et al., (2002), found that vitreousness of 14 mature U.S. hybrids, averaged 48.2% (range of 34.9 to 62.3%). They found that with advancing maturity, kernel vitreousness increased.

The predicted ruminal starch digestion (Table 3) for hybrids A, B, C and D, respectively, was 68.5, 67.9, 67, 66.7 with dry rolling, and 84.3, 82.7, 82.4 and 82.3 with steam flaking, averaging 67.5 and 82.9% for DRC and SFC, respectively). These values for ruminal digestion of DRC and SFC are in good agreement with previous studies (Rodriguez et al., 2001; Zinn, 1988, 1990a, 1990b and 1991). There was a close relationship between corn vitreousness and PRSD for DRC ( $r^2 = 0.95$ ,  $P < .01$ ) and SFC ( $r^2 = 0.88$ ,  $P < 0.06$ ; Figure 1). Correa et al., (2002), and Philippeau and Michalet-Doreau (1997) also observed close associations ( $R^2 = 0.93$  and  $0.86$ , respectively) between vitreousness and ruminal starch availability.

For DRC, corn vitreousness explained 64% of the variation in percent fecal starch. However, with SFC, percent fecal starch was low and not affected by corn vitreousness ( $r^2 = 0.04$ ,  $P > .79$ ; Figure 2). Vitreousness reflects the nature of the association between starch and protein in the endosperm. In the vitreous endosperm, starch granules are surrounded by protein bodies and are embedded in a dense matrix, which limits the action of hydrolytic enzymes. In contrast starch granules in the floury endosperm are more accessible to ruminal bacteria because of inclusion in a discontinuous protein matrix (Kotarski et al. 1992). The effect of vitreousness is minimized by steam flaking because the hard starch of the endosperm is directly exposed to the shear forces during flaking of corn grain. Increasing starch solubility from 5 to 10 percentage units will increase total tract starch digestion from 90 to over 99% (Zinn et al., 1998; Zinn et al., 2000).

## Implications

Vitreousness has an appreciable impact on the feeding value of dry processed corn for feedlot cattle. Measurements of soluble starch and insoluble starch are useful predictors of changes in starch digestion due to corn vitreousness. The effect of vitreousness is minimized when the grain is processed with steam flaking.

## Literature Cited

- AOAC, 1986. Official methods of analysis. 14<sup>th</sup> ed. Assoc. Offic. Anal. Chem. Arlington, VA.
- Chai, W., and P. Uden. 1998. An Alternative oven method combined with different detergent strengths in analysis of neutral detergent fiber. *Anim. Feed Sci. Technol.* 74:281-288.
- Correa, C.E.S., R.D. Shaver, M.N. Pereira, J.G. Lauer, and K. Kohn. 2002. Relationship between corn vitreousness and ruminal in situ starch degradability. *J. Dairy Sci.* 85: 3008-3012
- Goering, H.K., and P.J. Van Soest. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). *Agric. Handbook 379*. ARS-USDA, Washington, D.C.
- Hicks, C.R. 1973. *Fundamental Concepts in the Design of Experiments*. Holt, Rinehart and Winston, New York
- Hill, F.N., and D.L. Anderson. 1958. Comparison of metabolizable energy and productive determinations with growing chicks. *J. Nutr.* 64:587-603.
- Kotarski, S.F., R.D. Waniska, and K.K. Thurn. 1992. Starch hydrolysis by the rumen microflora. *J. Nutr.* 122:178-190.
- McAllister, T.A., C.J. Cheng, and L.M. Rode. 1990. Digestion of barley, maize, and wheat by selected species of ruminal bacteria. *Appl. Environ. Microbiol.* 56:3146-3153.
- NRC. 1996. *Nutrient Requirements of Beef Cattle*. 7<sup>th</sup> ed. Natl. Acad. Sci. Press. Washington, D.C.
- Philippeau, C., F. Le Deschault de Monredon, and B. Michalet-Doreau. 1999. Relationship between ruminal starch degradation and the physical characteristics of corn grain. *J. Anim. Sci.* 77: 238 - 243.
- Philippeau, C., and B. Michalet-Doreau. 1997. Influence of genotype and stage of maturity of maize on rate of ruminal starch degradation. *Anim. Feed Sci. Technol.* 68:25-35.
- Rodriguez, S., J.F. Calderón, and R.A. Zinn. 2001. Variation in ruminal starch digestion due to dry rolling versus steam flaking corn and sorghum can be reliably predicted based on changes in starch solubility and 6-h amylase reactive insoluble starch. *Proc. West. Sect. Am. Soc. Anim. Sci.* 52: 529-530.
- Rooney, L.W. and R.L. Plugfelder 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. *J. Anim. Sci.* 63:1067-1623.
- Zinn, R.A. 1990a. Influence of flake density on the comparative feeding value of steam-flaked corn for feedlot cattle. *J. Anim. Sci.* 68:767-775.
- Zinn, R.A. 1990b. Influence of steaming time on site of digestion of flaked corn in steers. *J. Anim. Sci.* 68:776-781.

- Zinn, R.A. 1991. Comparative feeding value of steam-flaked corn and sorghum in finishing diets supplemented with or without sodium bicarbonate. J. Anim. Sci. 69:905.
- Zinn, R.A., F.N. Owens, and R.A. Ware. 2002. Flaking corn: Processing mechanics, quality standards, and impacts on energy availability and performance of feedlot cattle. J. Anim. Sci. 80:1-12
- Zinn, R.A., C.F. Adams, and M.S. Tamayo. 1995. Interaction of feed intake level on comparative ruminal and total tract digestion of dry-rolled and steam-flaked corn. J. Anim. Sci. 73:1239-1245.
- Zinn, R.A., E.G. Alvarez, M.F. Montano, A. Plascencia, and J.E. Ramirez. 1988. Influence of tempering on the feeding value of rolled corn in finishing diets for feedlot cattle.. J. Anim. Sci. 76:2239-2246.

Table 1. Ingredient and nutrient composition of experimental diets

Ingredient, % (DM basis)	Treatments <sup>a</sup>	
	DRC	SFC
Alfalfa hay	4.00	4.00
Sudan hay	8.00	8.00
Steam-flaked corn	-	73.20
Dry Rolled Corn	73.20	-
Cane molasses	7.50	7.50
Yellow grass	3.50	3.50
Limestone	1.40	1.40
Magnesium oxide	0.20	0.20
Urea	1.00	1.00
TM salt <sup>b</sup>	0.95	0.95
Chromic oxide	0.30	0.30
Nutrient composition (DM)		
NE, Mcal/kg <sup>c</sup>		
Maintenance	2.12	2.12
Gain	1.46	1.46
Crude protein, %	12.07	12.07
NDF, %	14.76	14.76
Calcium, %	0.75	0.75
Phosphorus, %	0.33	0.33

<sup>a</sup> DRC = dry rolled corn and SFC = steam flake corn (variety A, B, C y D)

<sup>b</sup> Trace mineral salt contained: CoSO<sub>4</sub>, 0.68%; CuSO<sub>4</sub>, 1.04%; FeSO<sub>4</sub>, 3.57%; ZnO, 1.24%; MnSO<sub>4</sub>, 1.07; KI, .052%; and NaCl, 92.96%.

<sup>c</sup> Based on tabular NE values for individual feed ingredients (NRC,1996).

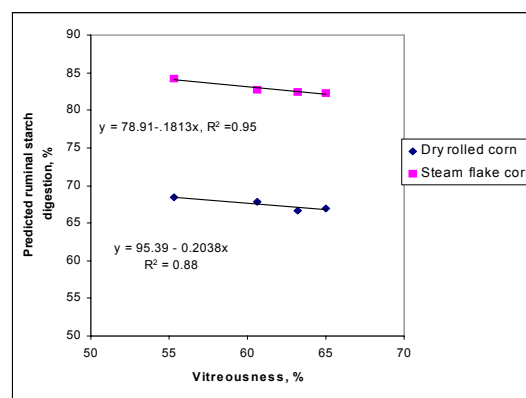


Figure 1. Relationship

between vitreousness and predicted ruminal starch digestion. Predicted ruminal starch digestion =  $78.91 - 0.1813 \times \text{Vitreousness}$ ;  $r^2=0.95$  ( $P < 0.02$ ) for dry-rolled corn and Predicted ruminal starch digestion =  $95.39 - 0.2038 \times \text{Vitreousness}$ ;  $r^2=0.88$  ( $P < 0.02$ ) for steam-flaked corn.

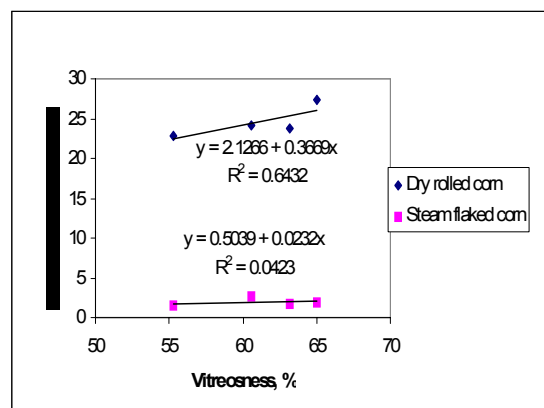


Figure 2. Relationship between vitreousness and fecal starch excretion. Fecal starch excretion =  $2.1266 + 0.3669 \times \text{Vitreousness}$ ;  $r^2=0.64$  ( $P > .19$ ) for dry-rolled corn and Fecal starch excretion =  $0.5039 + 0.0232 \times \text{Vitreousness}$ ;  $r^2=0.04$  ( $P > .79$ ) for steam-flaked corn.

Table 2. Characteristics of corn hybrids used in the experiment

Item	Dry Rolled Corn				Steam Flaked Corn			
	A	B	C	D	A	B	C	D
Flake thickness, mm					0.134	0.116	0.180	0.139
Density, kg/l	0.67	0.67	0.67	0.67	0.33	0.33	0.33	0.33
Corn particle size, (mm), % total <sup>a</sup>								
8					16.50	15.04	18.01	19.94
4	58.42	54.04	51.83	71.63	47.62	43.85	44.66	56.40
2	29.94	26.21	28.63	18.13	27.04	21.63	20.54	17.56
1	5.55	9.23	9.14	3.72	6.13	10.71	9.26	5.39
.5	2.71	5.51	5.58	2.70	1.43	5.23	4.41	2.12
.25	2.56	3.74	3.88	3.00	0.65	2.85	2.50	1.23
< .25	0.82	1.28	0.95	0.82	0.63	0.69	0.62	0.31
Dry Matter, %	89.22	87.90	87.57	87.71	89	87.57	87.05	86.84
CP (N x 6.25) %	8.1	8.7	8.4	8.6	7.7	8.4	8.2	8.3
Starch, %	73.1	73.2	73.6	71.4	76.8	75.6	76.9	74.5
NDF, %	6.63	7.55	8.01	7.38	5.39	5.89	6.19	5.71
ADF, %	1.89	2.07	2.23	2.02	1.58	1.73	1.89	1.70
Ash, %	0.99	1.18	1.27	1.30	0.96	1.03	1.14	1.03

<sup>a</sup> as-is basisTable 3. *In vitro* enzymatic starch digestion of corn hybrids and fecal starch excretion<sup>a</sup>

Item	Dry Rolled Corn				Steam Flaked Corn			
	A	B	C	D	A	B	C	D
Soluble Starch (AGR) <sup>b</sup> , %	9.61	10.29	10.47	8.89	36.98	36.04	33.57	31.90
Digestible Insoluble Starch (ISD) <sup>c</sup> , %	59.98	58.33	57.17	58.08	38.11	37.72	41.00	43.17
Predicted Ruminant Starch Digestible (PRSD) <sup>d</sup> , %	68.46	67.84	66.99	66.68	84.26	82.65	82.44	82.26
Fecal Sarch, % DM	22.77	24.18	23.85	27.27	1.53	2.60	1.66	1.89
Total Starch Digestion, % <sup>e</sup>	85.72	84.81	85.03	82.80	99.5	98.81	99.4	99.27

<sup>a</sup> Dry Matter basis<sup>b</sup>AGR = Amyloglucosidase reactivity, a measure of starch solubilization. Grains were ground to pass through a 20-mesh screen before enzymatic digestion 4-h (Zinn, 1990)<sup>c</sup>SD = Insoluble Starch digestible, amylase reactivity of insoluble starch. Grains were ground to pass through a 20-mesh screen before enzymatic digestion 6-h. (Rodriguez et al., 2001)<sup>d</sup> PRSD = Predicted ruminal starch digestion (1.32AGR + .93ISD)<sup>e</sup> TSD = -0.6489FS + 100.5, r<sup>2</sup> = 0.911 (Zinn et al., 2002)

# EFFECTS OF FEEDING *NEOTYPHODIUM COENOPHIALUM*-INFECTED TALL FESCUE STRAW ON LAMB PERFORMANCE

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**ABSTRACT:** An experiment was conducted to investigate the digestion responses and degradation of ergovaline and production of lysergic acid in the rumen of sheep offered *Neotyphodium coenophialum*-infected tall fescue straw at two ergovaline levels. Six crossbred wethers (56 +/- 3 kg BW) were randomly assigned to one of two treatment groups in a cross over design. Each experimental period consisted of 28 d feeding periods with a 14 d wash out between treatment periods. During the wash out period all animals received a diet devoid of ergovaline. Treatments were 1) <10 ppb ergovaline (E-) and 2) 500 ppb ergovaline (E+). Diets were isonitrogenous. Ergovaline levels were achieved with a combination of tall fescue straw and *Neotyphodium coenophialum*-infected tall fescue seed (>3,300 ppb ergovaline). Rumen ammonia, rumen pH, and rectal temperature were not influenced by alkaloid concentration (P<0.10). Digestion of DM, ADF and CP were not different between treatments (P<0.10). Water intake was reduced by treatment (P<0.05). Feed intake and body weight were not changed by ergovaline intake (P>0.10). Serum prolactin was reduced by 27% with ergovaline intake (P<0.05). Rumen fluid was sampled 3 times (d 0, 3, 28) during the 28-day period for ergovaline and lysergic acid. Samples were collected at time 0 (prior to feeding), 6, and 12 h post feeding. Ergovaline concentration in rumen fluid expressed as a percent of intake increased over sampling time and sampling day (P<0.05). Lysergic acid concentration in rumen fluid expressed as a percent of intake increased over time from d0 to d3 (P<0.05) but was not different on d28 between time (P>0.10). Ergovaline and lysergic acid detected in the feces was 35.82% +/- 4.45 and 112.53% +/- 24.88 of intake, respectively. The appearance of lysergic acid in the feces and rumen fluid is likely due to the degradation of ergovaline in the rumen due to microbial degradation and further hydrolysis in the lower digestive tract.

Key words: Sheep, Ruminants, Endophyte, Ergovaline, Tall fescue

## Introduction

There is a long history of problems associated with feeding tall fescue (Strickland et al., 1993; Bacon, 1995; Tor-Agbidye et al., 2001). These problems are associated with the endophyte fungus *Neotyphodium coenophialum*, which infects varieties of tall fescue in a symbiotic fashion. The endophyte produces ergopeptine alkaloids, with ergovaline being produced in the greatest quantity. Tall fescue toxicosis is estimated to cost the beef industry \$800 million dollars a year from reduction in reproductive performance and reduced gains. In Oregon grass seed

production is the fourth largest agriculture commodity and approximately 160,000 acres are planted in tall fescue mainly used for grass seed production (National Ag Statistic Service, 2002). After the seed is harvested, straw is left as a field residue. In the past, straw was eliminated by field burning, but increased restrictions on burning have left producers looking for other avenues to dispose of straw. The main market for this straw is Japan and other Pacific Rim countries as forage for livestock. Oregon currently exports 65% (600,000 tons) of the straw to Japan, 34% to Taiwan, 1% to Korea and 60,000 ton is used domestically in the US. For many years ergovaline has been believed to cause fescue toxicosis (Joost, 1995). However, recent work by Hill et al. (2001) implies the core ring structure of ergopeptine alkaloids, lysergic acid (Figure 1), crosses the rumen wall at a higher rate than any of the other alkaloids. To date no one has quantified lysergic acid in the feed or measured the metabolism of lysergic acid in relation to ergovaline levels in the diet. This study assessed the metabolic fate of ergovaline and lysergic acid.

## Materials and Methods

*Animals, Experimental Design, and Diets.* Six ruminally cannulated Poly Pay x Suffolk crossbred wethers (56 +/- 3kg BW) were randomly assigned to one of two treatment groups in a cross over design (Kuehl, 2000). Surgical and animal care procedures were approved by the Oregon State University Institution of Animal Care and Use Committee. Wethers were individually housed in metabolism crates within a barn during the duration of the study. Treatment periods were d 28 with total fecal collections on d 21 to 25. A 14d washout period was allowed between treatment periods. A two-week adaptation to the metabolism crates and voluntary intake was allowed before the first feeding period. Environmental temperatures were consistent with temperatures in the Pacific Northwest during August through November. Treatments were <10 ppb (E-) ergovaline and 500 ppb (E+) ergovaline. For both treatments chopped tall fescue straw was used. E- consisted of straw (95% AF) with <10 ppb ergovaline. E+ contained straw (88.5% AF) with 350 ppb ergovaline, the remainder of ergovaline was provided by endophyte infected tall fescue seed (>3,300 ppb ergovaline) (Table 1). The two treatment straws differed in CP and the addition of seed increased the CP of E+, therefore soybean meal (SBM) was added to ensure diets were isonitrogenous and CP requirements were fulfilled (NRC, 1985). Tall fescue straw was provided at 90% of previous 5d average intake at 0800, with feed refusals from the previous day determined prior to feeding. Prior to feeding straw (0730), SBM and

SBM/seed mix was provided (SBM: 10% and 7% of intake; seed 4.5% of intake on an as fed basis). Rectal temperatures were taken daily just after feeding via a handheld digital thermometer with probe placed approximately 3 cm into the rectum. Water intake was measured twice a day, first prior to feeding and then at 1700 and summed for daily water intake. Wethers were weighed at the start and end of each feeding period.

Rumen fluid was sampled on d 0, 3, and 28 of each period in a time course at 0 (prior to feeding), 6, and 12 h after feeding for ergovaline and lysergic acid analysis. Additional samples were collected at 0, 3, 6, 9, 12, and 24 hr for pH and ammonia analysis, pH measurements of rumen fluid were taken immediately after collection with a high performance combination probe (Corning, New York, 14831). Approximately 60 ml of rumen fluid was collected with a rumen suction strainer and aliquoted in the following fashion, 9 ml for ammonia was added to 25% HCl acid (3 ml); 13 ml for each ergovaline and lysergic acid, remaining rumen fluid was used for pH measurement. Rumen fluid was placed on ice immediately after collection then frozen and stored at -20°C. Blood samples for prolactin analysis were collected prior to feeding via jugular venipuncture with 10 ml vacutainers, tubes were allowed to coagulate at room temperature, centrifuged and serum was decanted and frozen for later analysis. Sheep were fitted with fecal bags at 0800 on d 19 of each treatment period for adaptation. Collection of total fecal samples commenced on d 21 to 25. Bags were changed twice in a 24 hr period, 0800 and 1700. The feces for each time was composited, weighed, hand mixed and 20% subsample (wet weight) was collected each day at 1700. Samples were dried in a freeze-drier for 7 days, reweighed for DM, ground, and composited by lamb. Fecal collections were used to estimate digestibility and calculate ergovaline and lysergic acid absorption. Intake and orts were monitored throughout the trial; however official measurements were taken on d 0, 3, 28 and each day during fecal collections. Diet grab samples collected on each sampling day and each fecal collection day were composited for analysis. Orts were collected during fecal collection and were individually analyzed for ergovaline, lysergic acid, DM, ADF and CP. Straw and ort samples ground to pass through a 1mm Wiley mill screen and were stored at -20°C until analyzed.

**Laboratory Analysis.** Feed, fecal, and rumen fluid samples were tested for ergovaline concentration by high performance liquid chromatography (HPLC) as described by Craig et al. (1994). Briefly, feed samples were with a Wiley mill to pass a 1mm screen. Approximately 1.0g (feed and feces) or 6 ml (rumen fluid) of sample were extracted in chloroform with ergotamine added as an internal standard (1µl/ml ergotamine), chloroform was buffered with NaOH (feed and feces) or KPO<sub>4</sub> (rumen fluid). Samples were rotated in the dark for 24 hours (feed and feces) or 5 h (rumen fluid), 5ml of the supernatant were eluted from an ergosil column. The ergovaline containing elute was then evaporated and residue was suspended in 500µl methanol and injected onto the HPLC. HPLC conditions were as follows: 30:60 ammonium carbonate (0.2mg/ml):acetonitrile mobile phase, injection volume was

20 µl, and the fluorometer setting was 250 nm excitation and 420 nm emission

Lysergic acid was determined by HPLC. Briefly, ground samples were extracted with 10 ml acetonitrile:water (50:50 v/v) for overnight, centrifuged 2000 rpm for 10 minute. Rumen fluid was concentrated (6.5 ml) in the rotovap on high temperature (65°C) the pellet resuspended in 3 ml water by vortexing. Supernatant pH was adjusted to 5.0 to 5.5 with 50% acetic acid. Three ml of supernatant was passed through a solid phase extraction column (Supelco DSC-SCX SPE column, 500 mg/3ml; Bellefonte, PA. 16823). Lysergic acid was eluted, evaporated, suspended in 200 µl of 0.05 M phosphoric acid:methanol (50:50) and placed in HPLC vials. Samples were injected onto HPLC under the following conditions: mobile phase was 94:6 0.05M phosphoric acid:acetonitrile, injection volume was 20 µl, and the fluorometer setting was 250 nm excitation and 420 nm emission.

Acidified rumen fluid samples were thawed, centrifuged (1,000 x g) for 15 minutes and analyzed for ammonia by the phenol-hypochlorite method Broderick and Kang 1980 using 96-well microtiter plate reader attached to UV/Vis Spectrometer (Elx808; Bio-Tek instruments, Winooski, VT. 05404). Straw, SBM and feces were analyzed for DM, ADF, and CP by NIR and seed was analyzed by wet chemistry at Dairy One Forage Laboratory (Dairy One, Ithaca, NY. 14850). NIR is recognized by the Association of Official Analytical Chemists (AOAC) as an official method of analysis. Serum prolactin was analyzed as described by Hockett et al. (2000) by the University of Tennessee (assay CV = 5%).

**Statistical Analysis.** Data was analyzed as a crossover by SAS GLM procedure. Animal, treatment (TRT), period, day and day X TRT were included in the model. Ruminal pH and NH<sub>3</sub>, serum prolactin and physiological variables were analyzed using the REPEATED statement with the MIXED procedure of SAS.

## Results and Discussion

No clinical signs of tall fescue toxicosis were observed during the treatment periods. Rumen ammonia and pH was not different between treatment groups ( $P = 0.896$  and  $P = 0.355$  respectively) at any time point. These results are consistent with diets formulated to be isonitrogenous. Contrary to some other published studies (Aldrich et al., 1993; Paterson et al., 1995), daily rectal temperatures were not influenced by alkaloid concentration ( $P = 0.395$ ). However, these results are similar to findings by Stamm et al. (1994) where no difference in rectal, tail and ear temperature was detected. The variation in observed rectal temperatures in response to E<sup>+</sup> feed between studies could result from the different alkaloid levels of alkaloid used in each study. Consistent with finding by Stamm et al. (1994) and Aldrich et al. (1993) apparent digestibility of DM, ADF, and CP was not different between treatment groups (see Table 2). Aldrich et al. (1993) also reported no difference in water disappearance; however, in this study water intake was decreased by E<sup>+</sup> diet ( $P < 0.05$ ) which is consistent with

findings by Fiorito et al. (1991) where E+ feed caused a drop in voluntary water intake. Serum prolactin was decreased by 27% (Table 2) indicating subclinical fescue toxicosis. This result is consistent with previous research (Stamm et al., 1994; Porter, 1995; Paterson et al., 1995) which shows depressed blood (serum or plasma) prolactin levels of animals on E+ diets.

Ergovaline to lysergic acid ratio in the feed was 3.00:1 while in the feces the ratio was 0.94:1 (Table 4). Ergovaline released in the rumen, as a percentage of intake, increased over time within a sampling day and over the treatment period (Table 3). Lysergic acid liberated in the rumen, as a percentage of intake, increased from d 0 to d 3 but no difference was detected between d 3 and d 28 (Table 3). Hill et al. (2001) theorized that lysergic acid crosses the ruminal wall at a greater rate than other ergopeptine alkaloids because it is more polar and can be transported across tissue easier. This implies that the apparent digestibility of ergovaline results from degradation not from direct absorption. No carryover effect of ergovaline or lysergic acid in the rumen fluid was detected; concentration of ergovaline and lysergic acid at 0hr on d0 of both treatment periods was undetectable (data not shown).

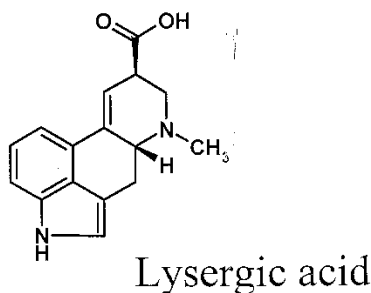
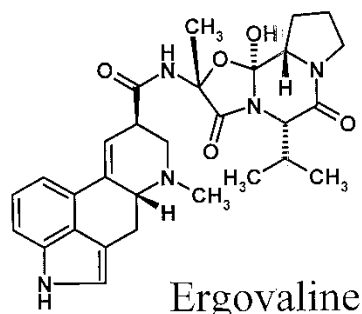
The appearance of lysergic acid in the feces implies the ergot alkaloids in the feed were degraded to lysergic acid by rumen microbial digestion and degradation in the lower gastrointestinal tract.

### Implications

This study is the first reported attempt to quantify the metabolism of lysergic acid in the ruminant digestive system using a HPLC assay. Results of this study may lead to a better understanding of ergovaline metabolism and the causes of fescue toxicosis.

### Literature Cited

- Alrich, C. G., M. T. Rhodes, J. L. Miner, M. S. Kerley, and J. A. Paterson. 1993. The effects of endophyte-infected tall fescue consumption and use of a dopamine antagonist on intake, digestibility, body temperature, and blood constituents in sheep. *J. Anim. Sci.* 71:158-163
- Bacon, C. W. 1995. Toxic endophyte-infected tall fescue and range grasses: historical perspectives. *J. Animal Sci.* 73:861-870
- Broderick, G. A. and J. H. Kang-Meznarich. 1980. Effects of incremental urea supplementation on ruminal ammonia concentration and bacterial protein formation. *J. Dairy Sci.* 63:64-75
- Cheeke, P.R. 1995. Endogenous toxins and mycotoxins in forage grasses and their effects on livestock. *J. Animal Sci.* 73:909-918
- Craig, A. M., D. Bilich, J. T. Hovermale, R. E. Welty. 1994. Improved extraction and HPLC methods for ergovaline from plant material and rumen fluid. *J. Vet. Diagn. Invest.* 6:348-352
- Fiorito, I. M., L. D. Bunting, G. M. Davenport, and J. A. Boling. 1991. Metabolic and endocrine responses of lambs fed *Acremonium coenophialum*-infected or noninfected tall fescue hay at equivalent nutrient intake. *J. Anim. Sci.* 69:2108-2114.
- Hill, N. S., F. N. Thompson, J. A. Stuedemann, G.W. Rottinghaus, H. J. Ju, D. L. Dawe, and E. E. Hiatt III. 2001. Ergot alkaloid transport across ruminant gastric tissues. *J. Anim. Sci.* 79:542-549.
- Hockett, M. E., F. M. Hopkins, M. J. Lewis, A. M. Saxton, H. H. Dowlen, S. P. Oliver, and F. N. Schrick. 2000. Endocrine profiles of dairy cows following experimentally induced clinical mastitis during early lactation. *Anim. Reprod. Sci.* 58:241-251.
- Joost, R. E. 1995. *Acremonium* in fescue and ryegrass: Boon or bane? A review. *J. Animal Sci.* 73:881-888
- Kuehl, R. O. 2000. Design of experiments: statistical principles of research design and analysis. 2<sup>nd</sup> Ed. Brooks/Cole Publishing Co. Pacific Grove, CA. 93950.
- National Ag Statistic Service web page 2002-2003. Available: [www.nass.usda.gov/or/annsum2003.htm](http://www.nass.usda.gov/or/annsum2003.htm)
- Paterson, J., C. Forcherio, B. Larson, M. Samford and M. Kerley. 1995. The effect of fescue toxicosis on beef cattle productivity. *J. Animal Sci.* 73:889-898.
- Porter, J. K. 1995. Analysis of endophyte toxins: fescue and other grasses toxin to livestock. *J. Animal Sci.* 73:871-880
- Stamm, M. M., T. DelCurto, M. R. Horney, S. D. Brandyberry, and R. K. Barton. 1994. Influence of alkaloid concentration of tall fescue straw on the nutrition, physiology, and subsequent performance of beef steers. *J. Animal Sci.* 72:1068-1075
- Strickland, J. R., J. W. Oliver, and D. L. Cross. 1993. Fescue toxicosis and its impact on animal agriculture. *Vet. Human Toxicol* 35:454-464
- Tor-Agbidye, J., L.L. Blythe, A.M. Craig. 2001. Correlation of endophyte toxins (ergovaline and Lolitrem B) with clinical disease: fescue foot and perennial ryegrass staggers.



**Figure 1 Structure of ergovaline and lysergic acid**

**Table 1 Feedstuff nutrient content (DM basis)**

Item	E- straw	E+ straw	Soybean meal	E+ seed
CP, %	5.5	6.5	49	16.2
NDF, %	68.0	73.0	N/A	41.7
EV <sup>a</sup> , ppb	<10	350	N/A	>3,300
LA <sup>a</sup> , ppb	<10	142	N/A	660

<sup>a</sup>EV = ergovaline; LA = Lysergic acid

**Table 2 Least square means for the physiological parameters during treatment**

	E- <sup>a</sup>	E+	SE
Rectal temperature, °C <sup>b</sup>	38.36	38.41	0.0615
Water Intake, L/d <sup>c</sup>	2.95	2.77	0.590
Rumen, pH <sup>d</sup>	6.86	6.97	0.0322
Rumen NH <sub>3</sub> , mMol <sup>e</sup>	4.72	4.70	0.128
Prolactin, ng/ml <sup>f</sup>	22.9	6.4	5.19

<sup>a</sup>E- = <10 ppb ergovaline, E+ = 500 ppb ergovaline; <sup>b</sup>P = 0.395; <sup>c</sup>P = 0.0398; <sup>d</sup>P = 0.355; <sup>e</sup>P = 0.896; <sup>f</sup>P = 0.023

**Table 3 Least square means for digestibility of DM, ADF and CP in treatment groups<sup>a</sup>**

	Treatment <sup>b</sup>		SE
	E-	E+	
DM	53.8	49.8	2.89
ADF	49.4	52.2	4.84
CP	61.4	63.7	1.62

<sup>a</sup> Values expresses as percent on a dry matter basis

<sup>b</sup> E- = <10 ppb ergovaline, E+ = 500 ppb ergovaline

**Table 4 Ergovaline and lysergic acid content of rumen fluid of animals consuming E+ diet presented as a percentage of intake<sup>a</sup>**

	Time	0	3	Day 28	SE
EV <sup>c</sup>	0 hr	0.00 <sup>b</sup>	5.12 <sup>c</sup>	7.60 <sup>d</sup>	0.451
	6 hr	1.42 <sup>b</sup>	5.33 <sup>c</sup>	7.25 <sup>d</sup>	0.511
	12 hr	2.67 <sup>b</sup>	6.20 <sup>c</sup>	8.42 <sup>d</sup>	0.601
LA <sup>c</sup>	0 hr	0.00 <sup>b</sup>	29.5 <sup>c</sup>	27.4 <sup>c</sup>	3.01
	6 hr	13.4 <sup>b</sup>	37.6 <sup>c</sup>	42.3 <sup>c</sup>	5.94
	12 hr	21.6 <sup>b</sup>	30.1 <sup>b</sup>	41.6 <sup>b</sup>	8.32

<sup>a</sup> Calculated as amount in rumen fluid/amount in feed

<sup>b,c,d</sup> Within a row, means without a common superscript letter differ (P<0.05)

<sup>c</sup>EV = ergovaline; LA = Lysergic acid

**Table 5 Ergovaline and lysergic acid in the diet and feces of animals consuming E+ diet**

	EV <sup>ab</sup>	SD	LA <sup>ab</sup>	SD	EV:LA <sup>b</sup>
Diet <sup>c</sup>	0.60	0.07	0.20	0.04	3.00
Feces <sup>d</sup>	0.48	0.04	0.51	0.15	0.94

<sup>a</sup> Actual means, n = 6

<sup>b</sup> EV = ergovaline; LA = Lysergic acid

<sup>c</sup> mg/kg of Intake

<sup>d</sup> mg/kg of Fecal output

# EFFECTS OF CONDENSED DISTILLERS SOLUBLE SUPPLEMENTATION ON IN SITU DISAPPEARANCE RATE OF SWITCHGRASS HAY IN STEERS CONSUMING LOW QUALITY HAY

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**ABSTRACT:** Four ruminally and duodenally cannulated steers ( $561 \pm 52.8$  kg initial BW) were used in a 4 x 4 Latin square to evaluate effects of corn distillers solubles (CCDS) supplementation on forage in situ disappearance rate (%/h) in beef steers fed low quality switchgrass hay. Steers were offered hay ad libitum at 0700 and 1900 daily and were allowed free access to water. Switchgrass hay (*Panicum virgatum* L.) offered was 5.05 % CP, 40.3% ADF, and 70.7% NDF. Corn condensed distillers solubles composition was 28.1% DM, 19.3% CP, 4.7% fat (DM basis). Treatments consisted of: 1) control, no CCDS; 2) 5% CCDS; 3) 10% CCDS; and 4) 15% CCDS supplementation (DM basis). Steers were adapted to diets for 9 d. Grass hay was incubated in situ beginning on d 10, for 0, 2, 5, 9, 12, 24, 36, 72, and 96 h. Linear, quadratic, and cubic contrasts were used to compare increasing CCDS levels. Rate of in situ disappearance for hay DM ( $2.4, 2.3, 2.5, \text{ and } 2.0 \pm 0.39$  %/h), NDF ( $2.6, 2.3, 2.7, \text{ and } 2.0 \pm 0.47$  %/h), ADF ( $2.4, 2.3, 2.4, \text{ and } 2.2 \pm 0.42$  %/h), and CP (2.1, 2.2, 2.9, and  $3.6 \pm 0.83$  %/h) were not affected ( $P \geq 0.65$ ) by CCDS supplementation. Also, hay CP soluble fraction, slowly degradable fraction, and effective degradability were not affected ( $P \geq 0.31$ ) by CCDS supplementation. Inclusion of up to 15% CCDS supplementation to steers consuming low-quality hay did not affect characteristics of in situ DM, ADF, NDF, and CP disappearance. These results suggest that in situ disappearance in steers consuming low-quality hay are not altered by CCDS supplementation.

Key Words: Corn Condensed Distillers Soluble, In Situ Digestion Rate, Cattle

## Introduction

The ethanol industry is expanding rapidly in the upper midwest. Consequently, availability of ethanol byproducts is also expanding. Interest among cow calf producers in using corn condensed distillers solubles (CCDS; commonly known as corn syrup) is growing. Corn condensed distillers solubles are high in protein (20 to 30%; DM basis), which makes the product an attractive supplement for low quality forages. Protein supplements may increase forage digestibility, forage intake, or both, resulting in increased animal performance and lowered production costs

(Caton and Dhuyvetter, 1997). Also, CCDS are high in fat. Even though fat increases NE of diets (Zinn 1992; Krehbiel et al., 1995), high levels of fats in ruminant diets can reduce fiber digestibility several ways. One is by physically coating the fiber particles in ruminant diets (MacLeod and Buchanan-Smith, 1972). This can impair or impede colonization of fiber by ruminal microorganisms. Some fatty acids, especially unsaturated fatty acids, are reportedly toxic to certain classes of ruminal microorganisms (Henderson, 1973; Chalupa et al., 1984). In most cases, added fat is generally limited to 3 to 4 percent of diet dry matter (Chalupa et al., 1984). At these levels, the above mentioned problems with fat are generally not encountered. Above these levels, fats may have negative effects on fiber digestibility. However, little is known about optimum levels of condensed distillers solubles in low-quality forage based diets and subsequent effects on ruminal fermentation, digestion, and ruminal metabolism. Therefore, the objectives of this study were to evaluate the influence of CCDS supplementation on in situ disappearance of grass hay in beef steers fed low-quality hay.

## Materials and Methods

Four ruminally and duodenally cannulated steers ( $561 \pm 53$  kg initial BW) were used in a 4 x 4 Latin square to evaluate effects of CCDS supplementation on apparent in situ disappearance rate (%/h) of grass hay in beef steers fed low-quality hay. Steers were housed in an enclosed barn in 1.2 x 2.2 m individual tie stalls. The experimental protocol was approved by North Dakota State University's Institutional Animal Care and Use Committee. Concentrated corn distillers soluble was offered at 0700 and 1900 h daily for one-half hour. Unconsumed CCDS was introduced to the rumen via the rumen cannulae. Steers were offered switchgrass hay at 110% of previous days consumption directly after CCDS and allowed free access to water. Grass hay was chopped (3.8 cm screen). Switchgrass hay (*Panicum virgatum* L.) offered was 5.1% CP, 40.3% ADF, and 70.7% NDF. Corn condensed distillers solubles composition was 28.1% DM, 19.3% CP, and 4.7% fat (DM basis). Treatments consisted of: 1) control, no CCDS; 2) 5% CCDS; 3) 10% CCDS; and 4) 15% CCDS supplementation (DM basis). Steers were adapted to diets for 9 d. In situ bags were incubated on d 10

through 13 of the experimental period. Ground grass hay (2 mm; 5 g, as is basis) was placed in Dacron bags (10 × 20 cm, 50 ± 15 µm pore size, Ankom, Fairport, NY) and ruminally incubated for 96, 72, 36, 24, 12, 9, 5, 2, and 0 h. All bags were removed at 0 h and rinsed with tap water to remove large particulate matter. In situ bags were then rinsed in a top-loading washing machine (General Electric, Louisville, KY) using the delicate cycle. The machine was filled with 45 L cold water. Bags were agitated for 1 minute, drained, and spun for 2 minutes. This cycle was repeated five times. Bags were dried in a forced-air oven at 50°C (Model: SB-350, The Grieve Corporation, Round Lake, IL) weighed and stored at room temperature for analysis. Samples were subjected to following analysis: DM (oven drying at 105°C until no further weight loss); Kjeldahl N (AOAC, 1997); ADF and NDF (Robertson and Van Soest, 1991). Ruminal DM, NDF, and ADF disappearance (%/h) of grass hay were estimated using the model described by Mertens and Loften (1980). The CP kinetic parameters of grass hay was estimated using the model proposed by Ørskov and McDonald (1979). Data were analyzed as a 4 × 4 Latin square using Mixed procedures of SAS. The model included CCDS level and period as fixed effects, and steer as random effects. Orthogonal contrasts for linear, quadratic, and cubic effects of CCDS level are discussed when a significant ( $P < 0.10$ ) treatment F-test was detected.

## Results and Discussion

Effect of CCDS level on rate of DM, NDF and ADF ruminal disappearance (%/h), and on CP kinetic parameters of grass hay in beef steers consuming low quality hay are presented in Table 1. Rate of in situ disappearance for hay DM ( $2.3 \pm 0.25$ ), NDF ( $2.4 \pm 0.35$ ), ADF ( $2.3 \pm 0.1$ ), and CP ( $2.7 \pm 0.75$ ) were not affected ( $P \geq 0.65$ ) by CCDS supplementation level. Also, hay CP soluble fraction, slowly degradable fraction, and effective degradability were not affected ( $P \geq 0.31$ ) by CCDS supplementation level. We hypothesized that CCDS supplementation would increase digestion rates of low quality hay because of increased ruminal ammonia levels and nitrogen supply to ruminal bacteria; however no differences were observed. Likewise, Arroquy et al. (2004) reported no effect of supplemental RDP (as NPN) on forage OM and NDF digestion; their hay quality was similar to ours. Certain microorganisms require amino acids for fiber digestion. Russel et al., (1992) observed the importance ammonia plays in the fermentation of structural carbohydrates; while non structural carbohydrate fermentation remained more responsive to peptides supplied in true proteins. In our study, ruminal ammonia did not change with CCDS supplementation ( $1.47 \pm 0.36$  mM, Soto-Navarro et al., in press) during incubation of grass hay. This may have limited microbial fiber fermentation rates.

Although fat supplementation (3 to 5% of DM) has been reported to decrease fiber digestibility (Chalupa et al., 1984), negative effects on rate of NDF and ADF disappearance were not observed. In fact, detrimental effects on fiber digestion were not expected because the 15% CCDS treatment, resulted in only 1.5 % of dietary fat supplied by the supplement (hay plus CCDS).

## Implications

These results suggest that supplementation of up to 15% CCDS to low-quality hay (5% CP) does not improved forage in situ disappearance. However, negative effects on in situ disappearance were not observed due to the high fat contents of CCDS.

## Literature Cited

- AOAC. 1997. Official Methods of Analysis. 16th ed. Assoc. Offic. Anal. Chem., Arlington, VA.
- Arroquy, J.I., R.C. Cochran, T.A. Wickersham, D.A. Llewellyn, E.C. Titgemeyer, T.G. Nagaraja, D.E. Johnson. Effects of supplemental carbohydrate and source of supplemental rumen degradable protein on low quality forage utilization by beef steers. Article in press.
- Caton, J.S., D.V. Dhuyvetter. 1997. Influence of energy supplementation on grazing ruminants: Requirements and responses. *J. Anim. Sci.* 75:533-542.
- Chalupa, W., B. Rickabaugh, D. S. Kronfeld and D. Sklan. 1984. Rumen fermentation in vitro as influenced by long chain fatty acids. *J. Dairy Sci.* 67:1439-1444.
- Henderson, C. 1973. The effect of fatty acids on pure culture of rumen bacteria. *J. Agric. Sci. (Camb.)* 81:107-112.
- Krehbiel, C. R., R. A. McCoy, R. A. Stock, T. J. Klopfenstein, D. H. Shain and R. P. Huffman. 1995. Influences of grain type, tallow level, and tallow feeding system on feedlot cattle performance. *J. Anim Sci.* 73:2916-2921.
- McLeod, G. L. K., and Buchanan-Smith., 1972. Digestibility of hydrogenated tallow, saturated fatty acids and soybean oil-supplemented diets by sheep. *J. Anim. Sci.* 35:890-895.
- Mertens, D. R., and J. R. Loften. 1980. The effect of starch on forage fiber digestion kinetics in vitro. *J. Dairy Sci.* 63:1437-1446.
- Ørskov, E. R. and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* 92:499-503.
- Robertson, J. B. and P. J. Van Soest. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.
- Russel, J. B., J. K. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net

- carbohydrate and protein system for evaluating Cattle diets: I. ruminal fermentation. *J. Anim. Sci.* 70:3551-3561.
- Soto-Navarro, S., T.C. Gilbery, G.P. Lardy, M. L. Bauer, J. S. Caton. 2004. Effects of corn condensed distillers soluble supplementation on intake and digestion in beef steers consuming low quality-hay. *J. Anim. Sci.* 82(Supp. 2):In press.
- Zinn, R. A. 1992. Comparative feeding value of supplemental fat in steam flaked corn and steam-flaked wheat-based finishing diets for feedlot steers. *J. Anim. Sci.* 70:2959-2969.

Table 1. Effect of CCDS level on rate of DM, NDF and ADF ruminal disappearance and on kinetic parameters of grass hay CP in beef steers consuming low quality hay

Component	CCDS (%)				SEM	P-Value	Contrast <sup>a</sup>		
	0	5	10	15			L	Q	C
Forage									
DM	2.4	2.3	2.5	2.0	0.39	0.80	0.61	0.62	0.53
NDF	2.6	2.3	2.7	2.0	0.47	0.80	0.58	0.77	0.48
ADF	2.4	2.3	2.4	2.2	0.42	0.99	0.80	0.52	0.60
Forage CP									
Soluble, %	43.3	44.8	44.6	45.9	0.78	0.31	0.10	0.94	0.42
Slowly degradable, %	30.9	33.5	28.4	26.1	5.60	0.83	0.49	0.69	0.71
Disappearance rate, %/h	2.1	2.2	2.9	3.6	0.83	0.65	0.26	0.73	0.89
Extent of degradability, %	74.2	78.4	73.0	72.0	5.28	0.86	0.65	0.66	0.60

<sup>a</sup>Probabilities for contrasts: linear (L), quadratic (Q), and cubic (C).

## NON-CONVENTIONAL PHOSPHATES IN SHEEP FEEDING

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**ABSTRACT:** To determine P bioavailability of two Venezuelan sedimentary phosphates, Riecito (RIO) and Monte Fresco (MONTE), and a fertilizer (triple super phosphate; TSP), an experiment was carried out with 24 crossbred West African lambs, using a dicalcium phosphate (DICAL) as reference control. The experimental diets were fed during 12 month period and were balanced to give 14% CP, 2.2 Mcal ME/kg and 0.37% total P (60% P from the different inorganic phosphates). Body weight and feed intake were recorded on monthly and weekly bases, respectively. At the end of the feeding period, P apparent (PAA) and true (PTA) absorption was determined using a total collection method, with four animals/treatment. Sheep were kept in metabolic crates, 14 days for adaptation and 7 days to record feed intake and fecal excretions. Endogenous fecal phosphorus was determined by isotope dilution technique with  $^{32}\text{P}$ . Each animal was dosed (i.v.) with 200  $\mu\text{Ci}$   $^{32}\text{P}$ . Ash, P, F content and specific activity of  $^{32}\text{P}$  in bone were measured. Body weight gain (g/day) of DICAL (112.8) and RIO (108.0) was higher ( $P<0.05$ ) than MONTE (90.3) and TSP (73.9). Feed intake of DICAL, RIO and MONTE was higher ( $P<0.05$ ) than TSP. Phosphorus apparent absorption (%) of DICAL (68.19) and TSP (65.72) was greater ( $P<0.05$ ) than RIO (55.62) and MONTE (51.89). Endogenous fecal phosphorus (%) was lower for RIO (8.17) and MONTE (6.39), intermediate for TSP (13.09) and higher ( $P<0.05$ ) for DICAL (20.79). Phosphorus true absorption (%) of DICAL (75.92) and TSP (70.78) was greater ( $P<0.05$ ) than RIO (58.53) and MONTE (54.04). Bone ash content ( $\text{mg cm}^{-3}$ ) was similar for DICAL and TSP and lower for RIO and MONTE. Bone fluorine accumulation (ppm) of TSP (3,133), MONTE (2,667) and RIO (2,433) was higher ( $P<0.05$ ) than DICAL (817). Bone specific activity of  $^{32}\text{P}$  ( $\times 10^{-4}$ ) of MONTE (29.28) and RIO (17.82) was higher than TSP (12.24) and DICAL (9.13). Relation between bone  $^{32}\text{P}$  and PTA was significant ( $y=82.53-1.04x$ ;  $r=-0.90$ ;  $P<0.05$ ). It is concluded that sedimentary phosphates and fertilizers can be used in ruminant feeding, partially substituting feed grade phosphates. Type of sedimentary phosphates, animal species and productive targets should also be considered.

Key Words: Phosphate, Phosphorus, Absorption, Sheep

### Introduction

Soils and forages of the majority of tropical grasslands are P deficient, being the concentration of the element lower than 0.15% in the available grasses (McDowell *et al.*, 1993). Studies in soils, forages and

blood samples of cattle carried out in Venezuela (Chicco and Godoy, 1987) indicate that P deficiency is dominant in well and poorly drained infertile savannas of the country. Registered average values indicate that soil (9 ppm) and forage (0.11%) P concentrations are lower than the levels required for plants and ruminants.

To correct P deficiencies, feed grade calcium, sodium and ammonium phosphates, and phosphoric acid are used. These P sources are not produced in Venezuela, and therefore they are imported for animal feeding. In addition, with some restrictions, sedimentary phosphates and P fertilizers are also used in the feed industry.

Venezuela has important sedimentary phosphate resources that could be used in animal feeding, previous adequate evaluation. Research conducted in poultry, swine (Godoy and Chicco, 2001, 2002) and ruminants (Godoy *et al.*, 2002) indicates that these non-conventional P sources can replace, partially or totally, the imported phosphates, depending upon animal species and productive goals.

The present article summarizes the results of productive performance, P apparent (PAA) and true absorption (PTA), bone mineralization, and F retention in lambs fed with two Venezuelan sedimentary phosphates and a P fertilizer.

### Materials and Methods

To determine P bioavailability of two Venezuelan sedimentary phosphates, Riecito (RIO) and Monte Fresco (MONTE), and a fertilizer (triple super phosphate; TSP), an experiment was carried out with 24 (six/treatment) crossbred West African lambs, with an initial average weight of 14.5 kg, using a dicalcium phosphate (DICAL) as reference control. RIO, MONTE and TSP contained 11.1, 11.0 and 16.0 %P, 24.6, 34.4 and 22.1% Ca, and 1.2, 2.5 and 2.8%F, respectively for sources and elements. For DICAL, the values (%) were 29.0, 22.7 and 0.01, respectively for Ca, P and F.

The experimental diets, composed of corn meal and cobs, soy meal, molasses, urea, vegetal oil, minerals and vitamins, were fed (1 kg/day) during 12 month period and were balanced to give 14% CP, 2.2 Mcal ME/kg and 0.37% total P (60% P from the different inorganic phosphates). Body weight and feed intake were recorded on monthly and on weekly bases, respectively.

At the end of the feeding period, P apparent (PAA) and true (PTA) absorption was determined with four animal/treatment, using a total collection method. Sheep were kept in metabolic crates, 14 days for adaptation and 7 days to record feed intake and fecal excretions.

Endogenous fecal phosphorus (EFP) was determined by isotope dilution technique with  $^{32}\text{P}$ . Each animal was dosed (i.v.) with 200  $\mu\text{Ci}$   $^{32}\text{P}$  as  $\text{Na}_2\text{HPO}_4$  (Amersham International®). The following formulas were used (Kleiber *et al.*, 1951; Underwood, 1981):

$\text{PAA, \%} = (\text{ingested} - \text{excreted} / \text{ingested}) \times 100$

$\text{PTA, \%} = [\text{ingested} - (\text{excreted} - \text{endogenous}) / \text{ingested}] \times 100$

$\text{EFP, \%} = (\text{specific activity feces} / \text{specific activity plasma}) \times 100$

One hundred and forty four hours post dosing, four animals/treatment were slaughtered and the seventh rib was removed for chemical analysis. Calcium concentration was assayed with atomic absorption spectrophotometry (AOAC, 1997), P was measured by a colorimetric procedure (Fiske and Subbarow, 1925) and F by an electrometric method (AOAC, 1997). Bone measurements were expressed as  $\text{mg cm}^{-3}$ . Bone volume was determined by weighing the displaced water when the bone was submerged in a 50 ml cylinder. Radioactive P was determined with a conventional liquid scintillation counter.

Data were analyzed by ANOVA of a completely randomized design. In the feeding trial two pens with three animals were assigned to each treatment. Treatment means were separated by Duncan's Multiple Range Test, in the case of a significant F-value ( $P < 0.05$ ). In addition, correlations and regressions were calculated between response variables (Steel and Torrie, 1988).

## Results and Discussion

Body weight at the end of the feeding period was higher ( $P < 0.05$ ) in lambs fed with DICAL and RIO than MONTE and TSP, averaging 53.8, 53.5, 44.2 and 42.8 kg, respectively. Daily gains (g/day) presented similar trend. Feed intake (g/day) was similar for DICAL (883.0), RIO (818.6) and MONTE (830.2), and lower for TSP (744.9). Same tendency was recorded for feed efficiency (gain:feed). Feed intake diminished progressively with time in animals fed with MONTE and TSP (Table 1). The lower performance of lambs fed with high F phosphates is probably due, on one side, to a lower P bioavailability of sedimentary phosphates, as shown previously in poultry and swine (Godoy and Chicco, 2001, 2002) and, on the other side, to the effect of F in lowering feed intake, as shown in the case of TSP. This effect is probably related to an alteration of carbohydrate metabolism by high F intake (Zebrowski *et al.*, 1964; Suttie *et al.*, 1974).

Table 2 summarizes digestibility data. Phosphorus AA (%) of DICAL (68.19) and TSP (65.72) was higher ( $P < 0.05$ ) than RIO (55.62) and MONTE (51.89), with no differences between raw rock phosphates. Higher values of P absorption of DICAL and TSP respond to a greater bioavailability of the element in relation to sedimentary phosphates (Vitti *et al.*, 1989; Lopes *et al.*, 1990). Similar results of PAA were obtained in previous studies with the same P sources (Godoy *et al.*, 2002).

Endogenous fecal P (%) was lower for RIO (8.17) and MONTE (6.39), intermediate for TSP (13.09)

and greater ( $P < 0.05$ ) for DICAL (20.79), supporting previous findings in sheep fed P deficient diets (Boxebeld *et al.*, 1983).

Phosphorus TA (%) of DICAL (75.92) and TSP (70.78) was greater than RIO (58.53) and MONTE (54.04), showing similar trend than PAA, with relatively higher values, due to the elimination of endogenous phosphorus from total fecal excretion of the element. Value of PTA of DICAL is similar to those reported by several authors (Lofgreen, 1990; O' Donovan *et al.*, 1965; Vitti *et al.*, 1989).

Table 1. Body weight and feed intake of lambs fed with different phosphates<sup>1</sup>

Item	DICAL	RIO	MONTE	TSP
Initial weight, kg	14.8	14.4	14.6	14.8
Final weight, kg	$\pm 3.27$	$\pm 4.51$	$\pm 4.16$	$\pm 2.86$
Body gain, g/day	53.8 <sup>a</sup>	53.5 <sup>a</sup>	44.2 <sup>b</sup>	42.8 <sup>b</sup>
Intake, g/day	$\pm 0.35$	$\pm 4.77$	$\pm 3.88$	$\pm 6.25$
Gain:feed <sup>2</sup>	112.8 <sup>a</sup>	108.0 <sup>a</sup>	90.3 <sup>b</sup>	73.9 <sup>b</sup>
g/kg	$\pm 0.35$	$\pm 4.80$	$\pm 3.88$	$\pm 6.25$
	883.0 <sup>a</sup>	818.6 <sup>b</sup>	830.2 <sup>b</sup>	744.9 <sup>c</sup>
	$\pm 185.7$	$\pm 175.9$	$\pm 243.9$	$\pm 287.6$
	127.7 <sup>a</sup>	131.9 <sup>a</sup>	108.8 <sup>b</sup>	99.2 <sup>b</sup>
	$\pm 33.4$	$\pm 36.9$	$\pm 31.9$	$\pm 43.4$

<sup>1</sup> Six animals/treatment

<sup>2</sup> Gain:feed = body gain (g):feed intake (kg)

<sup>a, b</sup> Average values with ( $P < 0.05$ ) different superscripts in the same row are significantly different

Table 2. Phosphorus apparent (PAA) and true absorption (PTA) of lambs fed with different phosphates<sup>1</sup>

Item	DICAL	RIO	MONTE	TSP
P ingested, g/day	2.60	1.97	2.22	2.32
P excreted, g/day	$\pm 0.52$	$\pm 0.74$	$\pm 0.78$	$\pm 0.72$
P endogenous, g/day	0.79	0.89	1.09	0.78
P endogenous, %	$\pm 0.11$	$\pm 0.40$	$\pm 0.45$	$\pm 0.24$
PAA, %	0.164	0.073	0.070	0.110
PTA, %	$\pm 0.05$	$\pm 0.03$	$\pm 0.04$	$\pm 0.03$
	20.79 <sup>a</sup>	8.17 <sup>c</sup>	6.39 <sup>c</sup>	13.09 <sup>b</sup>
	$\pm 4.17$	$\pm 2.27$	$\pm 0.80$	$\pm 2.54$
	68.19 <sup>a</sup>	55.62 <sup>b</sup>	51.89 <sup>b</sup>	65.72 <sup>a</sup>
	$\pm 6.60$	$\pm 5.66$	$\pm 8.99$	$\pm 5.97$
	75.92 <sup>a</sup>	58.53 <sup>b</sup>	54.04 <sup>b</sup>	70.78 <sup>a</sup>
	$\pm 7.00$	$\pm 5.33$	$\pm 5.20$	$\pm 4.45$

<sup>1</sup> Four animals/treatment

<sup>a, b</sup> Average values with different superscripts in the same row are significantly different ( $P < 0.05$ )

Bone measurements indicate that mineralization of the tissue was greater in lambs fed with DICAL and TSP than RIO and MONTE (Table 3). Bone density ( $\text{mg cm}^{-3}$ ) of DICAL (1,347) and TSP (1,333) was higher ( $P < 0.05$ ) than RIO (1,141), being MONTE (1,235) intermediate, with no differences with the other sources. Ash content ( $\text{mg cm}^{-3}$ ) was similar in lambs fed with DICAL (473.4) and TSP (512.9) and lower ( $P < 0.05$ ) for RIO (412.7) and MONTE (408.2). Calcium and P

concentrations in bone tissue ( $\text{mg cm}^{-3}$ ) showed similar trend than the above measurements, with higher values ( $P<0.05$ ) for TSP (72.74 and 204.03) and DICAL (78.71 and 165.85) than RIO (62.14 and 136.71) and MONTE (64.91 and 142.81). Bone density was significantly correlated ( $P<0.05$ ) with bone ash ( $r=0.66$ ), P ( $r=0.64$ ), and Ca ( $r=0.64$ ). Bone F accumulation (ppm) of TSP (3,133), MONTE (2,667) and RIO (2,433) was higher ( $P<0.05$ ) than DICAL (817), with no effect of F on bone mineralization measurements.

The lower mineralization of bone tissue registered in animals fed with sedimentary phosphates (RIO and MONTE) appears to be due to a lower P bioavailability and high F concentration of these sources. However, in the case of TSP, with high P availability and high content of soluble F, bone mineralization was not affected. This finding is in contradiction with previous results of the authors (Godoy and Chicco, 2001) and more recently with the data of Odongo *et al.* (2002) who reported that high levels of F in raw rock phosphates diminished bone ash and Ca content, Ca:P ratio and bone strength..

Bone specific activity of  $^{32}\text{P}$  ( $\times 10^{-4}$ ) of MONTE (29.28), and RIO (17.82) was higher than DICAL (9.13) and TSP (12.24). The higher  $^{32}\text{P}$  specific activity in the bone tissue corroborates previous findings (Chicco *et al.*, 1967) which indicate that  $^{32}\text{P}$  capture is greater in less mineralized bone. In addition, an inverse correlation was found between bone  $^{32}\text{P}$  and PTA ( $y = 82.53 - 1.04x$ ;  $r = 0.90$ ), which suggests that, in the difficult-to-metabolize compartment (bone), bone resorption process is lower at higher availability of the element.

Table 3. Bone tissue measurements of lambs fed with different phosphates<sup>1</sup>.

Item	DICAL	RIO	MONTE	TSP
Density, $\text{mg cm}^{-3}$	1,347 <sup>a</sup> $\pm 210$	1,141 <sup>b</sup> $\pm 160$	1,235 <sup>ab</sup> $\pm 80$	1,333 <sup>a</sup> $\pm 120$
Ash, $\text{mg cm}^{-3}$	473.4 <sup>a</sup> $\pm 52.6$	412.7 <sup>b</sup> $\pm 75.9$	408.2 <sup>b</sup> $\pm 115.6$	512.9 <sup>a</sup> $\pm 79.7$
Phosphorus, $\text{mg cm}^{-3}$	78.71 <sup>a</sup> $\pm 10.39$	62.14 <sup>b</sup> $\pm 8.68$	64.91 <sup>b</sup> $\pm 19.81$	72.74 <sup>a</sup> $\pm 14.24$
Calcium, $\text{mg cm}^{-3}$	165.85 <sup>a</sup> $\pm 22.86$	136.71 <sup>b</sup> $\pm 19.10$	142.81 <sup>b</sup> $\pm 43.58$	204.03 <sup>a</sup> $\pm 31.34$
Fluorine, ppm	817 <sup>c</sup> $\pm 76$	2,433 <sup>b</sup> $\pm 208$	2,667 <sup>b</sup> $\pm 152$	3,133 <sup>a</sup> $\pm 32$
SA <sup>2</sup> $^{32}\text{P}$ ( $\times 10^{-4}$ )	9.13 <sup>c</sup> $\pm 1.7$	17.82 <sup>b</sup> $\pm 3.4$	29.28 <sup>a</sup> $\pm 7.1$	12.24 <sup>bc</sup> $\pm 3.2$

<sup>1</sup> Four animals/treatment; <sup>2</sup> SA: specific activity

<sup>a, b, c</sup> Average values with different superscripts in the same row are significantly different ( $P < 0.05$ )

### Implications

Animal performance of lambs fed with RIO was similar to DICAL, although bone mineralization was lower, as also was the case of MONTE. Triple super phosphate promoted poor gains, affecting intake, independently of high P availability and adequate bone formation. Fluorine seems to be a limiting factor. It is

concluded that the possibility of using raw rock phosphates depends on type of P sedimentary deposits. Animal species and productive goals should also be considered. Therefore, evaluation with animals is required.

### Literature Cited

- AOAC. 1997. Official methods of analysis. 16<sup>th</sup> ed. Assoc. Offic. Anal. Chem., Arlington, VA.
- Boxebeld, A., L. Guenguen, G. Hannequart and M. Durand. 31983. Utilization of phosphorus in growing sheep fed a low phosphorus diet. *Reprod. Nutr. Dev.* 23:1043-1053.
- Chicco, C. F. and S. Godoy. 1987. Suplementación mineral de bovinos de carne a pastoreo. En *Plasse D, Peña N (Eds.) III Cursillo de Ganado de Carne*. Universidad Central de Venezuela. Facultad de Ciencias Veterinarias. Maracay, Venezuela. pp. 47-103.
- Chicco, C.F., C. B. Ammerman, P. A. Van Walleghem, P. W. Waldroup and R. H. Harms. 1967. Effects of varying dietary ratios of magnesium, calcium and phosphorus in growing chicks. *Poult. Sci.* 47: 368-373.
- Fiske, C. H. and E. Subbarrow. 1925. The colorimetric determination of phosphorus. *J. Biological Chem.* 66: 375-400.
- Godoy, S. and C. F. Chicco. 2001. Relative bioavailability of phosphorus from Venezuelan raw rock phosphates for poultry. *Animal Feed Science and Technology*. 94:103-113.
- Godoy, S. and C. F. Chicco. 2002. Bioavailability of venezuelan sedimentary phosphates in swine feeding. *Proceedings, Western Section, American Society of Animal Science*. 53:287-290.
- Godoy, S., C. F. Chicco and F. Requena. 2002. Fosfatos sedimentarios venezolanos en la alimentación de ovinos. *Interciencia*. 27: 482-488.
- Kleiber, M., A. H. Smith, N. P. Ralston and A. J. Black. 1951. Radiophosphorus ( $\text{P}^{32}$ ) as tracer for measuring endogenous phosphorus in cows feces. *J. Nutr.* 45:253-263.
- Lofgreen, G. P. 1960. The availability of the phosphorus in dicalcium phosphate, bone, meal, soft phosphate and calcium phytate for mature wethers. *J. Nutr.* 70: 58-62.
- Lópes, H. O., D. M. Vitti, E. A. Pereira, A. L. Abdalla, E. A. Moraes, J. C. Filho and S. S. Fichtner. 1990. Disponibilidade biológica do fósforo de fosfatos naturais para bovinos pele técnica de dilucao isotópica. *Pesq. Agropec. Bras.* 25:421-433.
- McDowell, L. R., J. H. Conrad and F. G. Hembry. 1993. Minerales para rumiantes en pastoreo en regiones tropicales. 2<sup>a</sup> ed. Boletín del Departamento de Zootecnia. Universidad de Florida. Gainesville. FL.
- O'Donovan, M., P. Plumlee, W. H. Smith and W. M. Beeson. 1965. Availability of phosphorus in

- dicalcium phosphates and defluorinated phosphate for steers. *J. Anim. Sci.* 24: 981-985.
- Odongo, N. F., J. Plaizier, P. Van Straatten and B. McBride. 2002. The effect of replacing dicalcium phosphate with BUSUMBU rock phosphate in performance and mechanical properties of bone in growing chicks. *Trop. Anim. Health Prod.* 34: 349-358.
- Steel, R. G. D. and J. H. Torrie. 1988. Principles and Procedures of statistics: A Biometrical Approach. 2nd. ed. New York. Mc Graw-Hill Book Co., New York.
- Suttie, J. M., M. P. Drescher, D. O. Quissell and K. L. Young. 1974. Trace Element Metabolism in Animal. 2<sup>nd</sup> ed. University Park Press. Baltimore, MD.
- Underwood, E. J. 1981. Sources of minerals. The mineral nutrition of livestock. Underwood, E.J. (Ed.) Commonwealth Agricultural Bureau. Farnham, UK.
- Vitti, D. M., A. L. Abdalla and J. C. Filho. 1989. Fontes alternativas de fósforo para ruminantes: Absorcao real e disponibilidade biológica. *Arq. Bras. Med. Vet. Zoot.* 41:503-512.
- Zebrowski, E. J., J. W. Suttie and P. H. Phillips. 1964. Metabolic studies in fluoride fed rats. *Fed. Proc.* 23:184-192.
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## EFFECTS OF MATERNAL NUTRIENT RESTRICTION DURING EARLY- TO MID-GESTATION ON COW AND FETAL VISCERAL ORGAN MEASUREMENTS

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**ABSTRACT:** Thirty multiparous beef cows (30 d pregnant; initial BW = 569 ± 62 kg, BCS = 5.4 ± 0.7) were used in one of two experiments to evaluate maternal nutrient restriction from early to mid-gestation on maternal and fetal visceral organs. In Exp. 1, cows were fed either native grass hay fortified with vitamins and minerals at recommendations for a mature cow to gain 0.72 kg/d during the first 120 d of gestation (C) or half the vitamins and minerals and millet straw at 68.1% of NEm requirements (NR). On d 125 of gestation, cows (n = 20) were slaughtered; after which, maternal and fetal visceral organs were removed and measured. In Exp. 2, C cows (n = 5) were fed as in Exp. 1 while NR cows (n = 5) were fed a diet to achieve the same BCS of C by 60 d prepartum. On d 250 of gestation, cows were slaughtered for collection of cow and fetal visceral tissue. In Exp. 1, cow heart, pancreas, liver, kidney, and digestive tract weights were less ( $P \leq 0.01$ ) for the NR than C cows. Eviscerated BW (EBW) was less ( $P < 0.01$ ) for the NR cows, which resulted in similar ( $P = 0.23$  to  $0.86$ ) relative weights (g/kg of EBW) of most visceral organs. Weights of fetal digestive tract components were not influenced ( $P = 0.14$  to  $0.90$ ) by maternal plane of nutrition. In Exp. 2, the omasum was lower (g/kg of EBW;  $P < 0.05$ ) for realimented cows. Other than shorter ( $P < 0.004$ ) colons for realimented cows, other visceral organs were similar ( $P = 0.16$  to  $0.97$ ) between treatments. Fetal ruminal and omasal mass (g/kg of EBW) was less ( $P \leq 0.05$ ) in realimented cows compared with controls. Maternal visceral organs generally decreased proportional to decreased EBW. Differential growth of the fetal stomach may occur when cows are realimented after being restricted of nutrients from early through mid-gestation.

**Key Words:** Beef Cows, Fetus, Visceral Organs

### Introduction

Quantity and quality of forage available to pregnant beef cows may not be sufficient to meet nutrient requirements (DelCurto et al., 2000), although, nutrient deficits may not persist if feed supplementation practices are implemented. Nutrient deficiencies have resulted in reduced visceral organ weights in sheep; however, losses in visceral organ mass were recovered when animals were realimented to eliminate nutrient deficits (Kabbali et al., 1982; Drouillard et al., 1991; Wester et al., 1995). Nutritional insults during early gestation may also affect digestive tract development. Winkler and Wille (1998) reported that the fetal bovine intestine begins to develop

crypts and villi by d 49 of gestation. Furthermore, fetuses subjected to maternal nutrient restriction during early- to mid-gestation experienced decreased growth of the gastrointestinal tract (Trahair et al., 1997). It is not known if growth retardation of the digestive tract associated with maternal nutrient restriction during early- to mid-gestation will persist if the cows are realimented from mid- to late gestation. We hypothesized that maternal and fetal visceral organs would respond to maternal plane of nutrition during pregnancy. Objectives were to evaluate the effects of maternal nutrient restriction during early- to mid-gestation and realimentation during mid- to late gestation on maternal and fetal visceral organ measurements.

### Materials and Methods

#### General

In accordance with an approved University of Wyoming Animal Care and Use Committee protocol, thirty multiparous beef cows (30 d pregnant; initial BW = 569 ± 62 kg, BCS = 5.4 ± 0.7) ranging in age from 3 to 13 yr were used in one of two experiments. Cows were sorted and blocked by initial BW, BCS, and age. Fifteen cows were fed native grass hay (12.1% CP, 70.7% TDN on a DM basis) fortified with vitamins and minerals at NRC (1996) recommendations for a mature cow to gain 0.72 kg/d during the first 125 d of gestation (Control). The other half of the cows were allotted to a nutrient restricted diet (NR), which consisted of feeding one-half of the control cow's vitamins and minerals and millet straw (9.9% CP, 54.5% IVDMD) to provide 68.1% NEm and 86.7% of metabolizable protein requirements during the first 120 d of gestation (NRC, 1996). Cows were weighed and assigned a BCS every 14 d to adjust rations for changes in BW throughout the experiment. For Exp 1, a subset of Control (n = 10) and NR (n = 10) cows were slaughtered on d 125 of gestation. Maternal and fetal visceral tissues were immediately removed, and digestive tracts were stripped of digesta, trimmed of fat, and measured. Additionally, cow liver, lung, heart, kidney and pancreas weights were also collected and recorded. For Exp 2, the remaining Control cows (n = 5) were fed the Control diet to maintain a BCS of 5.75 from d 125 to d 250 of gestation. The five remaining NR cows were fed the NR hay and the Control minerals and vitamins plus a corn-based supplement (Table 1) to achieve a BCS equal to their Control contemporaries by d 220 of gestation. On d 250 of gestation all cows (n = 10) were slaughtered and visceral tissues were collected as outlined for Exp 1.

All data were analyzed as a randomized complete block experiment using the GLM procedures of SAS (SAS

Inst. Cary, NC), and treatment means were calculated with the LSMEANS option.

## Results and Discussion

### Exp 1

Similar to our previous report with ewes (Vonnahme et al., 2003), control cows increased in BW by 4.2% whereas nutrient restricted cows lost 7.1% of initial BW during the feeding period. Nutrient restriction decreased ( $P \leq 0.04$ ) ruminal, omasal and total stomach weight in NR compared with control cows (Table 2). The 20% reduction in total stomach weight ( $P = 0.002$ ) resulted in decreased total digestive tract mass for NR compared with control cows. Dietary treatment did not affect ( $P \geq 0.26$ ) weight or length of the small or large intestines. Restricted cows had heavier ( $P \leq 0.03$ ) reticulum and abomasum when expressed as a g/kg of EBW, suggesting that the mass of these stomach compartments is preferentially retained during periods of nutrient restriction. Our results contrast those of Wilson and Osbourn (1960) who reported that the gastrointestinal tract was not greatly affected by nutrient restriction. In agreement with our data, Vonnahme et al. (2003) reported changes in stomach compartments in ewes restricted of nutrients from d 28 to d 78 of gestation, which is the same relative interval during gestation as that of d 30 to 125 of gestation in beef cows. Weights of the heart, pancreas, liver, and kidney for NR cows were less ( $P \leq 0.02$ ) than control cows; however, only the liver weighed less ( $P \leq 0.001$ ) for NR cows when expressed as g/kg of EBW. Drouillard et al. (1991) demonstrated that nutrient restriction decreased liver size with a concomitant decrease in liver oxygen consumption. Thus, decreased liver mass in NR cows may be attributed to the expected decrease in overall metabolic function of the liver for cows maintained on lower planes of nutrition.

Fetal digestive tract weights and lengths on d 125 of gestation were not different ( $P \geq 0.14$ ) between treatments (Table 3). Osgerby et al. (2002) also reported that nutrient restricting sheep did not affect fetal tissues at d 90 of gestation. Bassett (1986; 1991) suggested that, even though the dam is undernourished, placental systems compensate to provide the fetus with adequate nutrients. In a companion abstract, Vonnahme et al. (2004) noted that total placentomal weight was not affected by dietary treatment on d 125 of gestation. Moreover, Clarke et al. (1998) indicated that the placenta of nutrient restricted ewes compensated for reduced nutrient supply by increasing total number of caruncles.

### Exp 2

Realimenting the NR cows resulted in similar BCS between treatments (data not shown; Miller et al., 2004). Likewise, EBW of NR cows was similar ( $P = 0.42$ ) to controls on d 250 of gestation (Table 4). Other than reduced omasal weight ( $P \leq 0.05$ ) and colon length ( $P = 0.004$ ) for NR cows that were realimented, visceral tissues were not affected ( $P \geq 0.23$ ) by dietary treatment. Our results contrast those of Kabbali et al. (1992) who reported that after 20 d of feed restriction and 20 d of

refeeding, lamb visceral organ weight returned to 90% of their original weight. However, our data concur with Anugwa and Pond (1989) who reported that body and organ weights from nutrient restricted rats caught up with controls within 14 d of realimentation. Anugwa and Pond (1989) also noted that organ and body weights of realimented rats surpassed the controls after 28 d of realimentation. Differences in visceral tissues among treatments in the current study would not have been expected considering our objective to feed the cows to achieve similar BCS by d 220 of gestation.

Fetal omasal weight at d 250 of gestation was less ( $P = 0.05$ ) for NR compared with controls whereas the reticulum tended ( $P = 0.09$ ) to weigh more for NR fetuses (Table 5). Changes in reticular and omasal weights, however, did not influence ( $P \geq 0.20$ ) overall stomach weight. Nonetheless, the fetal rumen ( $P = 0.06$ ) and omasum ( $P = 0.03$ ) were less (g/kg) of EBW in NR fetuses. This is the first trial to our knowledge to investigate the influence of realimentation on fetal digestive tract growth and development. Therefore, it is difficult to ascertain the cause of this response, but our results indicate that the developing fetus may be responsive to maternal plane of nutrition. Likewise, in a companion abstract, Vonnahme et al. (2004) suggested that early maternal undernutrition leads to reduced placental growth; however, realimented cows had greater placental efficiency on d 250 of gestation.

## Implications

Loss of body and visceral tissue weight during nutrient restriction in beef cows that are 125 d pregnant can be replenished with realimentation by d 250 of gestation. Other than certain compartments of the stomach, fetal digestive tract tissues do not seem to be as responsive to level of maternal nutrition as the cow digestive tract. Although maternal nutrient restriction during early- to mid-gestation may not be detrimental to the development of the fetal digestive tract as a whole, more research is required to determine effects on functionality of these fetal tissues.

## Literature Cited

- Anugwa, F. O., and W. G. Pond. 1989. Growth of whole body and organs of growing rats during feed restriction and subsequent realimentation. *Growth Dev. Aging*. 53:167-174.
- Bassett, J. M. 1986. Nutrition of the conceptus: Aspects of its regulation. *Proc. Nutr. Soc.* 45:1-10.
- Bassett, J. M. 1991. Current perspectives on placental development and its integration with fetal growth. *Proc. Nutr. Soc.* 50:311-319.
- Clarke, L., Lindsay Heasman, Darren T. Juniper, and Michael E. Symonds 1998. Maternal nutrition in early-mid gestation and placental size in sheep. *Br. J. Nutr.* 79: 359-364.
- DelCurto, T., B.W. Hess, J.E. Huston, and K.C. Olson, etc. 2000. Optimum supplementation strategies for beef cattle consuming low-quality roughages in the western United States. *Proc. Am. Soc. Anim. Sci.* 1999. available at: <http://www.asas.org/jas/symposia/proceedings>.
- Drouillard, J. S., T. J. Klopfenstein, R. A. Britton, M. L. Bauer, S. M. Gramlich, T. J. Wester, and C. L. Ferrell.

1991. Growth, body composition, and visceral organ mass and metabolism in lambs during and after metabolizable protein or net energy restrictions. *J. Anim. Sci.* 69:3357-3375.
- Kabbali, A., W. L. Johnson, D. W. Johnson, R. D. Goodrich, and C. E. Allen. 1992. Effects of undernutrition and refeeding on weights of body parts and chemical components of growing Moroccan lambs. *J. Anim. Sci.* 70:2859-2865.
- NRC, 1996. Nutrient requirements for beef cattle. 7th revised ed. Natl. Acad. Press, Washington, DC.
- Trahair, J. F., T. M. DeBarro, J. S. Robinson, and J. A. Owens. 1997. Restriction of nutrition in utero selectively inhibits gastrointestinal growth in fetal sheep. *J. Nutr.* 127:637-641.
- Vonnahme, K. A., B. W. Hess, T. R. Hansen, R. J. McCormick, D. C. Rule, G. E. Moss, W. J. Murdoch, M. J. Nijland, D. C. Skinner, P. W. Nathanielsz, and S. P. Ford. 2003. Maternal undernutrition from early- to mid-gestation leads to growth retardation, cardiac ventricular hypertrophy, and increased liver weight in the fetal sheep. *Biol. Reprod.* 69:133-140.
- Wester, T. J., R. A. Britton, T. J. Klopfenstein, G. A. Ham, D. T. Hickok, and C. R. Krehbiel. 1995. Differential effects of plane of protein or energy nutrition on visceral organs and hormones in lambs. *J. Anim. Sci.* 73:1674-1688.
- Wilson, P. N. and D. R. Osbourn. 1960. Compensatory growth after undernutrition in mammals and birds. *Biol. Rev.* 35:324-363.
- Winkler, F., and K. H. Wille. 1998. Early fetal development of the small intestine mucosa in cattle (*Bos primigenius taurus*). *Anat. Histol. Embryol.* 27:335-343.
- Vonnahme, K.A., S.P. Ford, M.J. Nijland, and L.P. Reynolds. 2004. Alteration in cotyledonary (COT) vascular responsiveness to angiotensin II (ANG II) in beef cows undernourished during early gestation. *Proc. Soc. Study Reproduction*, Vancouver, B.C., Canada, (In Press).

Table 1 Realimentation supplements fed to NR cows from d-125 thru d-220 of gestation

Ingredient, %	
Corn	79.57
Soybean meal	6.12
Sunflower meal	5.30
Molasses	4.24
Safflower meal	2.65
Dried skim milk	1.59
Analyzed composition	----- %DM -----
CP	13.2
IVDMD	77.6

Table 2. Visceral measurements of d 125 pregnant cows fed to meet NRC requirements (Control) or nutrient restricted (NR)

Item	Treatments		SE	<i>P</i>
	Control	NR		
----- Measurements -----				
Stomach <sup>a</sup> , kg	16.6	13.2	0.43	<0.001
Rumen, kg	9.3	6.9	0.21	<0.001
Reticulum, g	1220.0	1155.0	41.5	0.30
Omasum, g	4350.0	3605.0	216.3	0.04
Abomasum, g	1677.0	1619.5	66.5	0.56
Small intestine, cm	3556.2	3669.2	266.4	0.77
Colon, cm	1233.5	1245.1	170.6	0.96
Small intestine, g	4232.5	3736.3	291.3	0.26
Cecum, g	361.1	340.0	28.8	0.62
Colon, g	2990.0	2810.0	259.8	0.64
Total digestive tract <sup>b</sup> , kg	23.8	19.8	0.66	0.002
Lung, g	3335.0	3190.0	121.6	0.42
Heart, g	2455.0	2115.0	75.4	0.01
Pancreas, g	959.9	785.2	36.1	0.01
Liver, g	5799.3	3850.0	160.5	<0.001
Kidney, g	1160.0	980.0	45.4	0.02
Eviscerated body, kg	389.9	316.7	6.8	<0.001
----- 100 g/kg eviscerated BW -----				
Stomach <sup>a</sup>	4.3	4.2	0.13	0.86
Rumen	2.4	2.2	0.06	0.03
Reticulum	0.32	0.37	0.02	0.03
Omasum	1.1	1.2	0.06	0.70
Abomasum	0.43	0.52	0.02	0.03
Cecum	0.10	0.11	0.01	0.27
Colon	0.77	0.90	0.07	0.23
Intestine	1.1	1.2	0.08	0.35
Total digestive tract <sup>b</sup>	6.1	6.3	0.20	0.48
Lung	0.86	1.0	0.05	0.03
Heart	0.63	0.68	0.03	0.24
Pancreas	0.25	0.25	0.01	0.77
Liver	1.5	1.2	0.04	0.001
Stomach <sup>a</sup> , kg	16.6	13.2	0.43	<0.001

<sup>a</sup>Stomach – rumen, reticulum, omasum, abomasum

<sup>b</sup>Total digestive tract – small and large intestine, rumen, reticulum, omasum, abomasum

Table 3. Digestive tracts measurements of d 125 fetuses collected from cows fed to meet NRC requirements (Control) or nutrient restricted (NR)

Item	Treatments		SE	<i>P</i>
	Control	NR		
	----- Measurements -----			
Stomach <sup>a</sup> , g	13.7	13.5	0.75	0.88
Rumen, g	6.3	6.1	0.35	0.74
Reticulum, g	1.3	1.2	0.09	0.66
Omasum, g	3.7	3.9	0.26	0.68
Abomasum, g	2.4	2.3	0.14	0.67
Small intestine, cm	242.0	230.4	19.5	0.73
Large intestine, cm	40.8	48.6	5.54	0.41
Total intestine, cm	282.8	279.0	18.7	0.90
Total intestine, g	10.6	10.3	0.67	0.80
Small intestine, g	8.0	7.7	0.51	0.67
Large intestine, g	2.6	2.6	0.26	0.84
Total digestive tract <sup>b</sup> , g	24.3	23.8	1.29	0.83
Cecum, g	0.14	0.18	0.02	0.15
Fetus, g	738.9	686.4	37.3	0.41
	----- 100 g/kg eviscerated BW -----			
Stomach <sup>a</sup>	1.9	1.9	0.05	0.33
Rumen	0.84	0.90	0.03	0.17
Reticulum	0.17	0.18	0.01	0.69
Omasum	0.53	0.52	0.02	0.84
Abomasum	0.32	0.34	0.01	0.14
Small intestine	1.1	1.1	0.04	0.67
Large intestine	0.36	0.38	0.02	0.54
Total intestine	1.5	1.5	0.05	0.53
Total digestive tract <sup>b</sup>	3.3	3.4	0.08	0.32
Cecum	0.02	0.02	<0.001	0.66

<sup>a</sup>Stomach= rumen + reticulum + omasum + abomasum

<sup>b</sup>Total digestive tract = small and large intestine, rumen, reticulum, omasum, abomasum

Table 4. Visceral measurements of d 125 pregnant cows fed to meet NRC requirements (Control) or nutrient restricted (NR) then realimented through d 220 of gestation

	Treatments			
Item	Control	NR	SE	P
	----- Measurements -----			
Stomach <sup>a</sup> , kg	16.6	13.2	0.43	<0.001
Rumen, kg	10.8	10.5	0.37	0.71
Reticulum, g	1552.0	1490.0	139.2	0.77
Omasum, g	4918.0	4309.8	114.7	0.02
Abomasum, g	1998.4	1733.0	188.5	0.38
Small intestine, cm	3764.7	4356.6	356.0	0.30
Colon, cm	1183.2	1043.8	16.5	0.004
Small intestine, g	4029.8	4293.4	173.2	0.34
Cecum, g	400.0	360.0	20.6	0.24
Colon, g	3440.0	3010.0	213.8	0.23
Total digestive tract <sup>b</sup> , kg	26.7	25.4	0.85	0.33
Lung, g	4470.0	4600.0	467.5	0.85
Heart, g	2620.0	2592.0	75.9	0.81
Pancreas, g	1124.5	1085.7	74.0	0.73
Stomach <sup>a</sup> , kg	16.6	13.2	0.43	<0.001
Kidney, g	1300.0	1194.0	58.3	0.27
Stomach <sup>a</sup> , kg	16.6	13.2	0.43	<0.001
	----- 100 g/kg eviscerated BW -----			
Stomach <sup>a</sup>	4.6	4.3	0.14	0.16
Rumen	2.6	2.5	0.08	0.49
Reticulum	0.37	0.35	0.03	0.69
Omasum	1.2	1.0	0.04	0.05
Abomasum	0.48	0.40	0.05	0.30
Cecum	0.10	0.09	0.01	0.27
Colon	0.82	0.71	0.06	0.26
Intestine	0.97	1.0	0.04	0.43
Total digestive tract <sup>b</sup>	6.4	6.0	0.15	0.27
Lung	1.1	1.1	0.12	0.97
Heart	0.63	0.61	0.02	0.57
Pancreas	0.27	0.26	0.02	0.68
Liver	1.5	1.5	0.05	0.88
Kidney	0.31	0.28	0.02	0.33

<sup>a</sup>Stomach = rumen, reticulum, omasum, abomasum

<sup>b</sup>Total digestive tract = small and large intestine, rumen, reticulum, omasum, abomasum

Table 5. Digestive tracts measurements of d 125 fetuses collected from cows fed to meet NRC requirements (Control) or nutrient restricted (NR) then realimented through d 220 of gestation

Item	Treatments		SE	P
	Control	NR		
	----- Measurements -----			
Stomach <sup>a</sup> , g	224.8	213.3	9.0	0.42
Rumen, g	74.1	70.0	2.1	0.23
Reticulum, g	15.6	17.6	0.65	0.09
Omasum, g	40.7	34.4	1.6	0.05
Abomasum, g	94.3	91.3	5.6	0.72
Small intestine, cm	1146.6	1068.8	57.8	0.40
Large intestine, cm	156.6	148.7	4.7	0.30
Intestine, cm	1303.2	1217.5	61.3	0.38
Intestine, g	438.7	431.4	29.6	0.87
Small intestine, g	349.3	339.0	23.8	0.78
Large intestine, g	89.4	92.4	6.0	0.74
Total digestive tract <sup>b</sup> , g	663.5	644.8	37.8	0.74
Cecum, g	7.1	7.8	0.40	0.26
Fetus, kg	20.0	21.0	0.79	0.52
	----- 100 g/kg eviscerated BW -----			
Stomach <sup>a</sup>	1.1	1.0	0.04	0.20
Rumen	0.37	0.34	0.01	0.06
Reticulum	0.08	0.09	0.003	0.16
Omasum	0.20	0.17	0.01	0.03
Abomasum	0.47	0.44	0.03	0.52
Small intestine	1.8	1.6	0.07	0.33
Large intestine	0.45	0.45	0.02	0.95
Intestine	2.2	2.1	0.10	0.43
Total digestive tract <sup>b</sup>	3.3	3.1	0.13	0.31
Cecum	0.04	0.04	0.002	0.57

<sup>a</sup>Stomach = rumen + reticulum + omasum + abomasum

<sup>b</sup>Total digestive tract = small and large intestine, rumen, reticulum, omasum, abomasum

## EFFECTS OF LOCOWEED ON SERUM CONSTITUENTS AND RUMEN PROFILES OF SHEEP\*

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**ABSTRACT:** Thirteen mixed breed wethers ( $47.5 \pm 9.3$  kg), fitted with ruminal and duodenal cannula, were used in a randomized design experiment to evaluate the effects of locoweed on serum constituents and ruminal fermentation. Locoweed treatments supplied: 1) 0.2 mg, 2) 0.4 mg, and 3) 0.8 mg of swainsonine·kg BW<sup>-1</sup>·d<sup>-1</sup>. Data were collected during 3 periods: pre-locoweed treatment (d -19 to d 0), locoweed treatment (d 1 to d 20), and post-locoweed treatment (d 21 to d 40). Blood and rumen samples were collected throughout each period; nutrient digestion (*in situ*) was assessed during the pre- and locoweed treatment periods. Serum swainsonine was detected in all treatments when locoweed was consumed, and was highest ( $P < 0.05$ ) for the 0.8 mg treatment. Serum alkaline phosphatase activity was elevated ( $P < 0.05$ ) due to locoweed treatment, but immediately returned to pre-locoweed values during the post-locoweed period ( $P = 0.13$ ). Locoweed concentration and duration of exposure increased ( $P < 0.05$ ) serum aspartate aminotransferase (AST). However, serum AST did not return to pre-locoweed values during the post-locoweed treatment period. Concentrations of blood urea N, NEFA, and AA were not affected by treatments ( $P > 0.11$ ). Although not affected when locoweed was fed, propionate was elevated ( $P = 0.05$ ) for the 0.2 mg treatment during the post-treatment period. Ammonia concentrations, ruminal pH, and DM digestion were not different ( $P > 0.13$ ) among treatments. Extent of ruminal DM, OM, and ADF digestion during the treatment period was greatest ( $P = 0.01$ ) in the 0.4 mg treatment. Wethers consuming locoweed exhibited subclinical toxicity. However, effects on nutrient metabolism were dependent upon the amount of locoweed consumed. Further, research is needed to fully determine the effects of swainsonine on nutrient metabolism.

**Key Words:** Sheep, Locoweed, Rumen digestion

### Introduction

Poisonous plants are a major detriment to livestock production worldwide. Several species of locoweed (e.g. those in the *Oxytropis* and *Astragalus* genera) are toxic to

livestock, resulting in production and economic losses to livestock producers (Nielsen et al., 1988). Locoweed intoxication generally occurs in animals having consumed the locoweed plants for 7 to 14 d (Taylor et al., 2000). The intoxication is characterized by slow growth rates, depression, emaciation, dull eyes, and reproductive problems (Broquist, 1986). Swainsonine, an indolizidine alkaloid, has been identified as the primary toxicant in locoweed (Molyneux and James, 1982).

Limited data is available concerning the effects of locoweed consumption under range conditions on nutrient digestion and metabolism by livestock. Altered nutrient digestion and metabolic processes due to locoweed consumption is evident in studies by Stavanja et al. (1992) and Taylor et al. (2000), who reported that serum cholesterol and triglycerides were depressed in rats and sheep consuming locoweed, respectively. In a recent preliminary report by our laboratory, Reed et al. (2003) found minimal effects of consuming locoweed on ruminal VFA, ammonia, and nutrient digestion (i.e., OM, ADF, NDF, CP) when sheep were fed at either 0.2 mg or 1.6 mg of swainsonine·kg BW<sup>-1</sup>·d<sup>-1</sup>. Given the mixed reports concerning the effects of swainsonine on nutrient metabolism, our objective was to further define the effects of feeding a forage-based diet containing different levels of locoweed on serum constituents and ruminal fermentation profiles of sheep.

### Materials and Methods

Experimental protocols were approved by the New Mexico State University Institutional Animal Care and Use Committee. Thirteen mixed breed wethers (BW =  $47.5 \pm 9.3$  kg) fitted with ruminal and duodenal cannula, were used in a randomized design and housed individually in metabolism crates. Data were collected during 3 periods: pre-locoweed treatment (d -19 to d 0), locoweed treatment (d 1 to d 20), and post-locoweed treatment (d 21 to d 40). Diets for all three periods were isocaloric/isonitrogenous and restricted to a DM intake of 1.84% of BW to minimize diet refusal. During the pre-locoweed treatment period, animals were fed a diet of blue grama and alfalfa hay

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(Table 1). During the locoweed treatment period, locoweed replaced alfalfa hay to deliver three levels of swainsonine. These levels of treatment were: 1) 0.2 mg, 2) 0.4 mg, and 3) 0.8 mg of swainsonine·kg BW<sup>-1</sup>·d<sup>-1</sup>. The locoweed (*Oxytropis sericea*) was analyzed using an  $\alpha$ -mannosidase inhibition assay described by Taylor et al. (2000), and contained 614  $\mu$ g swainsonine·g<sup>-1</sup> DM. Alfalfa hay was chopped to 2 to 4 cm in length. Locoweed (collected near Folsom, NM) was chopped to 1 to 1.5 cm in length. Taylor et al. (2000) demonstrated that sheep consuming 0.2 mg swainsonine·kg BW<sup>-1</sup>·d<sup>-1</sup> exhibited few signs of toxicity, whereas sheep consuming 0.8 mg had induced subclinical and clinical intoxication. Thus, the dose range of swainsonine exposure selected for the current study covers a range from limited toxicity to clinical intoxication. A salt solution (30 mL; 18.6% w/v non-iodized salt, 16.4% v/v molasses) was sprayed on the locoweed to improve palatability. Locoweed was offered at 0700 for 15 min and if not eaten, the remainder of locoweed was moistened with water and added directly to the rumen. The rest of the diet and 28 g of a trace mineral salt was offered to the sheep at 0715. Feed refusals were collected daily, weighed, composited for each sheep weekly, and a representative sample saved for nutrient analysis. During the post-locoweed treatment period, sheep were placed back on the pre-locoweed treatment diet and were not exposed to locoweed.

A 10-mL blood sample was collected (Corvac serum separator, Sherwood, St. Louis, MO) via jugular venapuncture from each animal on d -19, -12, 0, 1, 7, 14, 21, and 26. Blood samples were allowed 1 h to clot and then centrifuged at 1,850  $\times$  g for 15 min and stored (-20°C) in polypropylene tubes until analyses were performed. Serum swainsonine was analyzed using an  $\alpha$ -mannosidase inhibition assay (detection limit = 6 ng·mL<sup>-1</sup>, intraassay coefficient of variation = 13.4%; Taylor and Strickland, 2002). Serum alkaline phosphatase (ALK-P) and aspartate aminotransferase (AST) activities were determined using Sigma Diagnostic Kits (#104-LL and #505, respectively; Sigma-Aldrich Corp., St. Louis, MO). Diagnostic kits were modified using similar reagent ratios described for each kit, for use with a 96-well microtiter plate reader (MRX HD, Dynex, Chantilly, VA). Serum concentrations of NEFA were determined using the NEFA C (Wako Chemicals USA, INC., Richmond, VA 23237, Cat No. 994-75409E) modified endpoint protocol for use with a 96-well microtiter plate reader. Serum concentrations of urea N (Thermo DMA, Louisville, CO, 80027, Cat No. TR-12321) were determined using modified procedures for an endpoint assay similar for use with a 96-well microtiter plate reader. Rumen samples were collected from 0 to 48 h in 4 h interval on d -6 and -5; d 15 and 16; and d 39 and 40. Ruminal fluid (100 mL) was strained through four layers of cheesecloth and pH was immediately measured. A 10-mL aliquot was acidified with 0.5 mL of 6 N HCl and frozen for determination of ammonia N using 96 well microtiter plate reader. An additional 8 mL was acidified with 2 mL of 25% metaphosphoric acid and frozen for VFA analysis using gas chromatography (Star 3400, Varian, Walnut Creek, CA).

An *in situ* digestion experiment was conducted to evaluate the effects of swainsonine on the extent of nutrient digestion in the rumen. Duplicate nylon bags (5  $\times$  10 cm; 53  $\mu$ m pore size, Bar Diamond Inc., Parma, ID) containing 5 g of dietary treatments were incubated in the rumen for 3, 6, 9, 12, 24, and 48 h on d -3 and -2, and on d 17 and 18. After incubation in the rumen, bags were rinsed with water until the effluent was clear. Substrate remaining was analyzed for DM (100°C for 24 h), OM (500°C for 8 h), and ADF (Ankom 200 fiber analyzer, Ankom Technology Cooperation, Fairport, NY).

Data were analyzed with the mixed model procedures of SAS for repeated measures (sp [pow]). The model included treatment, day, and treatment  $\times$  day with animal as experimental unit.

## Results and Discussion

Serum swainsonine, ALK-P, and AST were used as indicators of subclinical toxicity (Figure 1). Serum swainsonine was detected in all locoweed treatments during the treatment period and was greater ( $P < 0.01$ ) for the 0.8 mg than both the 0.4 and 0.2 mg (Figure 1, Panel A). Mean serum swainsonine for all treatments were  $80.4 \pm 9.6$  ng·mL<sup>-1</sup>,  $27.4 \pm 9.9$  ng·mL<sup>-1</sup>,  $5.9 \pm 14.2$  ng·mL<sup>-1</sup> for 0.8, 0.4, and 0.2 mg treatments, respectively. Serum swainsonine decreased ( $P = 0.06$ ) as the study progressed for 0.8 and 0.4 mg treatments indicating differences in the rate of absorption, rate of elimination or rate of distribution. No differences ( $P = 0.28$ ) were found among treatments in ALK-P activity over the course of the study (Figure 1, Panel B). Serum ALK-P was increased ( $P < 0.05$ ) for all treatments during the locoweed treatment period vs pre-locoweed treatment and post-locoweed treatment period. Similar results for ALK-P were found when sheep and cattle consumed locoweed (Pulsipher et al., 1994; Taylor et al., 2000, Taylor and Strickland, 2002). Reed et al. (2003) also found that ALK-P activity increased when sheep consumed locoweed for 23 d at 0.2 and 1.6 mg treatments vs control group (no locoweed). Likewise, serum AST activity was increased ( $P < 0.05$ ) in all treatments during the locoweed treatment and post-locoweed treatment vs pre-locoweed treatment (Figure 1, Panel C). Further, unlike serum ALK-P, AST activity did not return to the pre-locoweed treatment values indicating that tissue damage (i.e., cell leakage) could be occurring and may continue to occur for a period after withdrawal from locoweed. Similar results were reported by Taylor et al. (2000) and Whittet et al. (2002).

Serum urea N and NEFA were analyzed as indicators of metabolic changes and nutrient metabolism. Serum NEFA concentrations were not affected ( $P > 0.11$ ) by treatment (Table 2). However, serum urea N was greater for 0.2 mg than 0.4 ( $P = 0.09$ ) and 0.8 mg ( $P = 0.01$ ) on d 1 and lower ( $P = 0.08$ ) for 0.2 mg than 0.4 mg on d 14, which likely does not reflect a treatment effect. Pulsipher et al. (1994) and Taylor et al. (2000) found no effects of locoweed consumption on serum urea N concentrations of sheep. Serum amino acid concentrations were not affected (data not shown;  $P > 0.11$ ) by locoweed consumption.

*In situ* DM, OM, and ADF digestion are presented in Table 3. No differences ( $P > 0.13$ ) were found for DM, OM, and ADF digestion during pre-locoweed treatment. However, during locoweed treatment, extent of ruminal DM, OM, and ADF digestion was greatest ( $P = 0.01$ ) in the 0.4 mg treatment compared to 0.2 mg and 0.8 mg treatment group. Reed et al. (2003) fed wethers with 0.0, 0.2, and 1.6 mg swainsonine/kg BW<sup>-1</sup>·d<sup>-1</sup> and found that *in situ* DM digestion was higher in 1.6 mg than 0.0 and 0.2 mg treatment with no differences in OM and ADF digestion. Authors speculated that the difference in the DM digestion is due to the diets, not to the swainsonine. However, in our study, it could be speculated that including locoweed at the level of 0.4 mg enhanced the nutrients degradation in the rumen.

Mean ruminal pH, ammonia, and VFA concentrations for 48 h collections are presented in Table 4. Ruminal pH and ammonia concentrations were not affected by treatments during the treatment period. These results agree with those of Reed et al. (2003) who found no differences among the control group (no swainsonine) and treatment groups at either 0.2 or 1.6 mg of swainsonine/kg BW<sup>-1</sup>·d<sup>-1</sup>. However, ruminal pH was lower ( $P = 0.05$ ) for 0.2 mg than 0.4 mg during the pre-locoweed treatment. Also, ruminal VFA were not different ( $P > 0.15$ ) among treatments, except for isovalerate and propionate. During the treatment period, isovalerate was greater ( $P = 0.06$ ) for the 0.8 mg than 0.2 and 0.4 mg treatment groups. Propionate concentrations were greater ( $P = 0.05$ ) for 0.2 mg than 0.4 mg during the post-treatment period.

In conclusion, serum swainsonine, ALK-P, and AST data indicate that sheep were subclinically intoxicated by locoweed consumption. However, serum urea N, NEFA, and amino acid concentrations appeared to be unaffected by adding locoweed to the diets. In addition to blood metabolite, a lack of consistent changes in ruminal digestion profiles indicates little effect of subclinical swainsonine intoxication on nutrient digestion in sheep.

### Implications

In locoweed infested rangelands, science based strategic grazing is required to minimize livestock production losses while optimizing nutrient intake. Current management strategies to minimize locoweed intoxication include: avoidance of locoweed infested areas, and removal of animals after visual observation of intoxication. Results of the current study indicate these types of management strategies may be safe if animals are not clinically intoxicated. However, additional research fully defining the effects of swainsonine on not only gross parameters of nutrient digestion and metabolism but also on nutrient transport and utilization is needed before a high degree of confidence can be obtained in recommending locoweed consumption at any level.

### Literature Cited

- Broquist, H. P. 1986. Livestock toxicosis, slobbers, locoism, and the indolizidine alkaloids. *Nutrition Reviews*. 44: 317-323.
- Molyneux, R. J. and L. F. James. 1982. Loco intoxication: Indolizidine alkaloids of spotted locoweed (*Astragalus lentiginosus*). *Science*. 216(9): 190-191.
- Nielsen, D. B., N. R. Rimbey, and L. F. James. 1988. Economic considerations of poisonous plants on livestock. In: *The ecology and economic impact of poisonous plants on livestock production*. (Eds.) James, L.F., M. H. Ralphs and D. B. Nielsen. Boulder: Westview Press, Inc. pp. 5-15.
- Pulsipher, G. D., M. L. Galyean, D. M. Hallford, G. S. Smith and D. E. Kiehl. 1994. Effects of graded levels of bentonite on serum clinical profiles, metabolic hormones and serum swainsonine concentrations in lambs fed locoweed (*Oxytropis sericea*). *J. Anim. Sci.* 72:1561-1569.
- Reed, M. M., B. S. Obeidat, J. R. Strickland, C. R. Krehbiel, J. B. Taylor, L. Chen, C. A. Loest, G. S. Bell, W. D. Bryan, J. D. Rivera, and J. L. Jim. 2003. Effects of swainsonine on digestion in wethers consuming locoweed. In *Proc: West Sec. Amer. Soc. Anim. Sci.* 54:108-112.
- Stavanja, M., G. S. Smith, T. Edrington, W. Velastegui, and G. M. Sothward. 1992. Organ weights and serum components of rats fed locoweed stems leaves or seedpods. In *Proc: West Sec. Amer. Soc. Anim. Sci.* 43:380-382.
- Taylor, J. B. and J. R. Strickland. 2002. Appearance and disappearance of swainsonine in serum and milk of lactating ruminants with nursing young following a single dose exposure to swainsonine (locoweed; *Oxytropis sericea*). *J. Anim. Sci.* 80:2476-2484.
- Taylor, J. B. J. R. Strickland, T. May, and D. E. Hawkins. 2000. Effect of subacute swainsonine (locoweed; *Oxytropis sericea*) consumption on immuno-competence and serum constituents of sheep in a nutrient-restricted state. *Vet. Human Toxicol.* 42:199-204.
- Whittet, K. M., H. B. Encinias, J. R. Strickland, J. B. Taylor, J. D. Graham, A. K. Clayshulte, and A. M. Encinias. 2002. Effect if ionophore supplementation of selected serum constituents of sheep consuming locoweed. *Vet. Human Toxicol.* 44(3):136-140.

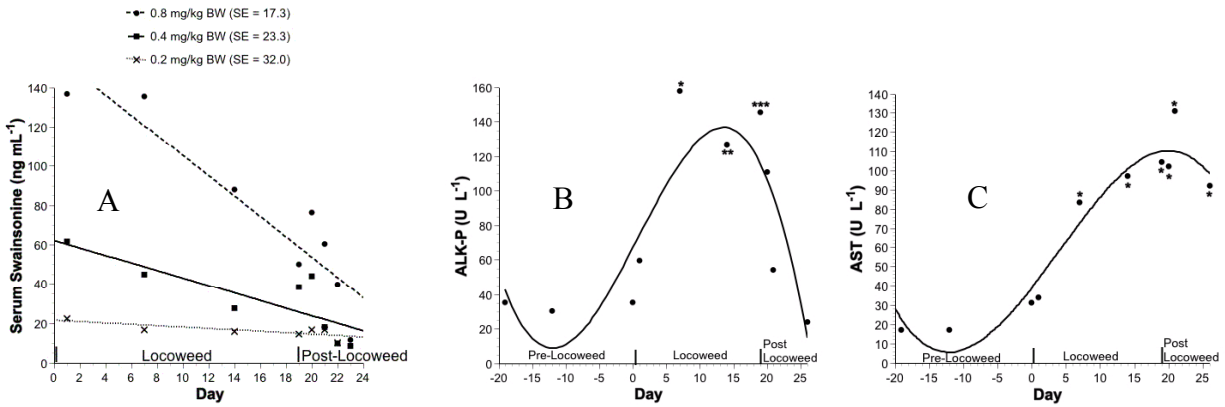


Figure 1: Effects of locoweed consumption on serum swainsonine (Panel A), alkaline phosphatase (ALK-P; Panel B), and aspartate aminotransferase (AST; Panel C) of sheep. Treatments were 0.2, 0.4, and 0.8 mg swainsonine·kg BW<sup>-1</sup>·d<sup>-1</sup> delivered by locoweed. Data were collected during 3 periods: pre-locoweed treatment (d -19 to 0), locoweed treatment (d 1 to 20), and post-locoweed treatment (d 21 to 40). Panel B: \* greater (P < 0.05) than d -19, -12, and 0. Panel C: \* greater (P < 0.05) than d -19, -12, 0, 1, 21, and 26; \*\* greater (P < 0.10) than d -19, -12, 0, and 26; \*\*\* greater d -19, -12, 0, 1, 21, and 26.

Table 1. Ingredient and nutrient compositions of diets fed to sheep.

Item	Pre-locoweed	Locoweed Treatment <sup>a</sup>		
	0.0 mg	0.2 mg	0.4 mg	0.8 mg
Ingredient				
Alfalfa, % DM	2.8	2.1	1.4	0.0
Blue grama, % DM	97.2	96.1	95.0	92.9
Locoweed, % DM	0.0	1.8	3.6	7.1
Nutrient				
Swainsonine, mg/kg BW	0.0	0.2	0.4	0.8
DM Intake, % BW	1.84	1.84	1.84	1.84
CP, % DM	11.1	11.1	11.1	11.1
TDN, % DM	59.1	59.1	59.1	59.1

<sup>a</sup>Treatments were 0.2, 0.4, and 0.8 mg swainsonine·kg BW<sup>-1</sup>·d<sup>-1</sup> delivered by locoweed.

Table 2. Effects of feeding locoweed on serum blood urea N and NEFA in sheep fed a forage-based diet.

Serum Constituent	Locoweed Treatments <sup>a</sup>				SE	P-Value
	Day	0.2 mg	0.4 mg	0.8 mg		
Blood urea N, mg/dL	0 <sup>b</sup>	18.88	18.23	15.53	2.3	> 0.14
	1	22.32 <sup>a</sup>	17.30 <sup>b</sup>	15.00 <sup>b</sup>	2.7	= 0.09
	7	18.00	16.38	15.13	2.7	> 0.33
	14	9.42 <sup>a</sup>	14.60 <sup>b</sup>	12.88 <sup>ab</sup>	2.7	= 0.08
	21	8.37	12.35	10.41	2.7	> 0.17
	26	16.50	16.89	16.24	2.8	> 0.80
NEFA, µEq/L	0 <sup>b</sup>	484.6	365.3	426.0	107.5	> 0.26
	1	524.2	406.3	379.9	125.9	> 0.28
	7	335.9	394.3	360.3	133.2	> 0.66
	14	327.2	338.3	401.8	125.9	> 0.57
	21	626.0	594.6	573.4	133.2	> 0.69
	26	668.8	758.8	647.7	125.9	> 0.32

<sup>a</sup>Locoweed treatments were 0.2, 0.4, and 0.8 mg swainsonine·kg BW<sup>-1</sup>·d<sup>-1</sup> delivered by locoweed (*Oxytropis sericea*, 614 µg swainsonine/g DM)

<sup>b</sup>d 0 is the average of pre-locoweed treatment (i.e., -19, -12, and 0)

Table 3. Effects of feeding locoweed on extent of ruminal digestion for *in situ* dry matter (DM), organic matter (OM), and acid detergent fiber (ADF) in sheep fed a forage-based diet.

Item	Locoweed Treatments <sup>a</sup>			SE	P-Value
	0.2 mg	0.4 mg	0.8 mg		
Pre-locoweed treatment <sup>b</sup>					
DM, % degraded	41.47	43.91	44.77	2.86	> 0.25
OM, % degraded	37.97	41.39	42.79	3.17	> 0.13
ADF, % degraded	40.50	42.67	44.41	3.41	> 0.26
Locoweed treatment <sup>b</sup>					
DM, % degraded	31.74 <sup>a</sup>	38.47 <sup>b</sup>	30.53 <sup>a</sup>	2.76	< 0.05
OM, % degraded	28.52 <sup>a</sup>	36.75 <sup>b</sup>	27.69 <sup>a</sup>	3.11	< 0.05
ADF, % degraded	28.45 <sup>a</sup>	35.14 <sup>b</sup>	25.22 <sup>a</sup>	2.86	< 0.05

<sup>a</sup>Locoweed treatments were 0.2, 0.4, and 0.8 mg swainsonine·kg BW<sup>-1</sup>·d<sup>-1</sup> delivered by locoweed (*Oxytropis sericea*, 614 µg swainsonine g DM)

<sup>b</sup>Data were collected during 3 periods: pre-locoweed treatment (d -19 to 0), locoweed treatment (d 1 to 20), and post-locoweed treatment (d 21 to 40)

Table 4. Effects of feeding locoweed on ruminal pH, ammonia, and VFA concentrations in sheep fed a forage-based diet.

Item	Locoweed Treatments <sup>a</sup>			SE	P-Value
	0.2 mg	0.4 mg	0.8 mg		
<b>Pre-locoweed treatment<sup>b</sup></b>					
Ruminal ammonia, mM	6.29	5.26	6.30	0.57	> 0.22
Ruminal pH	5.91 <sup>a</sup>	6.20 <sup>b</sup>	6.06 <sup>ab</sup>	0.14	= 0.05
Acetate, mM	71.20	66.53	74.98	6.70	> 0.25
Propionate, mM	16.75	15.54	17.85	1.76	> 0.23
Butyrate, mM	7.59	7.29	8.20	0.92	> 0.51
Isobutyrate, mM	0.70 <sup>a</sup>	0.54 <sup>b</sup>	0.70 <sup>a</sup>	0.08	= 0.07
Valerate, mM	0.87	0.72	0.83	0.14	> 0.29
Isvalerate, mM	0.97	0.69	0.99	0.12	> 0.11
Total VFAs, mM	98.08	91.29	103.56	6.93	> 0.26
<b>Treatment period<sup>b</sup></b>					
Ruminal ammonia, mM	5.01	4.09	4.99	0.67	> 0.32
Ruminal pH	5.79	5.82	5.73	0.11	> 0.38
Acetate, mM	69.41	67.74	70.28	3.32	> 0.56
Propionate, mM	18.20	16.25	18.06	1.26	> 0.28
Butyrate, mM	6.68	6.66	7.24	0.66	> 0.50
Isobutyrate, mM	0.46	0.55	0.58	0.06	> 0.22
Valerate, mM	0.76	0.89	0.89	0.05	> 0.15
Isvalerate, mM	0.60 <sup>a</sup>	0.65 <sup>a</sup>	0.83 <sup>b</sup>	0.07	= 0.07
Total VFAs, mM	96.10	92.73	97.89	4.93	> 0.43
<b>Post-locoweed treatment<sup>b</sup></b>					
Ruminal ammonia, mM	3.90	4.30	4.87	0.78	> 0.46
Ruminal pH	6.15	5.93	5.98	1.13	> 0.18
Acetate, mM	65.80	68.33	70.69	2.66	> 0.25
Propionate, mM	18.82 <sup>a</sup>	17.34 <sup>b</sup>	17.91 <sup>ab</sup>	0.40	= 0.05
Butyrate, mM	5.64	6.97	6.74	0.64	> 0.24
Isobutyrate, mM	0.18	0.29	0.28	0.05	> 0.17
Valerate, mM	0.69	0.84	0.80	0.08	> 0.29
Isvalerate, mM	0.42	0.57	0.58	0.13	> 0.43
Total VFAs, mM	91.54	94.31	96.97	3.60	> 0.34

<sup>a</sup>Locoweed Treatments were 0.2, 0.4, and 0.8 mg swainsonine·kg BW<sup>-1</sup>·d<sup>-1</sup> delivered by locoweed (*Oxytropis sericea*, 614 µg swainsonine g<sup>-1</sup> DM)

<sup>b</sup>Data were collected during 3 periods: pre-locoweed treatment (d -19 to 0), locoweed treatment (d 1 to 20), and post-locoweed treatment (d 21 to 40)

# INFLUENCE OF LEVELS OF FAT SUPPLEMENTATION ON BILE FLOW AND FATTY ACID DIGESTION IN CATTLE

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**ABSTRACT:** Three Jersey x Holstein steers (215 ± 4.8 kg) with cannulas in the rumen and proximal duodenum were used in a 3 x 3 Latin squares design experiment to evaluate the influence of level of fat supplementation (0, 4, and, 8% yellow grease) on characteristics of duodenal chyme, bile production, and digestion of fatty acids. Steers were fed an 88% concentrate, steam-flaked corn-based finishing diet. Dry matter intake was restricted to 2.15% of BW. A total of eighteen gall bladders obtained at a local abattoir were utilized to determine total lipid content of bile juice as a reference for calculating bile flow. Characteristics of bile were: total solids =  $8.32\% \pm 0.66$ ; pH =  $7.48 \pm 0.29$ ; density, g/mL =  $1.012 \pm 0.02$ ; total lipids, mg/dL =  $1468 \pm 82$ . Increasing level of fat supplementation decreased (linear effect,  $P < 0.07$ ) postprandial fatty acid digestion. Decreases in postprandial fatty acid digestion were due primarily to decreased digestion of saturated fatty acids (C16:0 and C18:0). The estimated NE<sub>m</sub> (Mcal/kg) of yellow grease averaged 5.87 and 5.46 for the 4, and 8% of level supplementation, respectively. There were no treatment effects ( $P > 0.20$ ) on pH and density of duodenal chyme. However, pH was lower (2.34 vs 3.81,  $P < 0.01$ ), and density was greater (1.3%  $P < 0.01$ ) for chyme at the proximal duodenum than at the distal duodenum. Bile production (averaging  $31.9 \pm 0.35$  mL/kg BW) increased (linear effect,  $P < 0.07$ ) with increasing lipid flow to the small intestine. However, as a proportion of lipid flow (mL bile/g lipid entering the small intestine), bile production decreased (linear effect,  $P < 0.01$ ) from 23.4 to 16.7. Bile production explained 69% of variation in intestinal fatty acid digestion.

**Key words:** Fat level, Fatty acid, Cattle, Bile flow, Digestion.

## Introduction

Current standards (NRC, 1996) for the NE<sub>m</sub> and NE<sub>g</sub> values of supplemental fats are 6.00 and 4.50 Mcal/kg. Estimates based on these values are consistent with empirically derived measures when total fatty acid (FA) intake did not exceed 0.86 g/kg of BW (Zinn, 1994). When FA intake has exceeded 0.86 g/kg of BW, the NE value of fat declined (Zinn and Plascencia, 2003). This decline has been largely attributable to decreased postprandial fatty acid digestion (Zinn, 1994) mainly stearic and palmitic acids (Enjalbert et al., 2000; Plascencia et al., 2003). Bouchart (1993) suggested that decreased fatty acid digestion might be attributable to insufficient bile flow at high levels of dietary fatty acid intake.

Early reports (Harrison and Hill, 1960; Harrison, 1962) indicated bile flows in sheep of from 0.6 to 1.45 mL/h/kg of BW. The basis for the wide discrepancy is not certain. It may be related to limitations of bile duct cannulation (Merchen, 1988). However, bile secretion has been shown to increase with increasing OM flow to the small intestine (Harrison, 1962). The objective of present study was to directly evaluate the influence of level of fat supplementation on bile flow and fatty acid digestion in cattle fed a finishing diet.

## Materials and Methods

Three Jersey x Holstein steers (215 ± 4.8 kg) with cannulas in proximal (6 cm of pyloric sphincter) and distal duodenum (10 cm forward of bile duct) were used in a 3 x 3 Latin square design to evaluate the influence of level of fat supplementation (0, 4 and 8%) on characteristics of bile flow and fat utilization in cattle fed finishing diets. Composition of experimental diets is shown in Table 1. Supplemental fat added to the mixer as the next to the last step in diet preparation, prior to adding molasses. Gall bladders were removed from eighteen feedlot steers at time of slaughter. Bile contents from each bladder were placed in individual plastic jars (500 mL). Jars were packed in ice, and promptly transported the laboratory for immediate determination of DM, pH, density, and total lipids.

Steers were maintained in concrete slotted-floor pens (3.9 m<sup>2</sup>) with access to water at all times. Dry matter intake was restricted to 2.15% of BW ( $4.6 \pm 0.056$  kg/d). Diets were fed in equal portions at 0800 and 2000 daily. Experimental periods consisted of a 10-d diet adjustment period followed by a 4-d collection period. During the collection period duodenal and fecal samples were taken from all steers, twice daily as follows: d 1, 0750 and 1350 h; d 2, 0900 and 1500 h; d 3, 1050 and 1650 h; and d 4, 1200 and 1800 h. Individual samples consisted of approximately 1,500 mL of duodenal chyme (750 mL from proximal and 750 mL from distal duodenal) and 200 g (wet basis) fecal material. Samples from each steer and within each collection period were composited for analysis. Feed and digesta samples were subjected to all or part of the following analysis: DM (oven drying at 105 °C until no further weight loss); ash (AOAC, 1986); fatty acids (Sukhija and Palmquist, 1988); lipid (acidified chloroform-methanol extraction; Zinn, 1994); pH (Orion, model 2345); density (w/v); and chromic oxide (Hill and Anderson, 1958). Lipid content and physical characteristics of bile used as a reference

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to calculate bile flow were determined as follows: density (w/v); total solids (oven drying at 65°C until no further weight loss); pH (Orion, model 2345); and total lipid (colorimetric method; TECO diagnostics, Anaheim, CA). Bile flow was calculated as the difference in lipid content in duodenal chyme divided by lipid concentration in bile reference:

$BF, L/d = (\text{lipid in distal duodenum} - \text{lipid in proximal duodenum}) / \text{lipid concentration in bile (reference), g/L}$ , where BL = bile flow (L/d)

Data for bile flow and digestion of lipid and fatty acids were analyzed using a 3 x 3 Latin square design (Hicks, 1973). Treatment effects were tested by means of orthogonal polynomials. Treatment effects on characteristics of duodenal chyme were assessed using a 3 x 3 Latin square design with repeated measures as outlined by Hicks (1973).

### Results and Discussion

The characteristics bile were as follows: total solids =  $8.32\% \pm 0.66$ ; pH =  $7.48 \pm 0.29$ ; density, g/mL =  $1.012 \pm 0.02$  and total lipids, mg/dL =  $1468 \pm 82$ . These results are close in agreement with those reported by others (Ruckebusch et al., 1991; Moore and Christie, 1984).

There were no treatment effects ( $P > .20$ ) on pH and density of proximal and distal duodenal chyme. However, pH was lower ( $2.34$  vs  $3.81$ ,  $P < 0.01$ ), and density was greater ( $1.3\%$   $P < 0.01$ ) for proximal than for distal duodenal chyme. Greater pH at the distal segment is expected due to bile and pancreatic secretions (Moore and Christie, 1984).

Treatment effects on intestinal fatty acid digestion are shown in Table 2. Postruminal total fatty acid digestion decreased (linear component,  $P < 0.10$ ) with increasing level of fat supplementation, averaging 80.2, 78.6, and 72.63% for the 0, 4, and 8% level of fat supplementation, respectively. Plascencia et al. (2003) observed a close relationship ( $R^2 = 0.89\%$ ) between the total fatty acid intake (FAI, g/kg BW) and postruminal fatty acid digestion: fatty acid digestion (%) =  $87.560 - 8.591\text{FAI}$ ;  $R^2 = 0.89$  ( $P < 0.01$ ). Accordingly, observed postruminal fatty acid digestion was 1.00 and 0.99 for the 4 and 8% fat level, respectively of expected values.

Applying digestibility values, the  $NE_g$  values for the yellow grease used in this study are 4.74, and 4.38 Mcal/kg for the 4, and 8% of level supplementation, respectively. Corresponding  $NE_m$  values are 5.87, and 5.46 Mcal/kg, respectively [where  $NE_m = (NE_g + 0.41)/0.877$ ; derived from NRC, 1984]. Thus, NE values obtained for yellow grease supplemented at the rate of 4, and 8% of dietary DM (fatty acid intake = 1.07, and 1.72 g/kg BW) were 98, and 91% of

the tabular value (NRC, 1996).

Consistent with previous studies (Enjalbert et al., 2000; Avila et al., 2000), intestinal digestion of unsaturated fatty acids was high, and not affected ( $P > 0.10$ ) by level of fat intake, averaging 85 and 87%. Decreased intestinal fatty acid digestion is explained by changes in digestion of C18:0.

Treatment effects on bile productions is shown in Table 3. Across the treatments, bile secretion per unit BW averaged 32 mL/kg, in close agreement with Studinsky and Bobowiec (1979; 32.4 mL/kg), and Harrison (1962; 34.8 mL/kg), but slightly higher than values reported by Bobowiec and Kosior-Korzecka (1999; 26.8 mL/kg).

Bile flow increased (linear effect,  $P < .10$ ) with increasing fat intake. However, the magnitude of the increase was comparatively small. Thus, bile flow as a proportion of fat entering the small intestine decreased markedly (linear effect,  $P < .01$ ) with increasing fat intake (60.7, 34.2, and 24.5% for 0, 4 and 8% fat level, respectively).

Numerous studies have demonstrated decreasing fat digestion with increasing levels of fat intake (Palmquist, 1991; Zinn, 1994; Plascencia et al., 2003). This limitation has been associated with the nature of the fat (fatty acid association with feed particles and degree of saturation; Wu et al., 1991; Pantoja et al., 1996). The basis for this experiment was to better understand the relationship between fat intake and bile production. Unsaturated fatty acids are physically much more bulky and less viscous than saturated fatty acids. Because lipids are highly insoluble in water, their absorption from the small intestine is dependent on emulsification and the formation of micelles mediated by bile acids. Børsting et al. (1992) observed that when cows were fed emulsified vegetable fat protected by means of formaldehyde-casein, the digestion of C18:0 was 92%. In the present study, bile production (expressed as mL of bile/g of lipid entering the small intestine) explained 69% of variation in intestinal fatty acid digestion (Figure 1).

### Implications

Depressions in fatty acid digestion with increasing level of intake are due primarily to decreasing intestinal absorption of palmitic and stearic acids. Bile production relative to the quantity of saturated fatty acids present in the duodenum is largely responsible for this reduction. Research to define physiological factors limiting digestibility of saturated FA has potential to increase the value of supplemental fat.

ruminants. J. Dairy Sci. 76:3864-3881.

### Literature Cited

- AOAC. 1986. Official methods of analysis. 14th ed. Association of Official Analytical Chemists, Arlington, VA.
- Avila, C.D., E.J. DePeters, H. Perez-Monti, S.J. Taylor, and R.A. Zinn. 2000. Influences of saturation ratio of supplemental dietary fat on digestion and milk yield in dairy cows. J. Dairy Sci. 83:1505-1519.
- Bauchart, D. 1993. Lipid absorption and transport in

- Bobowiec, R., and U. Kosior-Korzecka. 1999. Relationships amongst liver bile salt clearance, bile secretion and infusion of lipids in calves. J. Vet. Med. 46: 409-420.
- Børsting, C. F., and M. R. Weisbjerg. 1989. Fatty acid metabolism in the digestive tract of ruminants. PhD Thesis. The Royal Veterinary and Agricultural University, Copenhagen.
- Børsting, C.F., M.R. Weisbjerg, and T.H. Hvelplund. 1992. Fatty acid digestibility in lactating cows fed

- increasing amounts of protected vegetable oil, fish oil or saturated fat. *Acta. Agric. Scand., Sect. A. Animal Sci.* 42:148-156.
- Christiansen, M.L., and K.E. Webb, Jr. 1990. Intestinal acid flow, dry matter, starch and protein digestibility and amino acid absorption in beef cattle fed a high-concentrate diet with defluorinated rock phosphate, limestone or magnesium oxide. *J. Anim. Sci.* 68:2105-2118.
- Elliot, J. P., J. K. Drackley, A.D. Beaulieu, G.C. Aldrich, and N.R. Merchen. 1999. Effect of saturation and esterification of fat sources on site and extent of digestion in steers: Digestion of fatty acids, triglycerides, and energy. *J. Anim. Sci.* 77:1919-1929.
- Enjalbert, F., M.C. Nicot, C. Bayourthe, and R. Moncoulon. 2000. Effect of duodenal infusion of palmitic, stearic or oleic acids on milk composition and physical properties of butter. *J. Dairy Sci.* 83:1428-1433.
- Harrison, F.A. 1962. Bile secretion in the sheep. *J. Physiol.* 162:212-224.
- Harrison, F.A., and K.L. Hill. 1960. Bile secretion in conscious sheep. *J. Physiol.* 154:610-620.
- Hicks, C. R. 1973. Fundamental concepts in the design of experiments. Holt, Rinehart and Winston, New York.
- Hill, F. N., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive determinations with growing chicks. *J. Nutr.* 64:587-603.
- Merchen, N.R. 1988. Digestion, absorption and excretion in ruminants. Page 174 in *The ruminant animal: Digestive Physiology and Nutrition*. D.C. Church, ed. Prentice-Hall, New Jersey.
- Moore, J. H., and W.W. Christie. 1984. Digestion, absorption and transport of animal fats in ruminant animal. Page 123 in *Fats in Animal Nutrition*. J. Wiseman, ed. Butterworths, London, Engl.
- NRC. 1996. *Nutrient Requirements of Beef Cattle*. 7<sup>th</sup> ed. National Academy of Sciences Press. Washington, DC.
- Palmquist, D. L. 1991. Influence of source and amount of dietary fat on digestibility in lactating cows. *J. Dairy Sci.* 74:1354-1360.
- Pantoja, J., J. L. Firkins, and M. L. Eastridge. 1996. Fatty acid digestibility and lactation performance by dairy cow fed fats varying in degree of saturation. *J. Dairy Sci.* 79:429-437.
- Plascencia, A., G.D. Mendoza, C. Vazquez, and R. A. Zinn. 2003. Relationship between body weight and level of fat supplementation on fatty acid digestion in feedlot cattle. *J. Anim. Sci.* 81:2653-2659.
- Ruckebusch, Y., L.P. Phaneuf, and R. Dunlop. 1991. *Physiology of small and large animals*. Becker B.C., Inc. Philadelphia, PA.
- Studzinski T., and Bobowiec R. 1979. Effect of intra-duodenal administration of HCl and NaHCO<sub>3</sub> on the secretion and content of sodium, potassium and calcium in the pancreatic juice and bile of sheep. *Pol. Arch. Weter.* 1979:22:205-217.
- Sukhija, P., and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuff and feces. *J. Agric. Food Chem.* 36:1202-1206.
- Wu, Z., O. A. Ohajuruka, and D. L. Palmquist. 1991. Ruminal synthesis, biohydrogenation, and digestibility of fatty acids by dairy cows. *J. Dairy Sci.* 74:3025-3034.
- Zinn, R. A. 1989b. Influence of level and source of dietary fat on its comparative feeding value in finishing diets for steers: Metabolism. *J. Anim. Sci.* 67:1038-1049.
- Zinn, R. A. 1994. Effects of excessive supplemental fat on feedlot cattle growth performance and digestive function. *Prof. Anim. Sci.* 10:66-72.
- Zinn, R. A., and A. Plascencia. 2003. Influence of level and method of supplementation on the utilization of supplemental tallow fatty acids by feedlot steers. *J. Anim. Vet. Adv.* (Accepted).

Table 1. Ingredient and nutrient composition of experimental diets

Item	Fatty acids intake, g/kg BW		
	0.51	1.07	1.72
Ingredient, g/kg (DM basis)			
Alfalfa hay	60	60	60
Sorghum sudan hay	60	60	60
Steam-flaked corn	781	741	705
Yellow grease	---	40	80
Urea	7	8	9
Molasses	63	62	61
Limestone	13	13	13
Magnesium oxide	2	2	2
Trace mineral salt <sup>a</sup>	4	4	4
Chromic oxide <sup>b</sup>	4	4	4
Nutrient composition (DM basis)			
NE, Mcal/kg <sup>c</sup>			
Maintenance	2.13	2.27	2.41
Gain	1.47	1.59	1.72
Crude protein, %	11.6	11.50	11.41
Ether extract, %	3.6	7.4	11.2
Calcium, %	0.65	0.67	0.68
Phosphorus, %	0.30	0.29	0.29

<sup>a</sup>Trace mineral salt contained: CoSO<sub>4</sub>, 0.068%; CuSO<sub>4</sub>, 1.04%; FeSO<sub>4</sub>, 3.57%; ZnO, 1.24%; MnSO<sub>4</sub>, 1.07%; KI, 0.052%; and NaCl, 92.96%.

<sup>b</sup>Chromic oxide was added as a digesta marker.

<sup>c</sup>Based on tabular values for individual feed ingredients (NRC, 1996).

Table 2. Main effects of level of fat supplementation on fatty acid digestion in Holstein steers

Item	Supplemental yellow grease, %			SEM
	0	4	8	
Steers	3	3	3	
Intake, g/d	4586	4616	4613	
Fatty acid intake, g/d				
C16:0	25.3	88.5	104.5	
C18:0	4.2	46.2	56.7	
C18:1	36.7	164.9	240.3	
C18:2	54.6	54.2	39.4	
Total	109.6	228.9	369	
Fatty acid intake, g/kg BW	0.51	1.07	1.72	
Flow to duodenum, g/d				
C16:0 <sup>a</sup>	19.45	45.0	75.32	4.7
C18:0 <sup>a</sup>	52.8	117.6	206.5	9.7
C18:1 <sup>b</sup>	24.7	63.0	129.9	17.4
C18:2 <sup>a</sup>	12.2	15.3	18.3	0.4
Total fatty acid <sup>a</sup>	112.8	245.4	394.0	9.4
Fecal excretion, g/d				
C16:0 <sup>c</sup>	3.96	8.56	20.57	2.3
C18:0 <sup>c</sup>	10.5	31.0	67.8	0.6
C18:1 <sup>b</sup>	2.9	9.3	23.1	3.6
C18:2	1.2	1.7	3.0	0.4
Total fatty acid <sup>a</sup>	22.4	58.4	115.9	3.6
Postruminal digestion, %				
C16:0	79.5	79.9	72.6	4.3
C18:0	79.2	72.2	67.7	4.7
C18:1	87.4	88.8	86.5	2.0
C18:2	90.2	88.5	81.9	1.9
Total fatty acid <sup>b</sup>	82.7	78.6	72.6	1.9

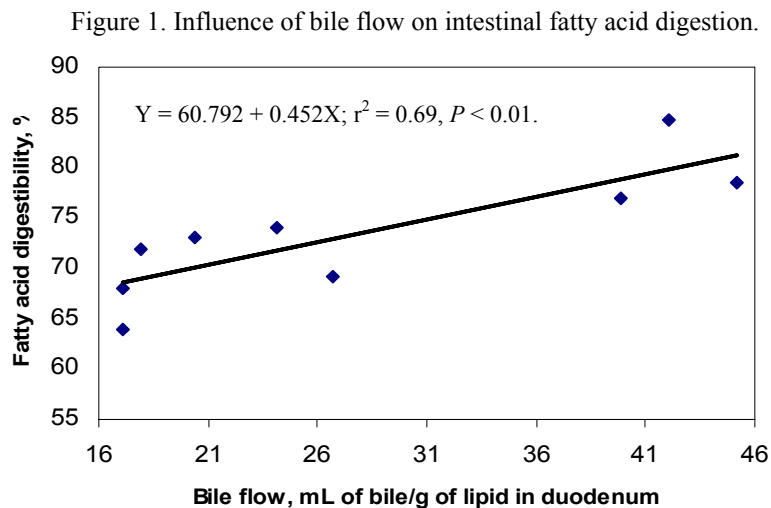
<sup>a</sup> Fat supplementation linear effect,  $P < 0.01$ .<sup>b</sup> Fat supplementation linear effect linear effect,  $P < 0.10$ .<sup>c</sup> Fat supplementation linear effect linear effect,  $P < 0.05$ .

Table 3. Calculated bile flow in steers (215 kg) fed diets with 3 level of supplemental yellow grease

Item	Supplemental yellow grease, %			SEM
	0	4	8	
Steers	3	3	3	
Lipids, g/d				
Intake	128.4	253.8	405.9	
Flow to proximal duodenum <sup>a</sup>	156.6	279.4	434.7	3.9
Flow to distal duodenum <sup>a</sup>	251.7	375.0	541.2	3.3
Difference	95.1	95.6	106.5	
Bile flow				
mL/d <sup>b</sup>	6483	6510	7254	149
mL/[kg BW] <sup>b</sup>	30.4	31.4	34.2	0.8
mL/[kg BW h] <sup>b</sup>	1.26	1.29	1.42	0.03
mL bile/lipid flow to duodenum <sup>a</sup>	41.2	23.4	16.7	1.07

<sup>a</sup> Fat supplementation linear effect,  $P < 0.01$ .

<sup>b</sup> Fat supplementation linear effect linear effect,  $P < 0.10$ .



# METHIONINE, AND AT LEAST ONE BRANCHED-CHAIN AMINO ACID, ARE LIMITING IN LAMBS

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**ABSTRACT:** Twelve ruminally cannulated Rambouillet wether lambs ( $36 \pm 4$  kg BW) were used in two  $6 \times 6$  Latin square experiments to determine if essential amino acids (AA) limit N retention. Lambs were limit-fed (0.63 kg DM/d) twice daily a diet (80% soybean hulls, 15% alfalfa hay, 3.5% molasses, 0.35% urea, 1.5% minerals/vitamins) low in rumen undegradable protein. Lambs received continuous infusions of acetate and propionate into the rumen to supply additional energy. Treatments for Exp. 1 were abomasal infusions of a solution (500 mL/d) containing 1) a mixture of 10 essential AA, 2 nonessential AA, and glucose (12AA), 2) 12AA with Met removed, 3) 12AA with Lys removed, 4) 12AA with His removed, 5) 12AA with Thr removed, and 6) 12AA with all AA removed. Treatments for Exp. 2 were also abomasal infusions of 1) 12AA, 2) 12AA with Arg removed, 3) 12AA with Phe removed, 4) 12AA with Trp removed, 5) 12AA with Leu, Ile, and Val removed, and 6) 12AA with all AA removed. Periods were 7 d with 3 d for adaptation to treatments, and 4 d for collections. For Exp. 1, N retention decreased ( $P < 0.05$ ) in response to removal of all AA, demonstrating that at least one AA was limiting. Also, N retention decreased ( $P < 0.05$ ) in response to Met removal, and tended ( $P = 0.09$ ) to decrease in response to Thr removal, but was not affected by removal of Lys and His. For Exp. 2, N retention decreased ( $P < 0.05$ ) when all AA were removed. Retained N also decreased ( $P < 0.05$ ) in response to removal of branched-chain AA, and tended to decrease in response to removal of Arg ( $P = 0.07$ ) and Trp ( $P = 0.05$ ). However, removal of Phe did not affect N retention. The results of this research demonstrates that Met, and at least one of the branched-chain AA, limit N retention of lambs when fed a diet low in ruminally undegradable protein.

Key Words: Amino acids, N retention, Lambs

## Introduction

For efficient use of metabolizable protein by growing lambs, all essential amino acids (AA) must be supplied in adequate amounts, because the under supply of a single essential AA may limit growth. When dietary CP is predominantly degraded by ruminal microorganisms, microbial protein is the major source of AA available for absorption by ruminants (Merchen and Titgemeyer, 1992). Storm and Ørskov (1984) reported that microbial protein of sheep maintained by intragastric nutrition was limiting in

Met, Lys, His and Arg. However, because of gastrointestinal tissue atrophy in sheep maintained by intragastric nutrition (Ørskov et al., 1979), their AA requirements may be somewhat different to those consuming diets.

Our objective was to determine if essential AA limit N retention of lambs when fed a diet low in ruminally undegradable protein.

## Materials and Methods

Procedures for these experiments were approved by the New Mexico State University Institutional Animal Care and Use Committee. Twelve ruminally cannulated Rambouillet wether lambs ( $36 \pm 4$  kg BW) were used in two  $6 \times 6$  Latin square experiments. For both experiments, lambs were housed individually in metabolism crates with free access to fresh water and were limit-fed (0.63 kg/d, DM basis) a soybean hull-based diet (Table 1) in equal portions twice daily. The diet was formulated to be low in ruminally undegradable protein so that the predominant source of AA entering the small intestine would be microbial protein. All lambs received continuous infusions of VFA (41 g/d acetate and 14 g/d propionate) into the rumen to supply additional energy. Infusions into the rumen were made by placing flexible tubing through the rumen cannula of lambs.

Treatments for Exp. 1 were continuous postprandial infusions of a solution (500 mL/d) containing 1) a mixture of 10 essential AA, 2 nonessential AA, and glucose (12AA), 2) 12AA with Met removed, 3) 12AA with Lys removed, 4) 12AA with His removed, 5) 12AA with Thr removed, and 6) 12AA with all AA removed. Treatments for Exp. 2 were also abomasal infusions of 1) 12AA, 2) 12AA with Arg removed, 3) 12AA with Phe removed, 4) 12AA with Trp removed, 5) 12AA with Leu, Ile, and Val removed, and 6) 12AA with all AA removed. The AA supplied by the 12AA treatments were (g/d): DL-Met (1.8), L-Lys (6.6), L-His (3.1), L-Thr (3.1), L-Arg (6.4), L-Phe (3.6), L-Trp (0.4), L-Leu (7.9), L-Ile (1.8), L-Val (4.4), L-Glu (10.9), and Gly (5.6). All treatments supplied 73 g/d of glucose postprandially to provide additional energy to the animal without altering rumen microbial growth. Postprandial infusions were made by extending flexible tubing through the rumen cannula and reticulo-omasal orifice of lambs.

Experimental periods were 7 d, which allowed 3 d for adaptation to treatments, and 4 d for collection of feces

and urine. Total feces and a representative sample of urine (5%) were saved, composited by period for each lamb, and frozen for later analysis. Urine was collected into bottles containing 50 mL 6 N HCl to prevent NH<sub>3</sub> loss. Feed and fecal samples were dried at 55°C in a forced-air oven and ground to pass a 1-mm screen. Dietary and fecal samples were analyzed for DM (105°C for 24 h) and OM (500°C for 8 h), and dietary, fecal, and urinary samples were analyzed for N (LECO FP-528, LECO Corporation, St. Joseph, MI) to calculate N retention.

Table 1. Diet composition

Item	% of DM
Ingredient	
Soybean hulls	79.6
Alfalfa hay	15.0
Cane molasses	3.5
Mineral/Vitamin premix <sup>a</sup>	0.80
Sodium bicarbonate	0.50
Urea	0.35
Salt	0.20
Elemental sulfur	0.05
Nutrient	
OM	91.1
CP	15.0
RDP <sup>b</sup>	12.8

<sup>a</sup> Composition: Ca (14.0 to 16.8%), P (>11.0%), NaCl (11.0 to 13.2%), Mg (>0.50%), K (>0.10%), Cu (5.0 to 7.0 ppm), Se (>15 ppm), Zn (>1980 ppm), Vit A (660 KIU/kg), Vit D (165 KIU/kg), Vit E (1.32 KIU/kg).

<sup>b</sup> Ruminally degraded protein, calculation based on table values.

Data were analyzed statistically using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model included effects of period and treatment, with lamb as a random effect. Data is presented as least squares means, and differences were considered significant when  $P < 0.05$ .

## Results and Discussion

To identify limiting AA, infusions of VFA into the rumen and glucose into the abomasum ensured that the basal supply of energy would not limit responses to changes in postruminal AA supply. Also, abomasal infusions of all essential AA (12AA) in a profile that matched the lamb's requirement as closely as possible, ensured that a single limiting AA could be evaluated without several essential AA being co-limiting. Then, a response in N retention to the removal of a single AA from the 12AA mixture would demonstrate that the basal supply of that essential AA was limiting, whereas no response would demonstrate that the basal supply of that AA was not limiting.

In Exp. 1, N retention of lambs decreased ( $P < 0.05$ ) when all the AA were removed from the 12AA mixture (Table 2). This demonstrated that the basal supply was limiting in at least one AA. Retained N also decreased ( $P < 0.05$ ) in response to the removal of Met, and tended ( $P = 0.09$ ) to decrease in response to the removal of Thr, but

was not affected when Lys and His were removed from the 12AA mixture. Similar to Exp. 1, N retention of lambs in Exp. 2 decreased ( $P < 0.05$ ) when all AA were removed from 12AA (Table 3). Retained N also decreased ( $P < 0.05$ ) in response to the removal of branched-chain AA, and tended to decrease in response to the removal of Arg ( $P = 0.07$ ) and Trp ( $P = 0.05$ ). However, the removal of Phe from the 12AA mixture did not affect N retention of lambs. Thus, the basal supply of postruminal essential AA was limiting in Met and at least one branched-chain AA (Leu, Ile, and/or) Val).

Our finding that Met was limiting is in agreement with several other reports for lambs (Nimrick et al., 1970; Storm and Ørskov, 1984) and steers (Richardson and Hatfield, 1978; Greenwood and Titgemeyer, 2000). However, the effects of branched-chain AA on N balance of growing lambs in this experiment have only recently been reported to also be limiting for growing steers (Löest et al., 2001). The tendencies for Thr and Arg to be limiting is in agreement with Nimrick et al. (1970), and Storm and Ørskov (1984), respectively, but the tendency for Trp to limit N retention of lambs in this experiment is not well documented in the literature for ruminants.

## Implications

The results of these experiments demonstrate that methionine, and at least one of the branched-chain amino acids, may limit the growth of lambs when fed a diet containing protein that is predominantly degraded in the rumen.

## Literature Cited

- Greenwood, R. H., and E. C. Titgemeyer. 2000. Limiting amino acids for growing Holstein steers limit-fed soybean hull-based diets. *J. Anim. Sci.* 78:1997-2004.
- Löest, C. A., E. C. Titgemeyer, B. D. Lambert, and A. M. Trater. 2001. Branched-chain amino acids for growing cattle limit-fed soybean hull-based diets. *J. Anim. Sci.* 79:2747-2753.
- Merchen, N. R., and E. C. Titgemeyer. 1992. Manipulation of amino acid supply to the growing ruminant. *J. Anim. Sci.* 70:3238-3247.
- Nimrick, K., E. E. Hatfield, J. Kaminski, and F. N. Owens. 1970. Qualitative assessment of supplemental amino acid needs for growing lambs fed urea as sole nitrogen source. *J. Nutr.* 100:1293-1300.
- Ørskov, E. R., D. A. Grubb, G. Wenham, and W. Corrigan. 1979. The sustenance of growing and fattening ruminants by intragastric infusion of volatile fatty acid and protein. *Br. J. Nutr.* 41:553-559.
- Richardson, C. R., and E. E. Hatfield. 1978. The limiting amino acids in growing cattle. *J. Anim. Sci.* 46:740-745.
- Storm, E., and E. R. Ørskov. 1984. The nutritive value of rumen micro-organisms in ruminants 4. The limiting amino acids of microbial protein in growing sheep determined by a new approach. *Br. J. Nutr.* 52:613-620.

Table 2. Effects of removing Met, Lys, His, Thr, or all AA from postruminal infusions on N balance of growing lambs (Exp. 1).

Item	Treatments <sup>a</sup>						SEM
	12AA	-MET	-LYS	-HIS	-THR	NC	
	----- g/d -----						
Feed N	15.5	14.7	15.9	15.3	14.1	14.3	-
Feed + Infused N	23.9	23.0	23.4	22.9	22.1	14.3	-
Fecal N	5.8	6.0	5.9	5.9	5.5	5.4	0.39
Urinary N	9.8	12.4 <sup>**</sup>	9.6	8.8	10.7	6.2 <sup>**</sup>	0.77
Retained N	8.2	4.6 <sup>**</sup>	7.9	8.2	5.8	2.7 <sup>**</sup>	0.88
	----- % -----						
Retained N Efficiency <sup>b</sup>	34.8	20.1 <sup>**</sup>	33.9	35.4	25.8	16.1 <sup>**</sup>	4.11

<sup>a</sup> 12AA = mixture of 10 essential AA, 2 nonessential AA, and glucose; -MET = Met removed from 12AA; -LYS = Lys removed from 12AA; -HIS = His removed from 12AA; -THR = Thr removed from 12AA; NC = negative control with all AA removed from 12AA.

<sup>b</sup> Retained N Efficiency = (N retained / N from feed plus infusion) × 100.

<sup>\*\*</sup> Different from 12AA ( $P < 0.05$ ).

Table 3. Effects of removing Arg, Phe, Trp, three branched-chain AA (Leu, Ile, and Val), or all AA from postruminal infusions on N balance of growing lambs (Exp. 2).

Item	Treatments <sup>a</sup>						SEM
	12AA	-ARG	-PHE	-TRP	-BCAA	NC	
	----- g/d -----						
Feed N	16.2	16.2	16.2	16.2	16.2	16.2	-
Feed + Infused N	24.6	22.6	24.3	24.6	23.1	16.2	-
Fecal N	5.5	5.3	5.7	5.4	5.8	5.5	0.23
Urinary N	10.5	10.0	10.8	12.1	10.5	8.3 <sup>**</sup>	0.64
Retained N	8.5	7.2	7.8	7.0	6.7 <sup>**</sup>	2.3 <sup>**</sup>	0.70
	----- % -----						
Retained N Efficiency <sup>b</sup>	34.2	32.0	32.3	28.7	29.0	13.4 <sup>**</sup>	3.28

<sup>a</sup> 12AA = mixture of 10 essential AA, 2 nonessential AA, and glucose; -ARG = Arg removed from 12AA; -PHE = Phe removed from 12AA; -TRP = Trp removed from 12AA; -BCAA = Leu, Ile, and Val removed from 12AA; NC = negative control with all AA removed from 12AA.

<sup>b</sup> Retained N Efficiency = (N retained / N from feed plus infusion) × 100.

<sup>\*\*</sup> Different from 12AA ( $P < 0.05$ ).

# INFLUENCE OF FORAGE SOURCE AND NDF LEVEL ON GROWTH PERFORMANCE OF FEEDLOT CATTLE

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**ABSTRACT:** One hundred sixty Holstein steers (222 kg) were used in a 261-d growing-finishing trial to evaluate the influence of forage source and NDF level on growth performance. Treatments consisted of a steam-flaked corn-based diet containing 4% and 8% forage NDF from: alfalfa (ALF), sudangrass (SG), ground rice straw (GRS) and pelletized rice straw (PRS). Steers were assigned to 32 pens, 5 steers/pen. Steers were allowed ad libitum access to experimental diets, fresh feed was provided twice daily. There were no differences ( $P > 0.10$ ) between ALF, SUD or GRS diets with respect to DMI, and ADG. However, DMI and ADG were 8% and 11% less ( $P < 0.01$ ), respectively, for PRS than for the other forage sources. The decreased ADG for steers receiving PRS diets was due entirely to depressed DMI, suggesting a lesser acceptability of the pelletized forage. As would be expected, increasing forage NDF level from 4 to 8% decreased ( $P < 0.05$ ) dietary NEm and NEg (2 and 3%, respectively) for ALF, GRS and PRS. However, there was an interaction ( $P < 0.05$ ) between forage NDF level and NE values for SUD, wherein dietary NE values were similar across forage NDF levels. For the ALF, GRS and PRS diets, observed dietary NE were in close (102%) agreement with expected. However, NEm and NEg values for SUD were respectively 4 and 5% greater (interaction,  $P > 0.05$ ) at the 8% vs 4% forage NDF level. Although, this effect has been observed in other trials using sudan, the basis for this response remains unclear. It is interesting to note that with the exception of the SUD interaction, the dietary NEm and NEg values were not different across forage NDF source. We conclude that when diets are formulated on the basis of forage NDF content within a range (4 - 8%) that does not limit rumen function, growth performance is not affected by forage source.

Key Words: Forage, Level, Source, NDF, Cattle

## Introduction

Forages are included in the diets of feedlot and dairy cattle primarily as "functional" feeds, and only secondarily for nutrient content. Although cattle have been finished on all-concentrate diets, the inclusion of small amounts of forage helps avoid digestive dysfunction, and improves performance. Benefits to the addition of small amounts of forage in the diet include reduced incidence of acidosis (Clark and Davis, 1984; Popp et al., 1997), and increased energy intake (Price et al., 1980), ADG (Wise et al., 1968; Stock et al., 1990), and gain efficiency (Stock et al., 1990). However, the inclusion of too much forage in

the diet may limit energy intake and ADG. In a summary of various experiments, Galyean and Defoor (2003) observed that increasing the forage NDF level above 10% decreased cattle performance. Grain processing may also play an important role in determining the optimum forage level (Gill et al., 1981). The objective of this trial was to evaluate the influence of forage source and NDF level on growth-performance in cattle fed a steam-flaked corn-based finishing diet.

## Materials and Methods

One hundred sixty Holstein steers (222 kg) were blocked by weight and randomly assigned within weight groups to 32 pens (five steers per pen). Pens were 43 m<sup>2</sup> with 22 m<sup>2</sup> overhead shade, automatic waterers and 2.4 m fence-line feed bunks. Treatments consisted of a steam-flaked corn-based diet containing 4% and 8% forage NDF from: alfalfa (ALF), sudangrass (SG), ground rice straw (GRS), and pelletized rice straw (PRS, .635 cm diameter). Sudangrass and rice straw were ground in a hammer mill to pass through a 2.6 cm screen prior to incorporation into complete mixed diets. Alfalfa hay was ground in a hammer mill with the screen removed. Composition of experimental diets is shown in Table 1. Steers had ad libitum access to feed and water. Fresh feed was added twice daily. Steers were implanted with Synovex-S<sup>®</sup> (Forte Dodge Animal Health, Forte Dodge, IA) on d 1 and d 56 of the trial. Energy gain (EG, Mcal/d) was calculated by the equation:  $EG = ADG^{1.095} \cdot .0493W^{.75}$  (NRC, 1984). Maintenance energy (EM) was calculated by the equation:  $EM = .077 W^{.75}$ . From the derived estimates of energy required for maintenance and gain, the NE<sub>m</sub> and NE<sub>g</sub> values of the diet were obtained

using the quadratic formula:  $x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2c}$  where a =

$-.41EM$ ,  $b = .877EM + .41DMI + EG$ , and  $c = -.877DMI$ , and  $NE_g = .877NE_m - .41$ . For calculating steer performance, initial and final full weights were reduced 4% to account for digestive tract fill. Final weight was adjusted for carcass weight by dividing carcass weights by the average dressing percentage. Pens were used as experimental units. The trial was analyzed as a randomized complete block design experiment. Comparisons among treatment means were analyzed using LSD (Hicks, 1973).

## Results and Discussion

There were no differences ( $P > 0.10$ ) between ALF, SUD or GRS diets with respect to DMI, and ADG. However, DMI and ADG were 8% and 11% less ( $P < 0.01$ ), respectively, for PRS than for the other forage sources. This was surprising, as pelletizing increases particle density, allowing for greater ruminal turnover rate, permitting increased DMI (Moore, 1964; Coleman et al., 1978; Mertens and Ely, 1979; Zinn, 1987; Ware et al., 2002). The decreased ADG for steers receiving PRS diets was due entirely to depressed DMI, suggesting a lesser acceptability of the pelletized forage.

As would be expected due to the dilution effect that forages have on dietary energy density, increasing forage NDF level from 4 to 8% decreased ( $P < 0.05$ ) dietary NEm and NEg (2 and 3%, respectively). However, there was an interaction ( $P < 0.05$ ) between forage NDF level and forage source. For the ALF, GRS and PRS diets, observed dietary NE were in close (102%) agreement with expected at both the 4 and 8% forage NDF levels. In contrast, with SUD the observed/ expected dietary NE was 5% greater at the 8% forage NDF level than at the 4% forage NDF level. The basis for this response is not clear.

### Implications

Energy intake of feedlot cattle is optimal when finishing diets are formulated to contain between 4 and 8% forage NDF. Differences among forage sources is minimized when diets are formulated to equivalent forage NDF levels. Pelletizing may limit functional NDF value of the forage, depressing energy intake and daily weight gain.

### Literature Cited

- Clark, J. H., and C. L. Davis. 1984. Forage and buffers. *Animal nutrition and Health*. September-October 1984.
- Coleman, S. W., O. Neri-Flores, R. J. Allen, and J. E. Moore. 1978. Effect of pelleting and of forage maturity on quality of two sub-tropical forage grasses. *J. Anim. Sci.* 46:1103-1112.
- Galyean, M.L. and P.J. Defoor. 2003. Effects of roughage source and level on intake by feedlot cattle. *J. Anim. Sci.* 81(E. Suppl. 2):E8-E16.
- Gill, D. R., F. N. Owens, J. J. Martin, D. E. Williams, R. A. Zinn and R. J. Hillier. 1981. Roughage levels in feedlot rations. *Anim. Sci. Research Report*. MP-108:141.
- Hicks, C. R. 1973. *Fundamental concepts in the design of experiments*. Holt, Rinehart and Winston, New York.
- Mertens, D. R., and L. O. Ely. 1979. A dynamic model of fiber digestion and passage in the ruminant for evaluating forage quality. *J. Anim. Sci.* 49:1085-1095.
- Moore, L. A. 1964. Symposium on forage utilization: Nutritive value of forage as affected by physical form. Part I. General principles involved with ruminants and effect of feeding pelleted or wafered forage to dairy cattle. *J. Anim. Sci.* 23:230-238.
- Popp, J. D., T. A. McAllister, and K. J. Cheng. 1997. Strategies to prevent digestive disturbances in feedlot cattle. Part 1. *Veterinary Clinical Nutrition*. 4:63-68.
- Price, M. A., S. D. M. Jones, G. W. Mathieson, and R. T. Berg. 1980. The effects of increasing dietary roughage level on the growth and productivity of intensively fed bulls. *Can. J. Anim. Sci.* 60:345-358.
- Stock, R. A., M. H. Sindt, J. C. Parrot, and F. Goedecken. 1990. Effects of grain type, roughage level and monensin level on finishing cattle performance. *J. Anim. Sci.* 68:3441-3455.
- Ware, R.A., E. G. Alvarez, M. Machado, M. F. Montano, S. Rodriguez, and R. A. Zinn. 2002. Influence of pelletizing on the feeding value of rice straw in growing-finishing diets. *Proc. West. Sec Am. Soc. Anim. Sci.* 53:637-641.
- Zinn, R. A. 1987. Comparative feeding value of alfalfa hay, sudangrass hay, bedding straw and pelletized bedding straw in finishing diets for feedlot cattle. *California Cattle Feeders Day Report*. p. 63-71.

Table 1. Composition of experimental diets fed to steers (Trials 1&amp;2)

Item	Forage Level					
	12 %			15 %		
	Sudan	Alfalfa	Straw	Sudan	Alfalfa	Straw
Ingredient Composition, % (DMB)						
Flaked Corn	86.50	82.95	80.40	80.30	73.40	75.30
Sudangrass hay	6.00	0.00	0.00	12.20	0.00	0.00
Wheat Straw	0.00	0.00	5.10	0.00	0.00	10.20
Alfalfa	0.00	9.55	0.00	0.00	19.10	0.00
TM Salt <sup>a</sup>	0.40	0.40	0.40	0.40	0.40	0.40
Limestone	1.35	1.35	1.35	1.35	1.35	1.35
Urea	1.00	1.00	1.00	1.00	1.00	1.00
MgO <sub>2</sub>	0.15	0.15	0.15	0.15	0.15	0.15
Ca <sub>2</sub> PO <sub>4</sub>	0.60	0.60	0.60	0.60	0.60	0.60
Canola Meal	0.00	0.00	2.00	0.00	0.00	2.00
Cane Molasses	0.00	0.00	5.00	0.00	0.00	5.00
Tallow	4.00	4.00	4.00	4.00	4.00	4.00

<sup>a</sup> Contains (%): CoSO<sub>4</sub>, 0.68; CuSO<sub>4</sub>, 1.04; FeSO<sub>4</sub>, 3.57; ZnO, 0.75; MnSO<sub>4</sub>, 1.07; KI, 0.052; and NaCl, 93.4.

Table 1. Treatment effects on feedlot cattle growth performance.

Item	Treatments <sup>a</sup>								Main Effects						
	4% Forage				8% Forage				Forage Source				Forage Level		SD
	ALF	SUD	GRS	PRS	ALF	SUD	GRS	PRS	ALF	SUD	GRS	PRS	4%NDF	8%NDF	
Pen Replicates	5	5	5	5	5	5	5	5							
Weight, kg															
IW	222	222	222	221	221	222	221	221	221	222	222	221	222	221	
FW <sup>bcd</sup>	605	600	601	583	588	603	603	575	597	601	602	579	597	592	
DM Intake, kg/d	8.07	8.16	8.02	7.54	8.24	8.13	8.30	7.63	8.16	8.15	8.16	7.58	7.95	8.08	.28
Avg Daily Gain, kg/d	1.46	1.49	1.47	1.34	1.45	1.49	1.46	1.31	1.46	1.49	1.47	1.33	1.44	1.43	.05
Feed/Gain	5.54	5.48	5.44	5.62	5.68	5.46	5.68	5.82	5.61	5.47	5.56	5.72	5.52	5.66	.21
Diet net energy, Mcal/kg															
Maintenance <sup>de</sup>	2.34	2.33	2.36	2.33	2.27	2.35	2.29	2.26	2.30	2.34	2.32	2.30	2.34	2.29	.06
Gain <sup>de</sup>	1.64	1.64	1.66	1.63	1.58	1.65	1.59	1.57	1.61	1.64	1.63	1.60	1.64	1.60	.06
Observed/Expected Net Energy															
Maintenance <sup>d</sup>	1.02	1.01	1.03	1.02	1.03	1.05	1.03	1.02	1.03	1.03	1.03	1.02	1.02	1.03	.03
Gain <sup>d</sup>	1.02	1.01	1.03	1.01	1.04	1.06	1.04	1.03	1.03	1.03	1.04	1.02	1.02	1.04	.04

<sup>a</sup> Treatment designations as follows: Alfalfa (**ALF**), Sudan (**SUD**), Ground Rice Straw (**RS-G**), Pelletized Rice Straw (**RS-P**)<sup>b</sup> Effect of Forage, (P<.01)<sup>c</sup> Main Forage Source Effect, (P<.05)<sup>d</sup> Variety x Processing Interaction, (P<.05)<sup>e</sup> Effect of NDF level, (P<.05)

## EFFECTS OF DIET FOR EARLY-WEANED CROSSBRED BEEF STEERS ON PERFORMANCE, METABOLIC PROFILES AND FEBRILE RESPONSE TO AN INFECTIOUS BOVINE HERPESVIRUS-1 CHALLENGE<sup>1</sup>

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**ABSTRACT:** Early-weaned crossbred steers (n = 33; initial BW = 106 ± 7.6 kg; average age at weaning = 132 d) were used to evaluate effects of protein supplementation of forage diets vs. a 70% concentrate diet fed during a backgrounding phase (d 0 to 84) on performance, metabolic profiles, and febrile response to an infectious bovine herpesvirus-1 (BHV-1) challenge during a receiving phase (d 84 to 112). The four treatments during backgrounding included a bermudagrass hay diet (CTRL); bermudagrass hay plus soybean meal (SBM) fed at 0.175% of BW (as-fed); bermudagrass hay plus SBM at 0.35% of BW; or a 70% concentrate (CONC) diet. During the receiving phase, all steers were fed CONC and intranasally challenged on d 85 with BHV-1. No differences (P = 0.69) were observed among treatments for G:F during the receiving phase. Treatment x day interactions (P < 0.01) were observed for serum concentrations of NEFA, total protein, urea nitrogen, glucose, immunoglobulin G (IgG), insulin, and for rectal temperature. On d 88 (P < 0.05) and 91 (P = 0.07), serum IgG was greater for steers fed forage diets vs. CONC, and NEFA and glucose were greater (P < 0.02) for CONC vs. forage diets. On d 88 and 89 (3 and 4 d after the BHV-1 challenge), rectal temperature was greater (P < 0.01) for protein supplemented steers vs. CTRL steers. We conclude that a higher quality diet fed during a backgrounding phase enhances performance of early-weaned steers and increases febrile response (as measured by rectal temperature) to an infectious BHV-1 respiratory challenge.

Key Words: Beef Steers, Feedlots, Protein, Early Weaning, Serum Chemistry, Respiratory Disease

### Introduction

Concerns such as rising production costs and drought have contributed to more beef cattle producers recognizing the benefits of early weaning (EW) their calves. These calves can be backgrounded on forages or placed directly in a feeding facility. Determining the most appropriate

protein supplementation regimen is a major interest in cattle backgrounded on forages since protein is a primary limiting nutrient, an integral part of growth and overall well-being, and an expensive diet constituent. A concern with young calves on non-supplemented, low-quality forage is that nitrogen may be insufficient, thus limit microbial synthesis and decrease intake, digestibility, and overall production (Owens et al., 1991; Russell, 1998). It is well recognized that supplementing forages with protein increases steer DMI and ADG (Bodine and Purvis, 2003).

In addition, steers backgrounded on low-quality forage may become more susceptible to immune challenges such as respiratory disease during a feedlot receiving phase. This is an industry concern (USDA-APHIS, 2000) because morbid feedlot steers have lower ADG (Hutcheson and Cole, 1986; Morck et al., 1993), require more days on feed, and have lower net returns than healthy steers (McNeill, 2001). It is not known what the effect of protein supplementation of low-quality forage is on the immune response during a feedlot receiving period. Therefore, this study analyzed diet effects for EW crossbred beef steers on metabolic profiles and febrile response to an infectious bovine herpesvirus-1 (BHV-1) challenge.

### Materials and Methods

*Animal Procedures and Measurements.* The following procedures were approved by the Institutional Animal Care and Use Committee of The University of Arizona. Crossbred steer calves (n = 33; initial BW = 106.1 ± 7.6 kg; average age at weaning = 132 d; Hereford, Angus, Charolais, Barzona, Tarentaise, and Shorthorn) used in this study were from the University of Arizona V Bar V Ranch, Camp Verde, AZ. Calves were weaned 8 d prior to this trial, placed in a drylot at the V Bar V ranch, and fed bermudagrass hay. They were then transported approximately 354 km from the V Bar V Ranch to The University of Arizona Beef Unit, Tucson. Calves were ranked by BW and randomly assigned to individual treatments including: bermudagrass hay diet (CTRL, negative control), bermudagrass hay plus soybean meal (SBM; 47.7% CP, DM basis) fed at 0.175% of BW, bermudagrass hay plus SBM fed at 0.35% of BW, or 70% concentrate (CONC) diet (contained 59.1% steam-flaked corn, 30% sudangrass hay, 3% soybean meal, 5% cane molasses, 0.40% urea, and 2.5% premix). Each calf was

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allocated to an individual, concrete surfaced covered pen (2.5 x 4.9 m) which had an automatic watering system and feed bunk. All calves were individually fed once daily between 0600 and 0700 at ad libitum intake. Feed refusals were weighed, measured, and discarded each morning before feeding and consumption was calculated daily. The study began (d 0) after a 4-d adaptation period on bermudagrass hay alone and consisted of a backgrounding phase (d 0 to 84) and a simulated feedlot receiving phase (d 85 to 112). Steers were observed daily for signs of morbidity and morbid steers were treated with appropriate medications according to label instructions (Pen-Aqueous, penicillin G procaine, Agripharm, Grapevine, TX; Micotil, Elanco, Indianapolis, IN; Nuflor and Banamine, Schering Plough, Union, NJ).

**Backgrounding Phase.** On d 0 of the study, all calves were treated for internal and external parasites (Ivomec, Merck, Rahway, NJ), implanted with 36 mg of zeranol (Ralgro; Schering-Plough), and started on their assigned diet. Bermudagrass hay (6.7% CP) was used as the basal diet for CTRL, 0.175% and 0.35% of BW SBM. Group CTRL was not supplemented and groups 0.175 and 0.35% received SBM fed daily. The quantity of SBM was adjusted every 28 d for each calf and fed separately before feeding the hay to ensure complete consumption. Group CONC (positive control) was transitioned from bermudagrass hay to the CONC diet over 12 d. Calves were also bled by jugular venipuncture and weights and rectal temperatures were recorded every 28 d (d 0, 28, 56, and 84).

**Receiving Phase.** On d 85, CONC continued to receive the 70% concentrate diet while CTRL, 0.175 and 0.35% BW SBM were transitioned over a 3-d period, from bermudagrass hay to the concentrate diet (Table 1). Each calf was bled by jugular venipuncture and a rectal temperature was recorded daily for 7 d (d 85 to 91). On d 85, calves were challenged intranasally with 2 mL (liquid) of an infectious dose of BHV-1. The Cooper strain of BHV-1 was prepared as previously described (Belknap et al., 1999) and diluted to 1 x 10<sup>6</sup> tissue culture infective doses in minimal essential medium just before inoculation. Steer activity was monitored daily for signs of morbidity such as nasal discharge, conjunctivitis, inflamed nares, staggering, anorexia, labored breathing, and/or coughing.

**Lab Analysis and Serum Collection.** Bermudagrass hay bales were stored under an open shed and random samples from individual bales were collected during the backgrounding phase. Random grab samples of the 70% concentrate diet were also collected when feed was delivered during the backgrounding phase. Samples were dried at 100 C for 24 hours (or until weights were constant), and stored at -20 C until analyzed for CP at Texas A & M Univ., College Station, ADF (Ankom Technol. Corp., Fairport, NY), and ash (AOAC, 1990). Samples were pooled every 28 d and ground in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 1-mm screen. Blood samples were collected before a.m. feeding on d 0, 28, 56, and 85 to 91 by jugular venipuncture. Blood samples were centrifuged (Sorvall RT600B refrigerated

centrifuge, DuPont, Wilmington, DE) at 970 g for 25 min. Serum was decanted and frozen at -20°C until analyzed for non-esterified fatty acids (NEFA), total protein (TP), serum urea nitrogen (SUN), glucose, immunoglobulin G (IgG), and insulin. Serum NEFA (μEq/L) concentrations were analyzed (NEFA C; Waco Chemicals, Neuss, Germany) with standard errors below 15.0. Total protein (g/dL) and glucose (mg/dL) were analyzed (Cat. No. 5412 and 510; Sigma Diagnostics, Inc., St. Louis, MO) and had standard errors below 0.54 and 0.6, respectively. The intra- and inter-assay CV for the two SUN (Cat. No. 640A; Sigma Diagnostics, Inc.) assays were assessed at 6.60 and 9.44%, respectively. Serum IgG concentrations were quantified in one assay using a RIA procedure as described by Richards et al. (1999) with modifications for bovine as previously discussed (Duff et al., 2000). The intra-assay CV for IgG was 18%. Serum insulin concentrations were also quantified in one assay as described by Reimers et al. (1982) with an intra-assay CV of 9% and 115% recovery rate.

**Statistical Analysis.** All data were analyzed using the Mixed procedure of SAS (SAS inst. Inc., Cary, NC) with steer as the experimental unit. Performance data (ADG, DMI, and G:F) were analyzed by 28-d periods with a model that included treatment. Steer within diet was used as the random error and differences in treatments were evaluated using the PDIF procedure within SAS. Serum metabolites and febrile response were analyzed as repeated measures. The model included effects due to treatment and treatment by day. Steer within diet was used as the random error and differences in effect levels were evaluated using the PDIF procedure within SAS. Covariance structures (heterogeneous autoregressive order-1 and heterogeneous compound symmetry) were compared to determine the most appropriate structure for each model. Heterogeneous autoregressive order-1 was used for NEFA and rectal temperatures, while heterogeneous compound symmetry was used for all other variables. Treatment by day interactions were observed for all serum metabolites and febrile response, therefore, all data were analyzed by day. Treatment means were compared using orthogonal contrasts. Contrasts included the negative control (CTRL) vs. the average of the protein supplements, SBM supplemented at 0.175% vs. 0.35% of BW, and concentrate (CONC) vs. forage-fed steers (CTRL, 0.175 and 0.35% of BW).

## Results and Discussion

**Growth Response.** As expected, steers fed CONC outperformed steers fed forage-based diets for the overall backgrounding period (data not shown). In addition, supplemental CP increased ( $P = 0.02$ ) total DMI during d 56 to 84, and protein supplemented steers had greater ( $P < 0.01$ ) hay intake than CTRL from d 56 to 84 (data not shown). Nutrient intake and diet quality are integral components of the immune system and increasing diet quality may improve animal health (McCoy et al., 1998). An effective immune system is critical, especially during a feedlot receiving period when steers are subjected to stress.

*Febrile Response and Metabolic Profiles.* Treatment by day interactions ( $P < 0.01$ ) were observed for febrile response and all serum metabolites. Therefore, all data were analyzed by day, and data are reported in Tables 1 and 2.

Greater rectal temperatures ( $P < 0.01$ ; Table 1) were observed for CONC steers on d 86, but the biological significance is questioned since this difference was only 0.34 C and observed 1 d after the immune challenge (Orr et al., 1988). Fever generally benefits the host animal since severity of some viral infections is decreased and immune responses become more effective at elevated temperatures (White, 1996). After the BHV-1 immune challenge (d 85), the average rectal temperatures of steers supplemented with SBM at 0.175 and 0.35% of BW were greater ( $P < 0.01$ ) than CTRL on d 88 and 89, respectively.

It is difficult to make definitive conclusions about the effectiveness of an immune response to a foreign antigen due to the many metabolic response pathways, metabolite interactions, disease severity, effectiveness of fever on recovery rate, and problems associated with visually detecting morbidity. Higher quality and/or quantity of dietary protein is needed during stress and infectious states due to protein catabolism (Orr et al., 1988). Even though morbidity was not observed (except in one HS steer) after steers were given an infectious BHV-1 dose, the present study supports Orr et al., (1988) conclusion because rectal temperatures (3 and 4 d after the challenge) were about 0.75 C greater ( $P < 0.01$ ) for the average of protein supplemented steers vs. CTRL.

Glucose and NEFA concentrations were greater ( $P < 0.02$ ) in CONC vs. the average of the forage-fed steers on d 28, 56, 85, 88, and 91. Steers supplemented with CP (0.175 or 0.35% of BW) had greater ( $P = 0.03$ ) NEFA on d 56 and greater ( $P < 0.02$ ) glucose on d 85 and 91 than CTRL. Furthermore, glucose was greater ( $P < 0.05$ ) on d 85 in steers fed SBM at 0.35 vs 0.175% of BW.

Steers fed CONC diet had greater serum insulin on d 56 ( $P = 0.05$ ) and 85 ( $P < 0.01$ ) vs. the average of the forage-fed steers. No differences ( $P > 0.17$ ) in insulin were detected between CTRL vs. SBM supplemented steers, or between steers fed SBM at 0.175 vs. 0.35% of BW. In contrast, insulin has been reported to be greater in steers supplemented (vs. non-supplemented) with CP (cottonseed meal and SBM) while grazing dormant forages (Barton et al., 1992; Bodine and Purvis, 2003).

The SUN of steers supplemented with SBM at 0.175 and 0.35% of BW was greater ( $P < 0.01$ ) on d 28 and 85 vs. CTRL. Increasing CP supplementation from 0.175 to 0.35% of BW also resulted in greater ( $P < 0.03$ ) SUN on d 28 and 85. Protein supplementation can increase circulating urea nitrogen concentrations (Bodine and Purvis, 2003).

No differences were observed for TP between CTRL vs. the average of SBM supplemented steers ( $P > 0.37$ ), or between steers supplemented at 0.175 vs. 0.35% of BW ( $P > 0.11$ ). Total protein was expected to be less in CTRL vs. the average of SBM supplemented steers because TP is primarily composed of albumin, which tends to decrease with sustained dietary protein deficiencies (Johnston and Morris, 1996). Steers fed CONC had greater ( $P < 0.01$ ) TP on d 91 than forage-fed steers.

In the present study, serum IgG concentrations were not greater ( $P > 0.34$ ) in steers fed higher quality diets. In fact, steers fed bermudagrass hay during the backgrounding phase had greater serum IgG concentrations on d 56, 85, 88 ( $P < 0.05$ ) than those fed CONC. Even though CONC had lower IgG than the forage fed steers, no visual morbidity signs were observed (except for one HS steer). Thus, CONC may have been more effective (vs. forage-fed steers) neutralizing BHV-1 virus at the site of infection before it could elicit a strong immune response. If a local immune response to a foreign antigen is effective, a systemic response (e.g. serum IgG) may not be required. Therefore, low serum IgG concentration are acceptable if the animal remains healthy and does not experience latent morbidity.

## References

- AOAC. 1990. Official Methods of analysis (15th ed.). Association of Official Analytical Chemists, Arlington, VA.
- Barton, R. K., L. J. Krysl, M. B. Judkins, D. W. Holcombe, J. T. Broesder, S. A. Gunter, and S. W. Beam. 1992. Time of daily supplementation for steers grazing dormant intermediate Wheatgrass pasture. J. Anim. Sci. 70:547-558.
- Belknap, E. B., L. M. Walters, C. Kelling, V. K. Ayers, J. Norris, J. McMillen, C. Hayhow, M. Cochran, D. N. Reddy, J. Wright, and J. K. Collins. 1999. Immunogenicity and protective efficacy of a gE and US2 gene-deleted bovine herpesvirus-1 (BHV-1) vaccine. Vaccine 17: 2297-2305.
- Bodine, T. N., and H. T. Purvis II. 2003. Effects of supplemental vitamin E and/or degradation intake protein on performance grazing behavior, intake, digestibility, and fecal and blood indices by beef steers grazed on dormant native tallgrass prairie. J. Anim. Sci. 81:304-317.
- Duff, G. C., D. A. Walker, K. J. Malcolm-Callis, M. W. Wiseman, and D. M. Hallford. 2000. Effects of preshipping versus arrival medication with tilmicosin phosphate and feeding chlortetracycline on health and performance of newly received beef cattle. J. Anim. Sci. 78:267-274.

- Hutcheson, D. P., and N. A. Cole. 1986. Management of transit-stress syndrome in cattle: Nutritional and environmental effects. *J. Anim. Sci.* 62:555-560.
- Johnston, J. K., and D. D. Morris. 1996. Alterations in blood proteins. Pages 489-497 in *Large Animal Internal Medicine* (2nd ed.). B. P. Smith, ed. Mosby-Year Book, Inc., St. Louis, MO.
- McCoy, R. A., R. A. Stock, T. J. Klopfenstein, D. H. Shain, and M. J. Klemesrud. 1998. Effect of energy source and escape protein on receiving and finishing performance and health of calves. *J. Anim. Sci.* 76:1488-1498.
- McNeill, J. W. 2001. Texas A&M Ranch to Rail-North/South Summary Report. Texas Agric. Ext. Serv., Texas A&M University.
- Moreck, D. W., J. K. Merrill, B. E. Thorlakson, M. E. Olson, L. V. Tonkinson, and J. W. Costerton. 1993. Prophylactic efficacy of tilmicosin for bovine respiratory tract disease. *J. Am. Vet. Med. Assoc.* 202:273-277.
- Orr, C., D. P. Hutcheson, J. M. Cummins, and G. B. Thompson. 1988. Nitrogen kinetics of infectious bovine rhinotracheitis-stressed calves. *J. Anim. Sci.* 66:1982-1989.
- Owens, F. N., J. Garza, P. Dubeski. 1991. Advances in amino acid and N nutrition in grazing ruminants. Pages 109-137 in *Proc. Grazing Livestock Nutr. Conf.*, OK Agric. Exp. Sta. MP-133, Stillwater.
- Reimers, T. J., R. G. Cowan, J. P. McCann, and M. W. Ross. 1982. Validation of a rapid solid-phase radioimmunoassay for canine, bovine, and equine insulin. *Am. J. Vet. Res.* 43:1274-1278.
- Richards, J. B., D. M. Hallford, and G. C. Duff. 1999. Serum luteinizing hormone, testosterone, and thyroxine and growth responses of ram lambs fed locoweed (*Oxytropis sericea*) and treated with vitamin E/selenium. *Theriogenology* 52:1055-1066.
- Russell, J. B. 1998. Strategies that ruminal bacteria use to handle excess carbohydrate. *J. Anim. Sci.* 76:1955-1963.
- USDA-APHIS. 2000. Part III: Health management and biosecurity in U.S. feedlots, 1999. USDA:APHIS:VS, CEAH, National Animal Health Monitoring System. Fort Collins, CO.
- White, S. L. 1996. Alterations in body temperature. Pages 35-45 in *Large Animal Internal Medicine* (2nd ed.). B. P. Smith, ed. Mosby-Year Book, Inc., St. Louis, MO.

Table 1. Effects of diet on febrile response (as measured by rectal temperature, °C) of early-weaned crossbred steers intranasally challenged with an infectious bovine herpesvirus-1<sup>a,b</sup>

Day	Soybean meal, % of BW			CONC <sup>d</sup>	SEM <sup>e</sup>	Contrast <sup>c</sup>		
	0	0.175	0.35			1	2	3
85	38.14	38.40	38.44	38.51	0.10	0.12	0.03	0.82
86	38.30	38.43	38.63	38.79	0.09	<0.01	0.04	0.14
87	38.67	39.11	38.93	38.86	0.21	0.86	0.17	0.56
88	39.21	40.22	39.73	39.58	0.22	0.60	<0.01	0.13
89	39.38	40.10	40.25	40.01	0.20	0.68	<0.01	0.62
90	39.17	39.44	39.46	39.13	0.20	0.32	0.25	0.94
91	39.16	39.33	39.24	38.97	0.18	0.20	0.55	0.70

<sup>a</sup>Rectal temperatures of all calves were greater than 39.44 °C by day 89 except one steer backgrounding on a 70% concentrate diet (CONC).

<sup>b</sup>Steers were intranasally challenged with infectious bovine herpes virus-1 on d 85.

<sup>c</sup>Orthogonal Contrasts: 1 = steers fed bermudagrass hay diets vs. concentrate-fed steers, 2 = steers fed bermudagrass hay alone vs. the average of steers fed soybean meal at 0.175 or 0.35% of BW, 3 = steers fed protein at 0.175 vs. 0.35% of BW.

<sup>d</sup>70% concentrate diet.

<sup>e</sup>Greatest SEM are reported.

Table 2. Effects of diet on serum metabolic profiles of early-weaned crossbred steer calves intranasally challenged with and infectious bovine herpes virus-1<sup>a</sup>

Item <sup>b</sup> /Day	Soybean meal, % of BW			CONC <sup>c</sup>	SEM <sup>d</sup>	Contrast <sup>e</sup>		
	0	0.175	0.35			1	2	3
NEFA, $\mu$ Eq/L								
d 28	72.03	87.61	81.02	133.55	11.45	<0.01	0.37	0.69
d 56	62.78	69.26	81.77	117.28	4.88	<0.01	0.03	0.08
d 85	69.80	79.54	69.72	116.35	11.36	<0.01	0.72	0.55
d 88	108.18	94.02	101.08	147.91	13.76	<0.01	0.52	0.72
d 91	97.64	129.85	109.58	173.90	19.60	0.01	0.35	0.47
Total Protein, g/dL								
d 28	5.77	5.87	5.69	6.00	0.29	0.50	0.97	0.67
d 56	5.61	5.75	5.62	6.03	0.19	0.11	0.74	0.63
d 85	6.12	6.21	6.16	6.33	0.14	0.31	0.71	0.82
d 88	5.22	5.37	5.43	5.90	0.28	0.09	0.57	0.89
d 91	5.40	5.42	4.96	5.86	0.19	0.01	0.37	0.11
SUN, mg/dL								
d 28	3.90	6.55	11.47	7.87	0.97	0.61	<0.01	<0.01
d 56	5.76	5.78	9.09	5.62	2.42	0.65	0.56	0.34
d 85	1.17	6.46	9.55	5.31	0.90	0.69	<0.01	0.02
d 88	7.26	8.26	5.28	5.20	1.42	0.30	0.77	0.15
d 91	8.74	11.04	10.55	6.21	1.15	<0.01	0.14	0.76
Glucose, mg/dL								
d 28	53.30	52.29	56.04	70.87	3.94	<0.01	0.85	0.51
d 56	59.29	60.68	67.46	84.98	4.54	<0.01	0.38	0.30
d 85	64.05	73.22	83.96	99.94	3.66	<0.01	<0.01	<0.05
d 88	73.66	74.35	85.20	107.51	4.44	<0.01	0.25	0.09
d 91	76.16	81.79	90.69	105.08	3.26	<0.01	0.01	0.06
IgG, mg/mL								
d 28	16.22	14.35	14.19	11.99	1.70	0.14	0.34	0.95
d 56	13.39	12.16	12.85	9.31	1.21	0.02	0.54	0.69
d 85	18.78	17.40	18.03	13.09	1.66	0.01	0.59	0.79
d 88	18.53	22.45	20.99	13.96	2.79	<0.05	0.34	0.71
d 91	15.59	15.13	13.18	10.74	1.80	0.07	0.50	0.45
Insulin, ng/mL								
d 28	1.36	1.20	1.04	1.28	0.15	0.67	0.20	0.47
d 56	1.50	2.29	1.51	3.01	0.54	0.05	0.54	0.31
d 85	1.08	1.63	1.51	2.66	0.39	<0.01	0.17	0.79
d 88	3.13	4.12	3.96	5.22	0.83	0.13	0.36	0.89
d 91	3.07	4.11	4.57	5.37	0.87	0.16	0.23	0.71

<sup>a</sup>Steers intranasally challenged with infectious bovine herpes virus-1 on d 85.

<sup>b</sup>NEFA = non-esterified fatty acids; SUN = serum urea nitrogen; IgG = immunoglobulin G.

<sup>c</sup>70% concentrate diet.

<sup>d</sup>Greatest SEM are reported.

<sup>e</sup>Linear Orthogonal Contrasts: 1 = steers fed bermudagrass hay vs. concentrate-fed steers, 2 = steers fed only bermudagrass hay vs. the average of steers fed soybean meal at 0.175 or 0.35% of BW, 3 = steers fed protein at 0.175 vs. 0.35% of BW.