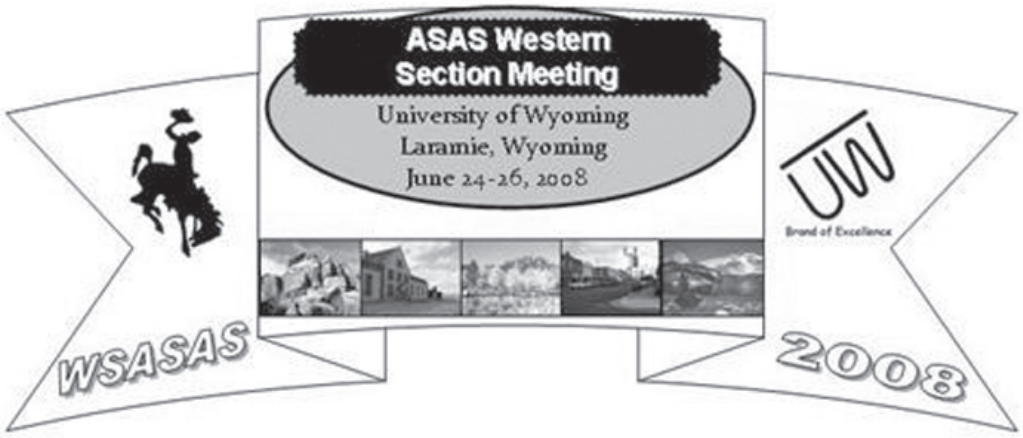
# PROCEEDINGS

**VOLUME 59**

# WESTERN SECTION

# American Society of Animal Science




## UNIVERSITY OF WYOMING

**LARAMIE, WYOMING**
**JUNE 24–26, 2008**

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# Western Section

# American Society of Animal Science

# Committee Assignments 2007-2008

*** Denotes Committee Chair**

**Executive**

K.C. Olson, President (09, S. Dakota State University)*
R. Battaglia, President-Elect (10, University of Idaho)
T.T. Ross, Past- President (08, New Mexico State University)
G. Moss, Secretary-Treasurer (11, University of Wyoming)
B. Hess, ASAS Board Director (10, University of Wyoming)
G. Tibbetts, Industry Director (09, Zinpro Corp.)
C. Loëst, A&C Chair (08, New Mexico State University)

**Awards**

R. Battaglia, President-Elect (08, University of Idaho)*
T. Engle (08, Colorado State University)
C. Mathis (08, New Mexico State University)
D. Bohnert (09, Oregon State University)
D. ZoBell (09, Utah State University)
M. Tess (09, Montana State University)

**Symposium**

J. B. Glaze (08, University of Idaho)*
B. Christensen (08, Virtus Nutrition)
R. Waterman (08, USDA-ARS Miles City)
T. Delcurto (09, Oregon State University)
T. Field (09, Colorado State University)
R. Endecott (09, Montana State University)

**Advising and Coordinating**

C.A. Loest (08, New Mexico State University)*
D. Drake (08, University of California, Davis)
R. Wiedmeier (08, Utah State University)
T. Bodine (08, Western Feed Supplements)
J. Stellflug (08, USDA-ARS, Dubois, ID)
D.H. Crews (09, AAFC, Edmonton)
M. Salisbury (09, Angelo State University)
S. Ivey (09, New Mexico State University)
G. Duff (10, University of Arizona)
P. Ludden (10, University of Wyoming)
J. Bowman (10, Montana State University)
C. Mueller (10, Oregon State University)
M. Enns (10, Colorado State University)
J. Carpenter (10, University of Hawaii)
A. Roberts (10, USDA-ARS Miles City)

**Paper Competition**

A. Ahmadzadeh (08, University of Idaho)*
J. Rumph (08, Montana State University)
S. Soto-Navarro (08, New Mexico State University)
M. Shipka (09, University of Alaska)
B. Taylor (09, USDA-ARS, Dubois, ID)
L. Baumgard (09, University of Arizona)
K. Walburger (10, University of Saskatchewan)
K. Cammack (10, University of Wyoming)

**Academic quadrathlon**

D.C. Rule (University of Wyoming)*
J.B. Lamb (BYU - Idaho)
S. Soto-Navarro (New Mexico State University)
Randy Wiedmeier (Utah State University
Hyungchul Han (Colorado State University)

**Extension**

J. Ahola (08, Univ. Idaho)*
S. Paisley (08, University of Wyoming)
J. Paterson (08 Montana State University)
B. Bruce (09, University of Nevada, Reno)
J. Sprinkle (09, University of Arizona)
R. Kott (10, Montana State University)
C. Parsons (10, Oregon State University)

**Necrology**

T.T. Ross (08, New Mexico State University)*

**Nominating**

T.T. Ross, Past- President (08, New Mexico State University)*
J. Thompson (08, Oregon State University)
P. Hatfield (08, Montana State University)

# Minutes of the Western Section of the American Society of Animal Science

## Business Meeting

**June 22, 2007**
**University of Idaho**
**Moscow, Idaho**

President Tim Ross called the meeting to order at 8:00 am.

**Acceptance of the minutes of the 2006 Business Meeting.**

The minutes of the 2006 business meeting were approved as printed in the 2007 Proceedings of the Western Section of the American Society of Animal Science.

### Advisory and Coordinating Committee Report

**Steve Paisley, chair**

**2006-2007 A & C Committee Members:**
S. Paisley (University of Wyoming)
M. Salisbury (Angelo State University)
S. Daugherty (Cal Poly)
D. Garrick (Colorado State University)
J.B. Glaze (University of Idaho)
D. Drake (University of California)
J. Sprinkle (University of Arizona)

**Committee Report:** The committee discussed approaches to maintaining support and increasing participation with Western Section ASAS Academic Quadrathlon competition. In addition the WSASAS Academic Quadrathlon advisors requested that the WSASAS Executive Committee discuss and vote to approve moving the Academic Quadrathlon back to the time and place of the WSASAS meetings beginning in 2008. Points of discussion and actions taken follow:

- The committee was not in favor of moving the AQ to conside with the WSASAS June meetings because several of the committee felt that it would be difficult to get members of the winning teams to attend June WSASAS. Many students are employed during the summer, or return home. Further, some schools incorporate AQ into semester activities, as semester credit. Tours and industry exposure are often incorporated into AQ travel as an educational component.
- The committee recommended developing an AQ Coordinator's Manual to be distributed to WSASAS participating institutions. The committee felt that perhaps some schools have dropped AQ because new faculty (potential AQ Coordinators) lack information on the Academic Quadrathlon.
- The committee recommended establishing a monetary award for the winning AQ team to encourage participation and competition. Recommended amount is $200/winning team member, $800 total.
- The committee also recommends encouraging participation by:
  a. Suggesting that the WSASAS president include a letter (along with AQ Coordinator's Manual) encouraging department heads to investigate ways of increasing student participation
  b. Develop list of Western universities and colleges that have Ag programs, and distribute AQ information and manuals to potential departments and advisors, encouraging participation in WS AQ contests.
  c. Continue current travel support for AQ teams

Committee Chair, Paisley, closed the A-C Committee report with the following recommendation. "The committee understands the current trend of reduced participation in AQ but unanimously feel that it is important to continue the AQ competition in the Western Section of ASAS."

**Action:** Discussion from Business Meeting attendees followed regarding Section support for the AQ. It was moved and seconded that we continue AQ support level of $600 per team, with an additional $500 support funding for the host institution (resulting in a total of $1100 for the host institution). In addition, WSASAS needs to pursue a national-level donor. Motion passed.

## Awards Committee Report

**Ken Olson, chair**

**Committee Members:**

Tom Geary, USDA-ARS, Miles City
Bret Hess, University of Wyoming
Jan Bowman, Montana State University
Terry Engle, Colorado State University
Clay Mathis, New Mexico State University

**Distinguished Teacher Award**

Sponsor: Elanco Animal Health
c/o Dr. Deana Hancock
2001 W. Main Street
Greenfield, IN 46140

Recipient; Dr. James R. Carpenter
University of Hawaii

Nominator: Dr. Halina Zaleski

**Young Scientist Award**

Sponsor: Ridley Block Operations
c/o Dr. Dan Dhuyvetter
424 N. Riverfront Drive
Mankato, MN 56002
ddhuyvetter@ridleyinc.com

Recipient: Dr. Lance H. Baumgard
University of Arizona

Nominator: Dr. Darrel E. Goll

**Extension Award**

Sponsor: Fort Dodge Animal Health
c/o Dr. Frank Prouty

9401 Indian Creed Parkway
Overland Park, KS 66225-5945

Recipient: Dr. Daniel J. Drake
University of California

Nominators: Dr. James Oltjen and Dr. Mary E. Delany

**Distinguished Service Award**

Sponsor: DSM Nutritional Products, Inc.
c/o Scot Williams
45 Water View Blvd.
Parsippany, NY 07054-1298

Recipient: Dr. Noelle E. Cockett
Utah State University

Nominators: Dr. Dale ZoBell and Dr. Mark Healey

Awards Chair, Olson acknowledged successful efforts to garner nominations in all categories, and encouraged early effort for 2008 nominations.

Awards Committee recommended that the presentation of the Service Award take place at the conclusion of the Graduate Student Competition.

## Applied Animal Science Award Report

**Bret Christensen, chair**

1. C.P. Mathis, "Low-input pasture backgrounding system is more profitable through harvest than high-input drylot system". New Mexico State University.
2. E.E. Snyder, "Sheep grazing winter wheat summer fallow and the impact on soil nitrogen, moisture, and crop yield". Montana State University.
3. M.L. Merrill, "The ability of a yeast-derived cell wall preparation to minimize toxic effects of high-alkaloid tall fescue straw in beef cattle". Oregon State University.

## Graduate Student Paper Competition Committee Report

**Janice Rumph, chair**

Committee Members
J.M. Rumph, Montana State University
D.W. Bohnert, Oregon State University
T. Bodine, Western Feed Supplements
S. Soto-Navarro, New Mexico State University
A. Ahmadzadeh, University of Idaho
M. Shipka, University of Alaska
B. Taylor, USDA-ARS, Dubois, ID
L. Baumgard, University of Arizona

**2007 Competition statistics**

18 submitted, 15 competing
- 1 moved – submitted in wrong section and changed to correct section
- 1 moved – committee opted to reject because proceedings paper was submitted past deadline and was moved to corresponding discipline's section
- 1 rejected – chair rejected because abstract was submitted several weeks past deadline and competitor was not a member of WSASAS

7 Universities represented
- Colorado State – 6*
- New Mexico State University – 2*
- University of Idaho – 2*
- University of Wyoming – 2*
- North Dakota State University – 1
- Oregon State University – 1
- Washington State University – 1

*eligible for institutional award

The 2007 Graduate Student Paper Competition was a great success. After an extremely competitive morning, the overall placings were as follows:

1. Price, Platt L., "Duodenal flow and intestinal disappearance of fatty acids in lambs fed safflower fatty acids in the form of whole seeds, cracked seeds, or oil extracted from seeds". University of Wyoming
2. Beckman, Devori W., Heterogenous variance of docility scores in Limousin cattle". Colorado State University
3. Stohrer, Rena M., "Expression and distribution of urea transporter-B in lambs fed increasing dietary protein". University of Wyoming

Zinpro Institutional Graduate Student Paper Competition: University of Wyoming.

The Graduate Student Paper Competition Committee also considered the following items:

*Graduating Students*. The committee decided to allow students who have defended, but not graduated at time of submission (i.e., graduating in spring) to compete.

*Rules on Website*. It was brought to the committee's attention that the rules and score sheet listed on the website were incorrect. On the website, the rules currently state: *3) Graduate Student Competition papers should be limited to four (4) pages. Tables, figures, etc. may go beyond the four (4) page limit.* This was previously changed so that tables, figures, etc. are included in the 4 pages and papers exceeding four pages would be discounted. The score sheet on the website currently reads: *1.8. Manuscript should be limited to 4 pages in length.* The score sheet currently being used by the committee reflects the page limit change mentioned above and states: *1.8. Manuscript should be limited to 4 pages in length.* For 2007, the committee opted to let the page limit reflect what is posted on the web; however this should be corrected in future years.

*Clarification of Rules Regarding First Author.* One paper was submitted in which a faculty member is the first author and the presenting student is the second/last author. There is no rule

against this, but the committee questioned who wrote the paper because the paper is worth 50% of the score. Should a rule be written to address this in future years or should it be allowed?

- A former chair of the committee thought it was against ASAS rules, but according to Paula, the ASAS office has no rule – leaves this up to the section
- Several committee members already thought that this was a rule
- Midwest section has had this happen in the past and decided against writing a formal rule, but assumed the judges would take off points on their own; however, Midwest section does not require a proceedings paper, so their scores are 100% based on presentation

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# EFFECT OF SUPPLEMENTAL ENERGY ON POST-RUMINAL UTILIZATION OF METHIONINE BY GROWING LAMBS

**S. A. Kuykendall, C. A. Löest, G. G. Gilliam, D. M. Griego, and D. M. Hallford**
New Mexico State University, Las Cruces, NM

**ABSTRACT:** Methionine is a limiting AA in growing lambs fed diets low in ruminally undegradable protein. Therefore, increasing Met utilization may improve N retention. The objective was to determine if supplemental energy affects post-ruminal utilization of Met in growing lambs. Six ruminally cannulated wethers (46.6 ± 2.8 kg initial BW) housed in metabolism crates were used in a 6 × 6 Latin square. Each period was 11 d, allowing for a 4-d rest, 3-d adaptation, and 4-d collection period to determine N balance. Lambs were limit-fed (0.78 kg DM/d) a soybean hull-based diet twice daily, and received continuous abomasal infusions (500 mL/d) of an L-AA solution devoid of L-Met. Treatments (2 × 3 factorial) were 2 amounts of supplemental energy (0 vs 0.40 Mcal ME/d) and 3 amounts of abomasally infused L-Met (0 vs 1 vs 2 g/d). Energy was supplied via ruminal infusions of acetate (35 g/d) and propionate (10 g/d), and abomasal infusions of glucose (60 g/d). Blood samples were collected 3 h after feeding on d 11 of each period. No energy × Met interaction ($P > 0.07$) was observed for N balance and blood metabolites. Also, infusion of Met and energy did not affect ($P > 0.17$) dietary intake, fecal excretion, and digestibility of NDF and N. Abomasal infusion of Met decreased (linear, $P < 0.01$) urinary N and increased (linear, $P < 0.01$) N retention. Similarly, urinary N decreased ($P < 0.01$) and N retention increased ($P = 0.03$) with supplemental energy. Plasma Met concentrations increased (linear, $P < 0.01$) and plasma concentrations of Leu, Val, Ala, Gly, Ser, and Pro decreased (linear, $P < 0.05$) in response to Met infusion. Energy supplementation decreased ($P < 0.05$) serum urea N and plasma concentrations of Val and Asn. Serum insulin concentrations increased in response to infusions of 2 g/d Met (quadratic, $P = 0.01$) and supplemental energy ($P = 0.04$). A decrease in plasma AA and an increase in N retention in response to supplemental Met confirm that Met is limiting for growing lambs. An increase in N retention due to supplemental energy regardless of the Met supply suggests that energy affects the efficiency with which metabolizable AA are utilized.

**KEYWORDS:** energy, methionine, sheep

## INTRODUCTION

Methionine is a limiting AA in growing lambs fed diets of which the major supply of metabolizable protein is microbial protein (Nolte et al., 2004). Additionally, Met absorbed from the small intestine is used for protein synthesis with variable and often low efficiencies. In growing cattle, the efficiency of Met utilization has been shown to range from 12.5% (Froidmont et al., 2000) to 63% (Löest et al., 2002). In growing lambs, abomasally infused Met was utilized for N retention with an efficiency of 25% (Nolte et al., 2004).

The efficiency of AA use for protein synthesis is, in part, affected by the need for those AA in other metabolic processes, such as glucogenic precursors. Schroeder et al. (2006) demonstrated that N retention of growing steers increased with supplemental energy when Met was limiting, and Kuykendall and Löest (2006) demonstrated that energy supply decreased urinary N excretion of lambs when Val was limiting. Because these studies suggest that the supply of dietary energy may affect AA utilization, we hypothesized that supplementation of additional energy might increase Met utilization for growth. Therefore, the objective of this study was to determine if supplemental energy affects post-ruminal utilization of Met in growing lambs.

## MATERIALS AND METHODS

***Animals and Design.*** Procedures were approved by New Mexico State University's Institutional Animal Care and Use Committee. Six ruminally cannulated wether lambs (46.6 ± 2.8 kg initial BW) housed individually in metabolism crates were used in a 6 × 6 Latin square. Periods were 11 d, allowing a 4-d rest, 3-d adaptation, and 4-d collection period to determine N balance. Lambs had free access to water and were limit-fed (0.78 kg DM/d) a soybean hull-based diet (Table 1) in equal portions twice daily. The diet was formulated to be low in ruminally undegradable protein. Lambs received continuous abomasal infusions (500 mL/d) of an L-AA solution devoid of L-Met to ensure that other essential AA did not limit protein accretion. This solution supplied (g/d): L-Arg (6.6), L-His (3.4), L-Ile (2.0), L-Leu (8.2), L-Lys (7.0), L-Phe (3.7), L-Thr (3.3), L-Trp (0.5), L-Val (4.0), L-Glu (11.0), and Gly (6.0). Abomasal infusions were made using a Manostat cassette pump, and by placing flexible tubing through the rumen cannula and reticula-omasal orifice.

***Treatments.*** Treatments were a 2 × 3 factorial, with 2 amounts of energy (0 vs 0.40 Mcal ME/d) and 3 amounts of Met (0 vs 1 vs 2 g/d). The energy was supplied via continuous ruminal infusions of acetate (35 g/d) and propionate (10 g/d), and abomasal infusions of glucose (60 g/d). The L-Met was dissolved with the basal L-AA solution and continuously infused into the abomasum.

***Collections.*** Feed samples, orts (if any), total feces, and total urine were collected on d 8 through 11 of each period.

Urine was collected into bottles containing 6 *N* HCl to minimize $NH_3$ loss. Daily feces and representative urine samples (5%) were immediately frozen, then later thawed and composited by period for each lamb before analysis. Blood samples were collected 3 h after feeding on d 11. Samples were collected from the jugular vein into vacuum tubes (Fisher Scientific, Pittsburg, PA) with and without sodium heparin. Blood samples for plasma were immediately chilled on ice, and samples for serum were allowed to coagulate at room temperature for 30 min. All blood samples were centrifuged at 1,300 × g for 15 min at 4°C, transferred to plastic vials, and frozen.

Table 1. Diet Composition

| Item | % of DM |
|---|---|
| *Ingredient* | |
| Soybean hulls | 79.6 |
| Alfalfa hay | 15.0 |
| Cane molasses | 3.5 |
| Mineral/Vitamin premix[1] | 0.80 |
| Sodium bicarbonate | 0.50 |
| Urea | 0.35 |
| Salt | 0.20 |
| Elemental sulfur | 0.05 |
| *Nutrient* | |
| NDF | 57.4 |
| CP | 13.8 |
| RDP[2] | 11.7 |

[1]Composition: Ca (14 to 17%), P (≥ 11%), NaCl (11 to 13%), Mg (≥ 0.5%), K (≥ 0.1%), Cu (5 to 7 mg/kg), Se (≥ 15 mg/kg), Zn (≥ 1980 mg/kg), Vit A (660 KIU/kg), Vit D (165 KIU/kg), Vit E (1.32 KIU/kg).
[2]Ruminally degradable protein, calculated based on table values (NRC, 2000).

***Sample Analysis.*** Feed, orts, and feces were dried in a forced-air oven at 55°C, allowed to air-equilibrate, then ground to pass a 2-mm screen (Wiley mill). Dietary and fecal samples were analyzed for DM (105°C for 24 h), and NDF (ANKOM 200, ANKOM Technology Corp., Fairport, NY). Dietary, fecal and urinary samples were analyzed for N (LECO FP-528, LECO Corporation, St. Joseph, MI). Serum samples were analyzed for urea N using a colorimetric assay (Infinity #TR15421, Thermo Scientific, Waltham, MA). Serum concentrations of insulin (Reimers et al., 1982) and IGF-I (Berrie et al., 1995) were determined by solid-phase RIA, and serum glucose concentrations were determined colorimetrically (enzymatic endpoint method, #TR12421, Thermo Scientific, Waltham, MA). Plasma was analyzed for AA by gas chromatography (EZ:FAAST #KGO-7165; Phenomenex, Torrance, CA).

***Statistical Analysis.*** Data was analyzed statistically using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model included period, energy, Met, and energy × Met, with lamb as a random effect. When there were no energy × Met interactions, linear and quadratic contrasts were used to determine incremental effects of Met. Data are presented as least square means, and differences were considered significant when $P < 0.05$.

## RESULTS

***Treatment Interactions.*** Energy × Met interactions ($P > 0.18$) were not significant for NDF and N intake, feces and digestibility, and for N retention. Also, no energy × Met interactions ($P > 0.07$) were observed for serum metabolites and plasma AA. Therefore, means for the effects of Met and energy are presented separately.

***Effects of Met.*** Abomasal Met infusion did not affect ($P > 0.27$) intake, fecal excretion and digestibility of NDF (Table 2). By design, Met infusion tended to increase (linear, $P = 0.06$) total N intake, but did not affect ($P > 0.39$) fecal N and N digestibility. Abomasal infusions of Met decreased (linear, $P < 0.01$) urinary N, and increased (linear, $P < 0.01$) N retention. Infusion of Met did not affect ($P > 0.28$) serum urea N, glucose, and IGF-I (Table 3). Serum insulin increased (quadratic, $P = 0.01$) with infusion of 2 g/d of Met. Supplemental Met increased (linear, $P < 0.01$) plasma Met, and decreased (linear, $P < 0.05$) plasma Ala, Gly, Leu, Pro, Ser, and Val.

***Effects of Energy.*** Infusion of energy did not affect ($P > 0.17$) NDF and N intake, feces, and digestibility, but decreased ($P < 0.01$) urinary N, and increased ($P = 0.03$) N retention (Table 4). Energy supplementation did not affect ($P > 0.67$) serum glucose and IGF-I, but decreased ($P = 0.01$) serum urea N, and increased ($P = 0.04$) serum insulin (Table 5). Energy infusions also decreased plasma Asn ($P < 0.01$) and Val ($P = 0.03$).

## DISCUSSION

***Supply of Met.*** Post-ruminal infusion of L-Met was effective at supplying absorbable Met, as evidenced by simultaneous increases in plasma Met concentrations of lambs. An improvement in N retention and decreases in many plasma AA in response to abomasal Met infusion indicate that the basal supply of Met from the soybean hull-based diet limited protein deposition in growing lambs. Decreases in plasma AA concentrations are indicative of an increase in their use for protein synthesis. Also, decreases in plasma Gly and Ser (interact with sulfur AA metabolism), and a tendency for greater plasma Cys, indicate that Met infusion increased transsulfuration (Löest et al., 2002). These findings are consistent with our previous research in growing lambs (Nolte et al., 2004).

Because N retention increased linearly with up to 2 g/d of infused Met, it can be interpreted that the lambs' requirements for supplemental Met was at least 2 g/d. However, a simultaneous linear increase in plasma Met suggests that 2 g/d of supplemental Met may have exceeded the lamb's requirement, or that other factors limited plasma Met utilization.

***Supply of Energy.*** An increase in serum insulin concentration indicated that ruminal infusion of VFA plus abomasal infusion of glucose increased ME supply. Infusion of energy improved N retention and decreased some plasma AA, indicating that energy limited protein

deposition of growing lambs. Additionally, a decrease in serum urea N in response to energy infusions is indicative of decreased AA deamination, probably because the additional supply of energy decreased the need for glucogenic precursors. Kuykendall and Löest (2006) also observed a decrease in urinary N excretion in response to energy (VFA plus glucose) infusion in growing lambs.

***Energy for AA Utilization.*** We hypothesized that additional energy will improve post-absorptive utilization of Met in growing lambs. The absence of an energy × Met interaction for N retention suggests that energy did not alter the efficiency with which Met was used for protein deposition. Nevertheless, an increase in N retention, and decreases in serum urea N and plasma AA in response to energy infusion suggests that post-absorptive AA metabolism as a whole was affected by energy supply.

Assuming protein accretion can be estimated from retained N × 6.25, and deposited protein contained 2% Met, then the incremental efficiencies of infused Met utilization was approximately 12% and 19% for lambs infused with 0 and 0.40 Mcal of energy, respectively. These efficiencies for Met utilization are lower than those calculated from the data of Nolte et al. (2004), likely because their lambs were infused with greater amounts of VFA and glucose.

***Conclusions.*** The results of this study demonstrated that energy supply alters protein accretion, even when Met was limiting. These findings imply that energy supply affects post-absorptive AA utilization in growing lambs. Energy supply may need to be considered when determining AA requirements for sheep.

## LITERATURE CITED


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Table 2. Effects of abomasal Met infusion on nutrient intake, digestibility and N retention of growing lambs

| | Methionine, g/d[1] | | | | *P*-value | |
|---|---|---|---|---|---|---|
| Item | 0 | 1 | 2 | SEM[2] | Linear | Quadratic |
| Intake, g/d | | | | | | |
| NDF | 443 | 443 | 448 | 3.33 | 0.27 | 0.47 |
| N | 25.7 | 25.8 | 26.1 | 0.14 | 0.06 | 0.31 |
| Fecal, g/d | | | | | | |
| NDF | 64.9 | 64.8 | 68.1 | 3.44 | 0.43 | 0.66 |
| N | 5.15 | 5.23 | 5.34 | 0.18 | 0.39 | 0.94 |
| Urinary, g/d | | | | | | |
| N | 13.5 | 11.9 | 11.2 | 0.56 | <0.01 | 0.20 |
| Digestibility, % | | | | | | |
| NDF | 85.3 | 85.3 | 84.8 | 0.78 | 0.56 | 0.71 |
| N | 79.9 | 79.7 | 79.5 | 0.71 | 0.60 | 0.95 |
| Retention | | | | | | |
| N, g/d | 7.1 | 8.7 | 9.6 | 0.61 | <0.01 | 0.54 |
| N, % | 27.5 | 33.6 | 36.8 | 2.33 | <0.01 | 0.45 |

[1]Continuous abomasal infusions of L-Met (0 vs 1 vs 2 g/d) dissolved in an essential AA solution.
[2]Standard error of the mean (n = 6).

Table 3. Effects of abomasal Met infusion on serum metabolites and plasma AA in growing lambs

| | Methionine, g/d | | | | *P*-value | |
|---|---|---|---|---|---|---|
| Item | 0 | 1 | 2 | SEM | Linear | Quadratic |
| Serum | | | | | | |
| Urea N, mg/dL | 15.9 | 15.6 | 15.3 | 0.81 | 0.28 | 1.00 |
| Glucose, mg/dL | 78.6 | 76.6 | 78.4 | 2.55 | 0.95 | 0.48 |
| Insulin, ng/mL | 1.21 | 0.94 | 1.38 | 0.14 | 0.22 | 0.01 |
| IGF-I, ng/mL | 201 | 207 | 194 | 23.3 | 0.57 | 0.35 |
| Plasma AA, µ*M* | | | | | | |
| Ala | 113 | 101 | 87 | 5.55 | <0.01 | 0.83 |
| Asn | 7.8 | 8.3 | 8.4 | 0.23 | 0.07 | 0.45 |
| Asp | 3.8 | 3.9 | 4.2 | 0.42 | 0.56 | 0.90 |
| Cys | 0.79 | 1.98 | 2.89 | 1.02 | 0.11 | 0.90 |
| Gln | 287 | 284 | 256 | 21.5 | 0.28 | 0.62 |
| Glu | 88.0 | 93.6 | 88.8 | 7.08 | 0.93 | 0.52 |
| Gly | 682 | 485 | 422 | 56.6 | <0.01 | 0.17 |
| His | 46.6 | 54.7 | 48.9 | 6.02 | 0.73 | 0.24 |
| Ile | 115 | 101 | 113 | 5.92 | 0.85 | 0.08 |
| Leu | 185 | 168 | 160 | 8.77 | 0.02 | 0.60 |
| Lys | 148 | 157 | 146 | 11.2 | 0.89 | 0.42 |
| Met | 8.9 | 14.5 | 19.5 | 0.99 | <0.01 | 0.80 |
| Orn | 124 | 125 | 101 | 10.6 | 0.07 | 0.21 |
| Phe | 50.3 | 52.7 | 50.1 | 2.40 | 0.94 | 0.35 |
| Pro | 81.6 | 74.5 | 65.5 | 3.13 | <0.01 | 0.79 |
| Ser | 236 | 122 | 90 | 23.5 | <0.01 | 0.10 |
| Thr | 206 | 220 | 187 | 27.8 | 0.38 | 0.23 |
| Trp | 34.0 | 35.5 | 33.5 | 1.98 | 0.85 | 0.48 |
| Tyr | 37.8 | 40.3 | 35.8 | 4.78 | 0.62 | 0.33 |
| Val | 272 | 250 | 228 | 11.8 | <0.01 | 0.95 |

Table 4. Effects of energy infusion on nutrient intake, digestibility and N retention of growing lambs

| | Energy, Mcal ME[1] | | | *P*- |
|---|---|---|---|---|
| Item | 0 | 0.4 | SEM[2] | value |
| Intake, g/d | | | | |
| NDF | 442 | 447 | 2.67 | 0.22 |
| N | 25.8 | 25.9 | 0.11 | 0.22 |
| Fecal, g/d | | | | |
| NDF | 64.4 | 67.7 | 2.98 | 0.31 |
| N | 5.1 | 5.3 | 0.15 | 0.26 |
| Urinary, g/d | | | | |
| N | 12.7 | 11.7 | 0.53 | <0.01 |
| Digestibility, % | | | | |
| NDF | 85.4 | 84.9 | 0.68 | 0.42 |
| N | 80.1 | 79.4 | 0.60 | 0.36 |
| Retention | | | | |
| N, g/d | 7.9 | 8.9 | 0.56 | 0.03 |
| N, % | 30.8 | 34.5 | 2.14 | 0.03 |

[1]Continuous ruminal infusions of acetate (35 g/d) and propionate (10 g/d), plus abomasal glucose (60 g/d) infusions (0.4 Mcal ME/d) vs infusion of water (0 Mcal ME/d).
[2]Standard error of the mean (n = 6).

Table 5. Effects of energy infusion on serum metabolites and plasma AA in growing lambs

| | Energy, Mcal ME | | | *P*- |
|---|---|---|---|---|
| Item | 0 | 0.4 | SEM | value |
| Serum | | | | |
| Urea N, mg/dL | 16.2 | 15.0 | 0.77 | 0.01 |
| Glucose, mg/dL | 77.3 | 78.4 | 2.21 | 0.67 |
| Insulin, ng/mL | 1.05 | 1.31 | 0.13 | 0.04 |
| IGF-I, ng/mL | 203 | 198 | 22.6 | 0.68 |
| Plasma AA, µ*M* | | | | |
| Ala | 99.6 | 101.3 | 4.60 | 0.79 |
| Asn | 8.58 | 7.78 | 0.20 | <0.01 |
| Asp | 4.12 | 3.83 | 0.35 | 0.55 |
| Cys | 2.12 | 1.65 | 0.88 | 0.65 |
| Gln | 284 | 268 | 18.3 | 0.50 |
| Glu | 95.5 | 84.8 | 6.03 | 0.16 |
| Gly | 496 | 564 | 52.2 | 0.14 |
| His | 50.4 | 49.7 | 5.38 | 0.89 |
| Ile | 110 | 109 | 4.83 | 0.79 |
| Leu | 174 | 168 | 7.82 | 0.47 |
| Lys | 158 | 144 | 9.80 | 0.21 |
| Met | 14.1 | 14.4 | 0.83 | 0.81 |
| Orn | 119 | 114 | 9.43 | 0.55 |
| Phe | 50.6 | 51.4 | 2.06 | 0.72 |
| Pro | 74.4 | 73.4 | 2.73 | 0.74 |
| Ser | 133 | 166 | 20.7 | 0.16 |
| Thr | 208 | 200 | 26.4 | 0.63 |
| Trp | 34.9 | 33.7 | 1.62 | 0.58 |
| Tyr | 36.3 | 39.6 | 4.48 | 0.32 |
| Val | 261 | 239 | 10.7 | 0.03 |

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# AN EVALUATION OF EXTRUDED-EXPELLED COTTONSEED MEAL AS A PROTEIN SUPPLEMENT TO BEEF COWS CONSUMING LOW QUALITY FORAGE

**S. J. Winterholler*, D. L. Lalman, M. D. Hudson, and C. L Goad**
Oklahoma State University, Stillwater, OK

**ABSTRACT:** Three experiments were conducted to evaluate the efficacy of extruded-expelled cottonseed meal (**ECSM**) as a protein supplement (**SUP**) to spring-calving beef cows (n = 102; 535 kg of initial BW; 5.4 initial BCS) consuming low-quality forage during late gestation and early lactation. Supplementation of ECSM was compared to two traditional cottonseed meal-based SUP. For all Experiments, SUP provided equal CP and included (DM basis): 1) 2.02 kg/d cottonseed meal and wheat midds-based SUP (**CSM20**); 1.02 kg/d 40% cottonseed meal-based SUP (**CSM40**); and 1.50 kg/d ECSM. In Exp. 1, cows were individually fed SUP 3 d/wk until calving and 4 d/wk during lactation; total SUP period was 96-d. Tall-grass prairie hay was provided *ad libitum* during the SUP period. Change in cow BW during SUP period was similar (-69 kg; $P = 0.23$). Cow BW was not different at weaning (508 kg; $P = 0.77$). Cow BCS was similar when SUP ended (4.54; $P = 0.55$) and at weaning (4.34; $P = 0.43$). Calf birth weight (35 kg; $P = 0.20$) and BW at weaning (213 kg; $P = 0.76$) were not different. Percentage of cows exhibiting luteal activity at beginning of breeding season (24%; $P = 0.59$) and pregnancy rate at weaning (84%; $P = 0.88$) were not different among SUP. In Exp. 2, cows (n = 20/trt) from Exp. 1, of similar d post-partum were machine-milked to evaluate the effect of SUP on milk production and composition. Butterfat (2.4%), protein (2.9%), lactose (5.0%), milk urea N (4.39 mg/dl) were not different ($P > 0.10$). Likewise, 24-h milk production was not different (6.34 kg/d; $P = 0.25$). In Exp. 3, 18 cows from Exp. 1 were used to determine the effect of SUP on hay intake and apparent digestibility. Hay intake was not influenced by SUP (2.14 kg/100 kg BW; $P = 0.10$). Digestibility of DM, NDF and CP were similar ($P > 0.10$) and averaged 60, 62, and 57%, respectively. Dry matter intake was greater for CSM20 ($P = 0.02$) compared to CSM40 and ECSM; likewise, digested DMI was 17% greater ($P = 0.01$) for CSM20. These results indicate that ECSM can be used as a winter protein SUP in place of traditional cottonseed meal-based SUP for beef cows.

**Key Words:** Beef cow, Byproduct, Supplementation

## Introduction

Delinted, extruded-expelled cottonseed meal (**ECSM**) is a byproduct of the cottonseed oil manufacturing industry. This mechanical process extracts less oil than the chemical extraction process, and results in a greater quantity of residual oil in ECSM compared to traditional solvent-extruded cottonseed meal. Previous research has been conducted with ECSM in the dairy industry (Noftsger et al., 2000; Meyer et al., 2001); however, we are aware of no studies that have evaluated ECSM as a protein supplement for beef cows. Therefore, the objectives of the present study were to determine the effects of supplementing ECSM to beef cows consuming low-quality forage on 1) cow performance and reproduction; 2) milk production and composition; 3) forage intake and diet digestibility.

## Materials and Methods

*Production of ECSM.* The ECSM used in this study was manufactured by Hollybrook Cottonseed Processing, Lake Providence, LA. Whole cottonseed was first delinted and then dry extruded, leaving 6-7% residual oil in the remaining meal. In this process, the meal is extruded for less than 30 s with an exit temperature of 121°C, followed by mechanical pressing for less than 30 s, with an exit temperature of 104°C. Last, the product is ground with a hammer mill to decrease particle size and improve uniformity. The average composition of the ECSM used in our experiment was: 33% CP, 44% NDF, 38% ADF, and 10.2 % oil.

*Experiment 1.* All experiments were conducted in accordance with an approved Oklahoma State University Animal Care and Use Committee protocol. In Exp. 1, spring-calving Angus and Angus x Hereford crossbred beef cows (n = 102) were used to evaluate the efficacy of ECSM as a protein supplement (**SUP**) for beef cows consuming low-quality forage during late gestation and early lactation. Cows were randomly assigned to 1 of 3 dietary SUP. Supplements (DM basis) included: 1) 2.02 kg/d during gestation and 3.88 kg/d during lactation of a cottonseed meal and wheat middlings based SUP (**CSM20**); 2) 1.02 kg/d during gestation and 1.94 kg/d during lactation of a 40% CP cottonseed meal based SUP (**CSM40**); 3) 1.50 kg/d during gestation and 2.79 kg/d during lactation ECSM. Supplements were formulated to provide similar amounts of CP (Table 1) based on chemical analysis and diets were formulated to meet CP requirements (NRC, 1996). Degradable intake protein (**DIP**), reported in Table 1, was determined in an *in situ* experiment (data not shown).

The SUP period began on January 2, 2007 and ended on April 6, 2007, resulting in a 95-d SUP period. The average calving d was March 22, 2007. Prior to calving, SUP were individually fed on Monday, Wednesday and Friday mornings. After calving, SUP frequency was increased to 4 times per week to meet nutrient demands for lactation and

cows were fed on Monday, Wednesday, Friday and Saturday mornings.

During gestation, cows were managed as a contemporary group in a single pasture with free choice access to prairie hay (4.5% CP, 57% TDN, 2.2% crude fat; DM basis) and a mineral supplement (28.6% NaCl; 12.8% Ca; 8.5% P; 1.2% Mg; 1044 ppm Cu; 12 ppm Se; 3117 ppm Zn; DM basis). At calving, cow/calf pairs were moved to an adjacent pasture and managed as a contemporary group. Cow/calf pairs had *ad libitum* access to the same native range forage, prairie hay and mineral supplement until green forage became available (April 7, 2007).

Individual cow BW and BCS were determined at initiation of SUP period, after the first 30 d of SUP, before calving, within 1 wk of calving, at trial termination, prior to breeding and at weaning. All weights were recorded after 16 h withdrawal from feed and water. Body condition scores (1 =emaciated, 9 = obese) were determined by the same two independent evaluators throughout the experiment.

The percentage of cows cycling at the start of the breeding season was determined by quantifying progesterone concentration (Vizcarra et al., 1997) in plasma samples obtained via tail ventipuncture 14 and 7 d prior to breeding and again on the first d of the breeding season. Cows with one or more plasma samples containing ≥ 0.5 ng/mL progesterone were considered to have luteal activity (interassay CV = 5.8%). Cows were bred via synchronization with a timed AI protocol on May 26, and cows were exposed to bulls from June 6 through July 20. First service conception rate was determined by transrectal ultrasonography 30 d following AI and confirmed using calving records. Pregnancy rate was determined by rectal palpation at weaning.

*Experiment 2*. Early lactation milk production and composition were determined using 20 cows from each treatment with a portable milking machine. Each d before milking, pairs were gathered at 1600. Calves were separated from their dams until 2200, when pairs were reunited and calves were allowed to nurse their dams *ad libitum*, but for ≤ 45 min. Cows and calves were separated again until milking was completed. Cows were provided free choice prairie hay and water during the separation period. Milking initiated at 0700 the following d and was completed by 1300. Before milking, a 1.0-mL injection of oxytocin (20 USP units/mL, i.m.; Phoenix Pharmaceutical Inc., St. Joseph, MO) was administered to facilitate milk let-down. When milk flow ceased to the milking machine, teats were hand-stripped and a sub-sample of total milk collected was obtained for analysis of milk urea N, protein, butterfat and lactose.

Twenty-four h milk production estimates were calculated with the following equation: P = (MW/MIN)*1440 where **P** = 24 h milk production **MW** = weight of milk obtained from milking procedure described above, **MIN** =minutes from calf-separation to termination of milking procedure and 1440 = minutes in 24 h period.

*Experiment 3*. During early lactation, 18 spring calving beef cows from Exp. 1 were used to determine the effects of SUP on hay intake and digestion. Based on calving date and treatment from Exp. 1, cows were assigned to one of two collection periods in a randomized complete block design, with 3 cows from each SUP group in each period. Cows were given *ad libitum* access to the same prairie hay that was fed in Exp. 1 and maintained the same feeding regimen as Exp. 1. Cows were housed in individual outdoor 3.7- x 9.1-m pens, so they would be exposed to the same environmental conditions as Exp. 1 herd mates.

**Table 1.** Supplement composition and nutrients supplied during gestation

| | Supplement, % of DM[1] | | |
|---|---|---|---|
| Item | CSM20 | CSM40 | ECSM |
| Cottonseed meal | 18.3 | 92.4 | -- |
| Extruded cottonseed meal | -- | -- | 93.2 |
| Wheat middlings | 76.9 | -- | -- |
| Calcium carbonate | 2.0 | 3.3 | 1.4 |
| Dicalcium phosphate | -- | 1.5 | 2.6 |
| Molasses | 2.7 | 2.7 | 2.7 |
| Vitamin A-30,000 IU[2] | 0.10 | 0.10 | 0.10 |
| ***Nutrient supplied*** | | | |
| DM, kg/d | 2.02 | 1.02 | 1.50 |
| CP supplied, kg/d | 0.460 | 0.460 | 0.460 |
| DIP[3] kg/d | 0.265 | 0.287 | 0.366 |
| TDN, kg/d | 1.55 | 0.77 | 1.11 |
| Crude fat, kg/d | 0.09 | 0.03 | 0.15 |

[1]CSM20 = 20% CP cottonseed meal and wheat midds supplement; CSM40 = 40% CP cottonseed meal supplement and ECSM = delinted, extruded cottonseed meal supplement.
[2]Provided 12,258 IU of vitamin A per kg of diet DM.
[3]Degradable intake protein, determined by separate *in situ* analysis.

Each 12-d period, consisted of 3-d of adaptation to the pens and hay feeders, and 9-d of data collection. Hay intake was measured from d 4 through 10 and fecal grab samples were collected twice daily at 0800 and 1600 from d 6 through 12 to predict fecal output from acid detergent insoluble ash concentration (**ADIA)**. Sub-samples of SUP, hay, and orts were dried at 100ºC to determine DM. Supplement, hay, ort, and fecal samples were dried at 50ºC and ground in a Wiley mill (Model-4, Thomas Scientific, Sweedesboro, NJ) to pass a 2-mm screen before analysis. After grinding, supplement and hay samples were composited within period; ort and fecal samples were composited by cow. Composite samples were analyzed for NDF, ADF, CP, and ADIA. Neutral detergent fiber and ADF content were determined using an ANKOM Fiber Analyzer (ANKOM Technology, Macedon, NY). Crude protein was determined using a Leco NS-2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Analysis of ADIA was determined as the residue following complete combustion of the ADF residue (Van Soest et al., 1991). Apparent DM, OM and CP digestibility and true NDF and ADF digestibility were calculated for each cow. Also, digested DMI (DMI kg/100kg of BW x DM digestibility) and digested OM intake was calculated for each cow.

*Statistics*. Cow was the experimental unit because SUP were individually fed. Continuous data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) and the Satterwaite approximation for degrees of freedom. The model for cow BW and BCS included SUP as a fixed effect and cow age and days on SUP prior to

calving as covariates. For milk production, SUP was a fixed effect and min from calf-separation to milking was a covariate. For milk composition, supplement was a fixed effect and d post-partum was a covariate. Intake and digestibility measurements were analyzed as a randomized complete block design using the MIXED procedure of SAS and the Satterwaite approximation for degrees of freedom. The model included SUP as a fixed effect, and collection period as a random variable. One cow was removed from the first period of the digestion experiment due to illness unrelated to SUP treatment. Treatment means were computed using the LSMEANS option, least squares means were separated ($P < 0.05$).

Data for reproductive performance were analyzed using the Glimmix procedure of SAS, assuming a binomial distribution with SUP as a fixed effect. Least squares means are reported in all tables, except for the percentage of cows exhibiting luteal activity, pregnancy rate, and first service conception rate which are raw means.

## Results

*Experiment 1.* Cow BW and BCS were similar among SUP groups throughout the trial ($P > 0.10$); at the end of the 95-d supplementation period, BW change was -68 kg and change in BCS was -0.84 (Table 2). Likewise, SUP did not effect calf BW or weaning BW ($P = 0.20$; Table 2). Cow BW (479 kg) and BCS (4.94) were similar at the beginning of the breeding season ($P = 0.19$-$0.89$; Table 2). Supplementation with ECSM did not affect the percentage of cows exhibiting luteal activity at the beginning of the breeding season (24%; Table 2). Likewise, AI conception rate (24%) and pregnancy rate at weaning (84%) were not impacted by SUP with ECSM (Table 2).

*Experiment 2.* Twenty-four h milk production (6.33 kg) was similar among SUP type ($P = 0.25$; Table 2). Lactose, butterfat, protein and milk urea N were also not affected by SUP ($P = 0.18$ to $0.81$; Table 2).

*Experiment 3.* As a percentage of BW, DM intake, OM intake, digestible DMI and digestible OM intake was greatest for CSM20 ($P = 0.01$ to $0.02$; Table 2). Hay intake was not different among SUP type and averaged 2.14% of BW (Table 2). Type of SUP did affect the digestibility of DM, NDF, ADF, CP, or OM (60, 62, 54, 57%, and 64%, respectively).

## Discussion

Data from this study indicate that ECSM is an efficacious SUP for beef cows consuming low-quality forage during late gestation and early lactation as measured performance characteristics were similar among SUP type. In Exp. 3, the increase in DM and OM intake for CSM20 cows was because these cows received the greatest quantity of SUP in order to balance SUP for CP.

The higher oil content of ECSM was not great enough to inhibit forage intake or diet digestibility. Research has shown that interval feeding of high fat SUP (5.7% diet DM fat or 3.8% diet DM fat) reduced cow performance, and decreased forage intake and digestibility (Banta et al., 2006; Steel et al., 2007). In this study, during lactation, when SUP feeding level was the highest, fat intake from ECSM represented 3.1% of diet DM at each feeding interval. This level of fat intake was evidently not great enough to hinder cow performance or diet digestibility.

In conclusion, SUP with ECSM to spring-caving cows during the winter months is an effective management practice to maintain cow BW and BCS without compromising other performance characteristics. With rising feed prices, SUP of ECSM may be more economically feasible compared to traditional cottonseed meal-based protein SUP, especially for cow/calf producers within close proximity to a manufacturing plant.

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**Table 2.** Effects of delinted-extruded cottonseed meal on cow and calf performance, cow reproductive performance, milk characteristics, hay intake and diet digestibility

| | Supplement[1] | | | | |
|---|---|---|---|---|---|
| Item | CSM20 | CSM40 | ECSM | SEM | *P*-Value |
| ***Cow performance*** | | | | | |
| n= | 34 | 31 | 31 | -- | -- |
| Initial BW (1/2/07), kg | 531 | 536 | 538 | 8.00 | 0.83 |
| BW at end of supplementation, kg | 470 | 465 | 466 | 7.41 | 0.89 |
| BW change (1/2 to 4/7/07) | -61 | -71 | -72 | 4.06 | 0.24 |
| BW at weaning (10/30/07) | 507 | 505 | 513 | 7.58 | 0.77 |
| Initial BCS (1/2/07), kg | 5.46 | 5.30 | 5.43 | 0.13 | 0.60 |
| BCS at end of supplementation, kg | 4.62 | 4.48 | 4.56 | 0.09 | 0.55 |
| BCS change (1/2 to 4/7/07), kg | -0.84 | -0.82 | -0.87 | 0.09 | 0.93 |
| BCS at weaning (10/30/07) | 4.30 | 4.30 | 4.41 | 0.07 | 0.43 |
| ***Calf-performance*** | | | | | |
| Calf BW, kg | 34 | 36 | 34 | 0.92 | 0.20 |
| Calf weaning weight, kg[2] | 211 | 215 | 213 | 3.95 | 0.76 |
| ***Reproductive performance*** | | | | | |
| Pre-breeding wt (5/17/07), kg | 482 | 477 | 479 | 8.65 | 0.89 |
| Pre-breeding BCS (5/17/07) | 5.02 | 4.79 | 5.01 | 0.10 | 0.19 |
| Luteal activity,[3] % | 18 | 26 | 29 | 0.51 | 0.59 |
| AI conception rate, % | 21 | 23 | 29 | 0.07 | 0.71 |
| Pregnancy rate at weaning, % | 82 | 84 | 87 | 0.59 | 0.88 |
| ***Milk characteristics*** | | | | | |
| n= | 20 | 20 | 20 | -- | -- |
| Butterfat, % | 2.41 | 2.26 | 2.55 | 0.31 | 0.81 |
| Lactose, % | 4.98 | 5.07 | 5.02 | 0.06 | 0.61 |
| Protein, % | 3.09 | 2.90 | 2.97 | 0.08 | 0.18 |
| Milk urea N, mg/dl | 4.36 | 4.35 | 4.46 | 0.53 | 0.48 |
| Milk production, kg[4] | 6.65 | 5.59 | 6.75 | 0.51 | 0.25 |
| ***Hay intake and diet digestibility*** | | | | | |
| n= | 5 | 6 | 6 | -- | -- |
| Hay intake, kg●100 kg of $BW^{-1}$●$d^{-1}$ | 2.13 | 2.27 | 2.03 | 0.14 | 0.10 |
| DMI, kg●100 kg of $BW^{-1}$●$d^{-1}$ | 2.79[a] | 2.55[b] | 2.45[b] | 0.16 | 0.02 |
| Fecal output, kg●100 kg of $BW^{-1}$●$d^{-1}$ | 1.25 | 1.24 | 1.19 | 0.06 | 0.70 |
| DM digestibility, % | 62.0 | 58.3 | 59.6 | 3.40 | 0.42 |
| NDF digestibility, % | 64.5 | 59.3 | 62.2 | 3.55 | 0.43 |
| ADF digestibility, % | 52.2 | 55.4 | 55.2 | 3.70 | 0.47 |
| CP digestibility, % | 57.2 | 55.9 | 59.8 | 2.29 | 0.51 |
| Digestible DMI, kg●100 kg of $BW^{-1}$●$d^{-1}$ | 1.79[a] | 1.54[b] | 1.53[b] | 0.06 | 0.02 |
| OM intake, kg●100 kg of $BW^{-1}$●$d^{-1}$ | 2.60[a] | 2.36[b] | 2.28[b] | 0.14 | 0.02 |
| OM digestibility, % | 66.7 | 63.1 | 63.9 | 3.50 | 0.36 |
| Digestible OM intake, kg●100 kg of $BW^{-1}$●$d^{-1}$ | 1.76[a] | 1.52[b] | 1.50[b] | 0.07 | 0.01 |

[a, b] Within a row, means without a common superscript letter differ ($P \leq 0.05$).

[1]Supplements (DM basis) included: 1) 2.02 kg/d during gestation and 3.88 kg/d during lactation of a 20% CP cottonseed meal and wheat midds based supplement (CSM20); 2) 1.02 kg/d during gestation and 1.94 kg/d during lactation of a 40% CP cottonseed meal based supplement (CSM40); 3) 1.50 kg/d during gestation and 3.72 kg/d during lactation of an extruded, expelled cottonseed meal based supplement that has been delinted (ECSM).

[2]Weaning weight reported as 205-d weight adjusted for calf sex.

[3]Percentage of cows exhibiting luteal activity at the beginning of the breeding season.

[4]Calculated 24 h milk production from machine milking procedure.

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# TRADITIONAL AND SELF-FED CULL COW FEEDING PROGRAMS: EVALUATION OF PERFORMANCE AND ECONOMICS

**F.D. Niemela, C.L. Loehr, and S.I. Paisley**
Department of Animal Science, University of Wyoming, Laramie, 82071

**ABSTRACT:** Non-pregnant cows (n=72, BW 554±20 kg, BCS 4.95±0.16) from University herds (17) and auction (55) were assigned to nine pens to determine effects of three feeding strategies on feed performance, carcass traits and economics. Initial weight, age and BCS were similar ($P$ = 0.12) across pens, with one of three treatments randomly assigned to each pen. Two treatments consisted of corn-based diets formulated to provide 1.32 Mcal $NE_g$/kg and 11.5% CP utilizing mixed hay and barley straw (**HAY**) or corn silage (**SILAGE**) as the roughage source. The third treatment, a self-fed ration using Purina Mills® controlled intake system (**LIMIT**), was included to evaluate the potential for range-based cull cow finishing programs. After a 91-d feeding study, animals were harvested at Gibbon Packing, L.L.C., Gibbon, NE. Data were analyzed in a completely randomized design with pen as the experimental unit. Although all treatments achieved similar ($P$ = 0.38; 156 kg, 1.71 kg/d) weight gain during the study, cows assigned to HAY and SILAGE rations attained more rapid gains in the first month ($P$ = 0.05), while animals on LIMIT diet had greater gains ($P$ = 0.03) in the final period. Slower gains during the initial period were partially attributed to lower intake ($P$ < 0.01) as LIMIT cows were slow to adapt to self-feeders. While performance was similar, LIMIT cattle consumed less feed ($P$ < 0.01; 13.4 vs. 16.3 and 15.7 kg/d for LIMIT, HAY and SILAGE, respectively) and had more efficient gains ($P$ = 0.02; 12.5 vs. 10.2 kg/100 kg) than HAY treatment, with SILAGE intermediate (11.3 kg/100 kg). Hot carcass weights and 12th rib backfat were similar ($P$ = 0.50; 362 kg, 11.7mm) across treatments. Marbling tended ($P$ = 0.08) to be higher for HAY and SILAGE groups compared to LIMIT pens. Fat color and final carcass price were similar ($P$ = 0.55; 2.80, $2.34/kg) across treatments. Despite similar performance and improved feed efficiency, higher feed costs resulted in LIMIT cattle having higher ($P$ < 0.01; $2.00/kg) feed cost/kg of gain than HAY or SILAGE treatments ($1.43 and $1.35, respectively).

**KEYWORDS**: cull cows, self-fed

## Introduction

Cull cows are responsible for a significant portion of annual income for beef cattle producers (15-30%), while also providing a source of meat for the packing industry (Feuz, 1999). The National Cow and Bull Beef Quality Audit (Roeber et al., 1999) identified management techniques available to producers to increase the quality and value of beef from cull cows, but research investigating additional income opportunities from cull cows is also important. Historically spring calving herds tend to sell open or other cull cows after weaning, flooding the fall market and creating seasonally low prices. One possibility for optimizing income may be feeding cull cows, taking advantage of relatively efficient gains (Matulis et al., 1987) and postponing the sale of cull cows until the market is less saturated (Yager et al., 1979). The length of the feeding period for cull cows has been suggested as 45 to 60 d to optimize feed conversion to gain (Sawyer et al., 2004), though Wooten (et al., 1979) cautions that while more time on feed improves both fat and lean deposition, initial body condition also contributes, and so deposition is not constant. Boyles (2001) suggests a feeding period of at least 60 d to allow yellow fat from forage diets to be converted to white fat. Corn has been a reasonably priced energy source for several decades, but increasing grain prices, drought conditions in the Midwest and Western High Plains, and rising input costs have impacted cull cow enterprise budgets and potential profitability. Re-evaluating the economic profitability of various feeding strategies has become necessary for determining feasibility of cull cow programs at the production level. Our objective was to evaluate traditional drylot diets versus range-based cull cow finishing programs in performance, carcass traits, and economics. Our hypothesis was that feed performance and carcass traits would be similar across feeding methods, but that self-fed range-based programs may potentially decrease feed and overall costs.

## Materials and Methods

### *Animals*

All procedures for the following experiment were approved by the University of Wyoming Animal Care and Use Committee. Seventeen Angus cross cull cows from the University of Wyoming Beef Unit, Laramie, and 55 Angus cross cull cows (average price of $0.946/kg) purchased from livestock auction in Torrington, WY, were shipped to the James C. Hageman Sustainable Agriculture Research and Extension Center in Lingle, WY. All cows were weighed, body condition scored (BW 554 ± 20 kg, BCS 4.95 ± 0.16), and had age estimated by dentition. Cows were vaccinated for respiratory and clostridial diseases, dewormed, and assigned to nine pens based on similar ($P$ = 0.12) pen weights, BCS, and age status. Each pen was then randomly assigned to one of three treatments.

### *Diets*

Specific nutritional information of the diets used is summarized in Tables 1 and 2. Animals on all treatments were provided ad libitum fortified trace mineral salt. Treatments were based on diets available in the High Plains Region in the United States, and included two traditional corn-based diets and one self-fed diet using a controlled

intake system (Accuration and Impact Finisher, Purina Mills, St. Louis, Missouri). The corn-based diets used mixed alfalfa and grass hay with barley straw (**HAY**) or corn silage (**SILAGE**) as the roughage source to accompany rolled corn and a commercial feedlot supplement containing Rumensin (Elanco, Greenfield, IN). Two step-up diets were formulated to acclimate the cull cows to the final diet over a month-long period. The final ration for both HAY and SILAGE were formulated to provide 1.32 Mcal $NE_g$/kg and 11.5% CP. The controlled intake system (**LIMIT**) used self-feeders and little or no alfalfa grass mix hay, potentially simulating range-based cull cow finishing programs. The use of the LIMIT diet followed the protocol developed by Purina nutritionists. Animals on the LIMIT treatment required two weeks to adapt to the self-feeder, initially, free-choice hay was provided via round bale feeders, and the limit-fed grain diets were gradually introduced while removing available forage from the pen.

**Table 1. Composition of HAY and SILAGE diets**

| Item | HAY FINAL | SILAGE FINAL |
|---|---|---|
| Ingredient composition, % | | |
| Alfalfa Hay | 9.9 | 7.8 |
| Corn Silage | - | 20.0 |
| Barley Straw | 5.8 | - |
| Corn Grain | 80.8 | 68.7 |
| 40-30 FDLT SU | 3.2 | 3.2 |
| Nutrient composition | | |
| DM, % | 89.2 | 66.9 |
| CP, % DM | 11.5 | 11.5 |
| $NE_m$, Mcal/kg DM | 2.22 | 2.22 |
| $NE_g$, Mcal/kg DM | 1.32 | 1.32 |
| C:P | 1.60 | 1.72 |

**Table 2. Composition of LIMIT self-fed diets**

| | ACCURATION[a] | | IMPACT[b] |
|---|---|---|---|
| **Item** | **d 1-16** | **d 17-29** | **d 30-92** |
| Ingredient composition | | | |
| Mixed Hay | Ad lib | - | - |
| Barley Straw | Ad lib | Ad lib | - |
| Corn Grain | 40% | 70% | 90% |
| Ration | 60% | 30% | 10% |
| Nutrient composition | | | |
| CP, min % | 32.0 | 32.0 | 44.0 |
| Crude Fat, min % | 9.00 | 9.00 | 1.50 |
| C:P | 2.00 | 2.00 | 6.66 |

[a] Contains 130.0 g/T Monensin as Monensin sodium.
[b] Contains 227.0 g/T Monensin as Monensin sodium, and 90.0 g/T Tylosin, as Tylosin phosphate.

***Measurements and Analysis***

Animals were weighed two weeks before the start of the feeding trial, and then on d 1, 2, 29, 60, 90, and 91 of the feeding trial. Both initial and final weights were determined by averaging weights taken on consecutive days. Cows were also implanted with Finaplix-H implants (trenbolone acetate, Intervet, Millsboro, DE) on d 29 to increase feedlot performance as demonstrated by Cranwell (et al., 1996) and Wright (2005). Cattle were harvested on d 94 at Gibbon Packing, L.L.C., Gibbon, NE, with final carcass data collected at the plant on d 95 and 96. Prices of feedstuffs were based on a daily USDA report and averaged across the duration of the study, and are reported in Table 3. Hay, straw, and corn nutrient analyses were completed by SDK Laboratories, Inc. (Hutchinson, Kansas), while Accuration and Impact Finisher data were based on information provided by Purina Mills (St. Louis, Missouri).

***Statistical Analysis***

Cattle performance, carcass traits, and economic data were analyzed in a completely randomized design using the GLM procedures of SAS (Version 9.1, SAS Inst., Inc., Cary, NC), with pen as the experimental unit. Least square means are reported, with means separated by least squares procedure when overall $P$-value < 0.05. Proc FREQ and CHISQ were used to interpret USDA quality grade distribution for individual cows.

## Results and Discussion

***Feedlot Performance***

Performance data for cull cows managed under three feeding strategies are summarized in Table 4. Although all treatments achieved similar ($P$ = 0.63; 156 kg, 1.71 kg/d) weight gain during the entire 91 d study, cows assigned to HAY and SILAGE rations attained more rapid gains in the first month ($P$ < 0.01), while animals on LIMIT diet had greater gains ($P$ = 0.02) in the final period. Average daily gain (ADG) for the first month was greater ($P$ < 0.01) for HAY and SILAGE treatments than for LIMIT treatment (2.71 kg/d, 2.65 kg/d, and 1.02 kg/d, respectively), partially due to the adaptation period required for cows on the LIMIT treatment. Cattle assigned to LIMIT diets were initially offered a grass/alfalfa mix hay in round bale feeders, a higher quality hay than recommended in diet transition guidelines, partially explaining the group's slow adaptation to the limit-fed grain diet. On d 13, the mixed hay was replaced with barley straw and cattle began consuming the self-fed diets. Although DMI for that same period was less ($P$ < 0.01) for SILAGE cows than for both HAY and LIMIT animals (12.3, 16.0, and 15.7 $kg \cdot hd^{-1} \cdot d^{-1}$), the animals on the LIMIT feeders were initially consuming the free-choice mixed hay instead of the higher energy supplement, affecting overall energy intake and ADG. While performance was similar, LIMIT cattle consumed less total feed per head per day ($P$ < 0.01; 13.4 vs. 16.3 and 15.7 $kg \cdot hd^{-1} \cdot d^{-1}$ for LIMIT, HAY and SILAGE, respectively) and had more efficient gains ($P$ < 0.01; 12.5 vs. 10.2 kg/100 kg) than HAY treatment, with SILAGE intermediate (11.3 kg/100 kg). Despite similar total performance and improved feed efficiency, higher total feed costs (Table 3) resulted in LIMIT cattle having higher ($P$ < 0.01; $2.00/kg) feed cost/kg of gain than HAY or SILAGE treatments ($1.43/kg and $1.35/kg, respectively).

***Carcass Characteristics***

Of the 72 cull cows sent to harvest, one cow was injured during transport, was deemed a downer animal, and did not have carcass data collected. Results of carcass data collection are found in Table 5 for cull cows on the three

feeding strategies. Hot carcass weights, 12th rib backfat, and Longissimus area were similar ($P > 0.58$; 362 kg, 11.7 mm, 73.8 $cm^2$) across treatments. These results are expected based on all cows having the same harvest date. Studies by Wooten (et al., 1979), and Matulis (et al., 1987) indicate that both HCW and backfat increased as days on feed increased, to the end of their studies (108 and 84 days on feed, respectively). Marbling tended ($P = 0.08$) to be greater for HAY than for LIMIT treatment, with SILAGE intermediate (all Slight; 366, 318, and 356, respectively). Dressing percentage was also higher ($P < 0.01$) for HAY cattle than for either SILAGE or LIMIT diets (53.2% vs. 51.3% and 51.5% respectively). Fat color and final carcass price were also similar ($P = 0.55$; 2.80, $2.34/kg) across treatments.

***Economic Analysis***

Initial November average value for the cull cows used in this study was $0.946/kg, or $524.08/hd. Feeding cull cows for an additional 91 d on the three strategies increased the average cow value to $845.86 ± 26.48, with no difference between treatments ($P = 0.87$). Holding cull cows for an additional 91 d demonstrated an average of $321.78/hd increased income. Feed costs for the entire trial period, based on the prices found in Table 3, for the three treatments were greater for LIMIT animals (data not shown, $P < 0.01$, $306.24/hd) than for those in HAY and SILAGE groups ($216.40/hd and $217.93/hd, respectively). Therefore, while both HAY and SILAGE treatments had the potential to capture $104.61/hd profit by feeding cull cows for 91 d, the use of the LIMIT diet could only augment profit by an average of $15.54/hd.

**Table 3. Prices used for estimation of feedstuffs**

| Feed | Cost, $/unit |
|---|---|
| Corn Silage | $46/metric ton |
| Alfalfa/Grass Hay | $121/metric ton |
| Straw Hay | $61/metric ton |
| Corn | $3.52/bu. |
| 40% Protein Supplement | $397/metric ton |
| Self-fed rations: | |
| Accuration | $324/metric ton |
| Impact | $272/metric ton |

## Conclusions

Despite the initial lag in performance, cattle assigned to the LIMIT treatment responded quickly and eventually achieved similar weight gain compared to cattle assigned to HAY and SILAGE treatments. Cows receiving the silage-based and hay-based finishing rations had similar feed efficiencies and similar feed cost of gains. In comparison, LIMIT cows consumed less feed and had more efficient gains than HAY and SILAGE treatments; however, overall cost of gains were higher, contrary to our initial hypothesis. While there were differences in lean maturity, skeletal maturity and lean color, carcass prices were similar for all treatments.

## Implications

As a significant portion of income for the beef producer, cull cow profitability is a key concern for both producers and researchers. Using available resources to add additional value and improve market timing for cull cows may increase profit for the producer. This profit potential is dependent upon the initial body condition, cost and availability of feed, length of feeding period, and final carcass traits. Traditional methods may be more expensive in the future, as grain prices and fuel costs continue to increase, making these strategies less appealing. Range-based methods explored in this study were equally effective, but were more expensive on a per kg gain basis than traditional methods. Further studies in this area should investigate other low-cost feeds, alternative marketing opportunities, and the development of value-added by-products from cull cows to improve overall profitability.

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**Table 4. Impact of cull cow feeding and management strategies on feeding performance and costs**

| Item | HAY[a] | SILAGE[b] | LIMIT[c] | SE | *P*-Value[d] |
|---|---|---|---|---|---|
| Cows | 24 | 24 | 24 | - | - |
| Pens (8 hd/pen) | 3 | 3 | 3 | - | - |
| Pre-trial BW, kg | 550.5 | 567.3 | 546.4 | 11.7 | 0.45 |
| Initial BCS (1-9) | 5.05 | 4.84 | 4.95 | 0.09 | 0.29 |
| **First Month, 29 d** | | | | | |
| ADG, kg/d | 2.71[g] | 2.65[g] | 1.02[f] | 0.16 | <0.01 |
| DMI, kg·hd$^{-1}$·d$^{-1}$ | 16.0[g] | 12.3[f] | 15.7[g] | 0.16 | <0.01 |
| G:F, kg/kg | 0.170[g] | 0.214[h] | 0.064[f] | 0.013 | 0.04 |
| Feed cost/gain, $/kg | 0.73[f] | 0.66[f] | 2.86[g] | 0.12 | <0.01 |
| **Second Month, 31 d** | | | | | |
| ADG, kg/d | 1.14 | 1.21 | 1.60 | 0.23 | 0.37 |
| DMI, kg·hd$^{-1}$·d$^{-1}$ | 16.9[g] | 17.9[g] | 11.6[f] | 0.43 | <0.01 |
| G:F, kg/kg | 0.070[f] | 0.069[f] | 0.143[g] | 0.013 | 0.01 |
| Feed cost/gain, $/kg | 2.33 | 2.75 | 1.94 | 0.60 | 0.65 |
| **Third Month, 31 d** | | | | | |
| ADG, kg/d | 1.22[f] | 1.56[f] | 2.45[g] | 0.22 | 0.02 |
| DMI, kg·hd$^{-1}$·d$^{-1}$ | 16.2[g] | 16.8[g] | 13.2[f] | 0.71 | 0.02 |
| G:F, kg/kg | 0.074[f] | 0.090[f] | 0.178[g] | 0.013 | 0.01 |
| Feed cost/gain, $/kg | 2.22 | 1.74 | 1.74 | 0.21 | 0.24 |
| **Total Test, d 91** | | | | | |
| Initial BW, kg[e] | 535.9 | 550.5 | 536.4 | 12.6 | 0.67 |
| Final BW, kg | 687.7 | 713.2 | 690.5 | 17.6 | 0.57 |
| 91 d gain, kg | 151.8 | 162.7 | 154.1 | 8.0 | 0.63 |
| ADG, kg/d | 1.67 | 1.79 | 1.70 | 0.09 | 0.63 |
| DMI, kg·hd$^{-1}$·d$^{-1}$ | 16.3[g] | 15.7[g] | 13.4[f] | 0.39 | <0.01 |
| G:F, kg/kg | 0.102[g] | 0.113[f,g] | 0.125[f] | 0.005 | 0.03 |
| Feed cost/gain, $/kg | 1.43[f] | 1.34[f] | 2.00[g] | 0.07 | <0.01 |

[a] Dry finishing ration consisting of alfalfa/grass hay, straw, corn and commercial feedlot protein supplement, calculated to provide 1.32 Mcal $NE_g$/kg and 11.5% CP

[b] Silage-based finishing ration consisting of silage, alfalfa/grass hay, corn and commercial feedlot protein supplement, calculated to provide 1.32 Mcal $NE_g$/kg and 11.5% CP

[c] Self-fed finishing ration, offered ad-libitum via self-feeders placed in pens

[d] Data analyzed as a completely randomized design using GLM of SAS. Least square means are reported, with means separated by least squares procedure when Overall *P*-value < 0.05.

[e] Initial and final weights determined by averaging two consecutive day weights.

[f,g,h] Means with different superscripts differ ($P < 0.05$).

**Table 5. Impact of cull cow feeding and management strategies on carcass traits**

| Item | HAY[a] | SILAGE[b] | LIMIT[c] | SE | *P*-Value[d] |
|---|---|---|---|---|---|
| Cows | 24 | 23[l] | 24 | - | - |
| Pens (8 hd/pen) | 3 | 3 | 3 | - | - |
| Final live BW[e], kg | 687.7 | 713.2 | 690.5 | 17.6 | 0.57 |
| HCW, kg | 366.8 | 365.9 | 356.4 | 10.4 | 0.74 |
| Dressing % | 53.2[k] | 51.3[j] | 51.5[j] | 0.01 | <0.01 |
| Lean maturity[f] | 452 | 434 | 471 | 8.9 | 0.07 |
| Skeletal maturity[f] | 576[k] | 560[j,k] | 548[j] | 5.9 | 0.04 |
| Marbling[g] | 366 | 356 | 318 | 12.8 | 0.08 |
| 12$^{th}$ rib backfat, mm | 11.9 | 11.9 | 11.2 | 2.5 | 0.90 |
| Longissimus area, cm$^2$ | 74.2 | 74.8 | 72.3 | 1.9 | 0.58 |
| Muscling score (1-5) | 3.54[k] | 2.84[j] | 3.08[j,k] | 0.15 | 0.02 |
| Fat color[h] | 2.62 | 2.95 | 2.83 | 0.21 | 0.56 |
| Lean color[i] | 5.58[j] | 5.58[j] | 6.20[k] | 0.12 | 0.01 |
| Carcass price, $/kg | 2.32 | 2.32 | 2.34 | 0.01 | 0.55 |
| Calc. quality grade, % ≥ Utility | 70.83 | 69.57 | 50.00 | - | 0.25 |
| Total cow value, $ | 852.13 | 851.13 | 834.32 | 26.48 | 0.87 |

[a] Dry finishing ration consisting of alfalfa/grass hay, straw, corn and commercial feedlot protein supplement, calculated to provide 1.32 Mcal $NE_g$/kg and 11.5% CP

[b] Silage-based finishing ration consisting of silage, alfalfa/grass hay, corn and commercial feedlot protein supplement, calculated to provide 1.32 Mcal $NE_g$/kg and 11.5% CP

[c] Self-fed finishing ration, offered ad-libitum via self-feeders placed in pens

[d] Data analyzed as a completely randomized design using GLM of SAS. Least square means are reported, with means separated by least squares procedure when Overall *P*-value < 0.05.

[e] Initial and final weights determined by averaging two consecutive day weights.

[f] A = 100 to 199, B = 200 to 299, C = 300 to 399, D = 400 to 499, and E = 500 to 599.

[g] Slight = 300 to 399.

[h] 1 = white; 5 = yellow.

[i] 1 = bright cherry red; 8 = dark brown.

[j,k] Means with different superscripts differ ($P < 0.05$).

[l] During transport to harvest, one cow was injured and arrived as a downer cow, therefore no data was collected.

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# EFFECTS OF BRANCHED-CHAIN AMINO ACID SUPPLEMENTATION ON GROWING STEERS DURING AN ENDOTOXIN CHALLENGE

**G. G. Gilliam, B. C. Graham, J. W. Waggoner, K. L. DeAtley, D. M. Hallford, and C. A. Löest**
New Mexico State University, Las Cruces, NM

**ABSTRACT:** Steers exposed to an endotoxin may require additional branched-chain AA (**BCAA**) to support acute-phase response protein synthesis. This study evaluated effects of bacterial lipopolysaccharide (**LPS**) and BCAA supplementation on N retention and blood metabolites of 20 ruminally cannulated steers (177 ± 4.2 kg BW). The experiment was a randomized block design, with 14-d adaptation to metabolism stalls and diet (DM fed = 1.5% BW), and 6-d collection. Treatments were a 2 × 2 factorial of LPS (0 vs ≥1.0 µg/kg BW; **-LPS** vs **+LPS**) and BCAA (0 vs 35 g/d; **-BCAA** vs **+BCAA**). The LPS in 100 mL sterile saline was infused (1 mL/min via i.v. catheter) on d 15. The BCAA in an essential AA solution were abomasally infused (900 mL/d) 3 times daily in equal portions beginning on d 7. Blood was collected on d 15 at h 0, 2, 4, 8, 12, and 24 after LPS infusion. Feces and urine were collected from d 16 to 20. No LPS × BCAA × h interactions ($P > 0.23$) were observed for blood metabolites. Serum cortisol increased 2 h after LPS infusion, peaked at h 4, and was greater for +LPS than -LPS steers at 8, 12, and 24 h thereafter (LPS × h, $P < 0.01$). Serum cortisol was greater for +BCAA than -BCAA steers at 12 h, and not different at 0, 2, 4, 8, and 24 h after LPS infusion (BCAA × h, $P < 0.05$). Plasma Ile, Leu, and Val were lower, and plasma His was greater in +LPS than -LPS steers (LPS, $P < 0.05$). Plasma Met, Lys, Thr, and Trp decreased at 4 h and was lower at 8, 12, and 24 h after infusion for +LPS than -LPS steers (LPS × h, $P < 0.05$). Steers infused with +BCAA had greater plasma Ile, Leu, and Val, and lower Met, Thr, His, Phe, and Trp than -BCAA steers at 0 h and 24 h after LPS infusion (BCAA × h, $P < 0.05$). No LPS × BCAA interactions ($P > 0.24$) were observed for N balance. Intake, fecal, digested, and retained N were lower ($P < 0.05$) for +LPS than -LPS steers. Also, intake and fecal N were greater ($P < 0.05$), and retained N tended to be greater ($P = 0.11$) for +BCAA vs -BCAA steers. These results imply that BCAA potentially limit N retention of growing steers, but BCAA supplementation does not alleviate negative effects associated with endotoxin-challenged steers.

**Key Words:** branched-chain amino acid, endotoxin, steers

## INTRODUCTION

Morbidity due to exposure of newly received feedlot calves to infectious agents, such as bovine respiratory disease complex, has a negative impact on performance and gross income (Waggoner et al., 2007b). Clinical and metabolic changes in morbid cattle have been shown to alter protein requirements (Waggoner et al., 2007a), possibly due to the repartitioning of AA away from synthesis of tissue proteins and towards synthesis of immune system proteins (Le Floc'h et al., 2003).

Waggoner et al. (2006) demonstrated that N excretion increases and plasma concentrations of branched-chain AA (**BCAA**) decrease in growing steers exposed to a bacterial lipopolysaccharide (**LPS**). According to Calder (2006), the immune system is dependent on BCAA for synthesis of acute-phase response proteins. Also, BCAA have been shown to "preserve" N balance by decreasing muscle protein catabolism in rats (Choudry et al., 2006). Therefore, we hypothesized that BCAA would improve N retention of endotoxin-challenged steers. The objective was to study the interactions of bacterial LPS exposure and supplemental BCAA on N balance and blood metabolites in growing steers.

## MATERIALS AND METHODS

***Animals, Facilities, and Diet.*** New Mexico State University's Institutional Animal Care and Use Committee approved all procedures. Twenty ruminally cannulated Angus steers (177 ± 4.2 kg initial BW) were housed in individual tie stalls of a metabolism barn with evaporative cooling. Steers were allowed free access to fresh water and were fed a wheat-based diet (Table 1). The diet was fed in 2 equal portions twice daily at 0700 and 1900, and daily DM offered was limited to 1.5% of BW to represent low intakes for newly received feedlot calves (NRC, 2000).

***Experimental Design.*** The experiment was a randomized block design, and the duration of the study was 20 d. This allowed for a 14-d period for adaptation to facilities and diet, 1 d for collection of blood, and a 5-d period for feces and urine collections. Beginning on d 7, all steers received abomasal infusions (900 mL/d) of an essential AA solution in equal portions 3 times daily (at 0900, 1500, and 2100). The AA solution supplied 5 g L-Arg, 5 g L-His, 10 g L-Lys, 5 g L-Met, 5 g L-Phe, 5 g L-Thr, and 2.5 g L-Trp daily. The AA solution was infused via a flexible tube (3.2 mm i.d.) that was placed through the rumen cannula and reticulo-omasal orifice. On d 14 jugular catheters (J-457A; Jorgenson Laboratories, Loveland, CO) were inserted for the infusion of LPS and blood collection.

***Treatments.*** Treatments were arranged as a 2 × 2 factorial, and included 2 doses of LPS infusions (0 vs ≥1.0 µg/kg BW; **-LPS** vs **+LPS**) and 2 amounts of BCAA supplementation (0 vs 35 g/d; **-BCAA** vs **+BCAA**). The LPS (*E. coli* 055:B5; Sigma Chem. Co., St. Louis, MO) was dissolved in 100 mL of sterile saline and infused (1 mL/min via i.v. catheter) at 3 h after feeding on d 15. The dose of

LPS was initially 1.5 µg/kg BW (block 1), but after the death of a steer the LPS dose was lowered to 1.0 µg/kg BW (block 2). Sterile saline was infused into -LPS steers. The BCAA (10 g Ile, 15 g Leu, and 10 g Val) were dissolved in the essential AA solution that was infused into the abomasum.

Table 1. Diet composition

| Item | DM basis |
|---|---|
| *Ingredient, %* | |
| Wheat grain | 30.0 |
| Corn silage | 21.3 |
| Alfalfa hay | 20.0 |
| Soybean hulls | 20.0 |
| Molasses | 4.0 |
| Tallow | 2.5 |
| Minerals[1] | 1.83 |
| Urea | 0.30 |
| Vitamins[2] | 0.04 |
| Ruminsin-80[3] | 0.02 |
| *Nutrient* | |
| $NE_g$, (Mcal/kg) | 1.19 |
| CP, % | 14.23 |
| Ca, % | 0.87 |
| P, % | 0.39 |

[1]Supplied (% of DM): limestone (0.50), dicalcium phosphate (0.50), sodium bicarbonate (0.50), salt (0.30), and trace minerals (0.03).
[2]Supplied 1,500 IU Vit A, and 150 IU Vit E per kg DM.
[3]Supplied 33 mg monensin per kg DM.

***Collections.*** Dietary samples, feed refusals, and fecal and urinary excretions were collected from each steer on d 16 through 20 to determine N balance. For collection of urine, steers were fitted with vacuum pouches connected to vessels containing 600 mL of 3 *M* HCL (to minimize $NH_3$ loss). For collection of feces, steers were fitted with fecal collection bags. Representative samples of feces (10%) and urine (1%) were frozen for later analysis.

On d 15 at 0, 2, 4, 8, 12, and 24 h after LPS infusion, blood samples were collected via the jugular catheter into vacuum tubes (Corvac serum separator and Monoject Sodium Heparin, Kendall, Ontario, CA). Blood samples for serum were allowed to coagulate at room temperature for 30 min, and samples for plasma were immediately chilled on ice. All samples were centrifuged at 1,500 × g for 20 min at 10ºC, and the supernatant transferred to plastic vials and frozen. Rectal temperatures were measured (Cooper TM99A digital thermometer, Cooper Atkins Corp., Middlefield, CT) at similar times of blood sampling.

***Sample Analysis.*** Diet, feed refusals, and fecal samples were dried at 55ºC in a forced air oven, then ground to pass a 2-mm screen. Samples were analyzed for DM (105ºC for 24 h) and ash (550ºC for 8 h). Also, all dried samples and urine were analyzed for N by total combustion (LECO FP-528, LECO Corp., St. Joseph, MI). Serum cortisol concentrations were analyzed by RIA (Kiyma et al., 2004) with assay CV < 4.5%. Plasma AA concentrations were analyzed by gas chromatography (Varian CP-3800, Varian, Walnut Creek, CA) using a commercially available kit (EZ:FAAST; Phenomenex, Torrance, CA).

***Statistical Analysis.*** Data was analyzed statistically as a randomized block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The metabolism facility had only 12 tie-stalls, therefore steers were blocked by date of collection (8 steers in block 1, and 12 steers in block 2). For all dietary measures, the statistical model included effects of BCAA, LPS, and the interaction. For rectal temperature and blood metabolites, the model included all combination of BCAA, LPS, and h using repeated measures subjected to autoregressive order one covariance structure. Means were least squares, and significance was declared at $P < 0.05$.

## RESULTS

***Rectal Temperature and Blood Metabolites.*** No LPS × BCAA × h interactions ($P > 0.23$) were observed for rectal temperature, serum cortisol, and plasma AA concentrations. Rectal temperatures (Figure 1) increased from 2 to 4 h, then decreased at 8 h, and were not different at 12 and 24 h after LPS infusion for +LPS vs -LPS steers (LPS × h, $P < 0.01$). Serum cortisol concentrations (Figure 1) increased 2 h after LPS infusion, peaked at h 4, and was greater for +LPS than -LPS steers at 8, 12, and 24 h thereafter (LPS × h, $P < 0.01$). Also, serum cortisol was greater for +BCAA than -BCAA steers at 12 h, but not different at 0, 2, 4, 8, and 24 h after LPS infusion (BCAA × h, $P < 0.05$; data not shown). Steers infused with +LPS had lower plasma Ile, Leu, and Val concentrations, and higher His concentrations than -LPS steers (LPS, $P < 0.05$; Figure 2). Plasma concentrations of Met, Lys, Thr, and Trp in +LPS steers decreased 4 h after LPS infusion and remained lower at 8, 12, and 24 h thereafter (LPS × h, $P < 0.05$). Steers infused with +BCAA had greater plasma concentrations of Ile, Leu, and Val, and lower concentrations of Met, Thr, His, Phe, and Trp concentrations than -BCAA steers at 0 and 24 h after LPS infusion (BCAA × h, $P < 0.05$; Figure 3).

***Digestibility and N Balance.*** No LPS × BCAA ($P > 0.24$) interactions were observed for N balance (Table 2). Infusion of LPS lowered (LPS, $P < 0.05$) intake, fecal, digested, and retained N of +LPS vs -LPS steers. Steers infused with +BCAA had greater intake and fecal N (BCAA, $P < 0.05$) and tended to have greater N retention (BCAA, $P = 0.11$) than -BCAA steers.

## DISCUSSION

***Endotoxin Challenge.*** Steers exposed to purified LPS exhibited symptoms of an inflammatory response to bacterial infection, as evidenced by increased serum concentrations of cortisol and altered rectal temperatures. These responses are similar to those reported for endotoxin-challenged steers by Waggoner et al. (2006, 2007a). Also, Waggoner et al. (2006) reported that LPS-challenged steers had increased levels of cytokines (tumor necrosis factor alpha) that stimulate synthesis of immune system proteins (e.g. haptoglobin). Lower concentrations of plasma BCAA (Ile, Leu, and Val), and decreases in plasma concentrations

of Lys, Met, Thr, and Trp in steers exposed to LPS suggest that these AA were potentially utilized to support synthesis of immune system proteins.

Lower N retention for steers infused with LPS was in part due to lower N intake and N digestibility, and in part due to a tendency for greater urinary N excretion. This experiment was designed to minimize differences in feed intake by limit-feeding steers at 1.5% BW (DM basis) to represent low intakes for newly received feedlot calves (NRC, 2000). In this study, DM intakes of endotoxin-challenged steers averaged only 1.0% of BW. Low intakes and digestibility are possibly due to negative effects of LPS on passage rates and gastrointestinal motility (Waggoner et al., 2006). Regardless of lower N intakes, urinary N excretion tended to be greater in LPS-challenged steers, possible because of an increase in tissue protein degradation for the mobilization of AA to support synthesis of immune system protein. According to Reeds and Jahoor (2001), catabolism of AA due to imbalances between tissue AA supply and immune system AA requirements may explain greater urinary N excretion.

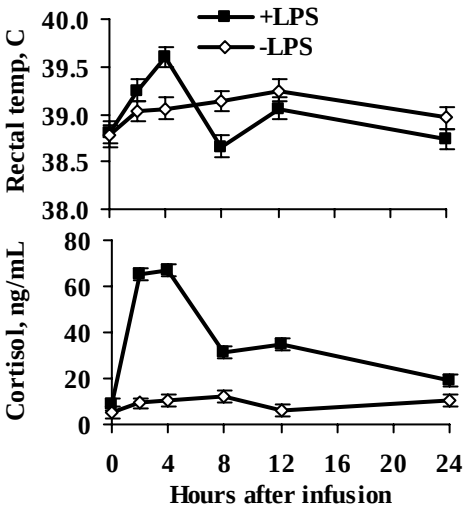


Figure 1. Effects of intravenous infusion of sterile saline vs ≥1.0 μg lipopolysaccharide per kg BW (-LPS vs +LPS) on rectal temperatures and serum cortisol of growing steers (LPS x h, $P < 0.01$).

***Supply of BCAA.*** Greater plasma Ile, Leu, and Val concentrations indicate that our abomasally infused treatment effectively supplied absorbable BCAA. Lower concentrations of His, Met, Phe, Thr, and Trp, and a tendency for greater N retention in BCAA-supplemented steers suggests that at least one of the BCAA were limiting, and therefore increased the utilization of plasma AA for protein synthesis. The lack of BCAA × LPS interactions on N balance and blood metabolites suggests that BCAA supplementation does not alleviate negative effects associated with an endotoxin-challenge. However, the death of two +BCAA steers infused with LPS suggest that BCAA may have affected immune responses that we did not measure.

***Conclusions.*** These findings imply that BCAA limit N retention in growing steers, but do not alleviate the negative effects of an endotoxin.

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Table 2. Effects of lipopolysaccharide (LPS) and branched-chain AA (BCAA) supplementation on N retention of steers

| | Treatments[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | -LPS | | +LPS | | | *P*-value | | |
| N, g/d | -BCAA | +BCAA | -BCAA | +BCAA | SEM[2] | LPS × BCAA | LPS | BCAA |
| Intake | 64.4 | 71.2 | 47.4 | 63.5 | 4.58 | 0.30 | 0.01 | 0.02 |
| Feces | 15.7 | 18.0 | 10.2 | 14.5 | 1.50 | 0.49 | <0.01 | 0.02 |
| Urine | 29.7 | 29.0 | 30.4 | 33.5 | 1.31 | 0.24 | 0.11 | 0.45 |
| Digested | 48.7 | 53.1 | 37.2 | 49.0 | 3.30 | 0.29 | 0.04 | 0.03 |
| Retained | 18.9 | 24.0 | 6.5 | 15.6 | 3.56 | 0.64 | 0.02 | 0.11 |

[1]Intravenous infusion of sterile saline vs ≥1.0 μg LPS per kg BW (-LPS vs +LPS), and abomasal infusion of 0 vs 35 g/d branched-chain AA (-BCAA vs +BCAA).

[2]Standard error of the mean (n = 5).

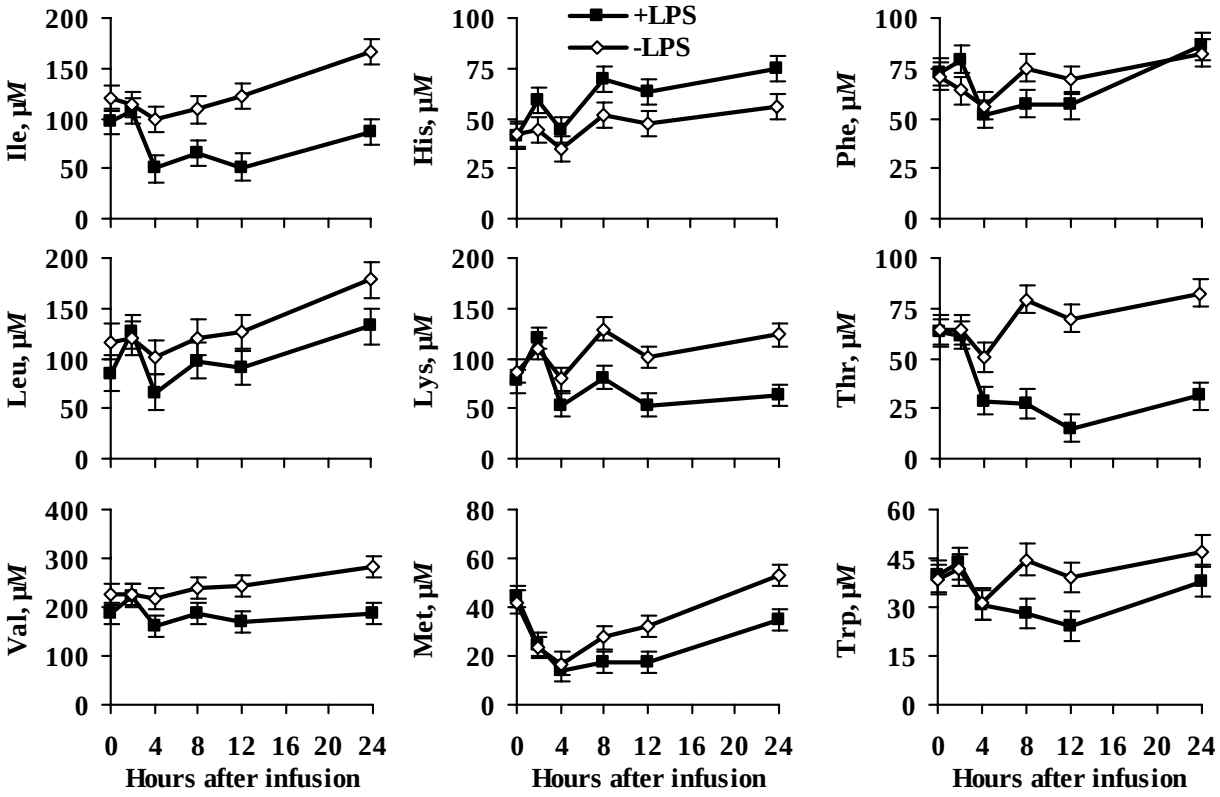


Figure 2. Effects of intravenous infusion of sterile saline vs ≥1.0 µg lipopolysaccharide per kg BW (-LPS vs +LPS) on plasma AA concentrations in growing steers. Effect of LPS × h ($P < 0.05$) for Lys, Met, Thr, and Trp; effect of LPS ($P < 0.05$) for Ile, Leu, Val, and His.

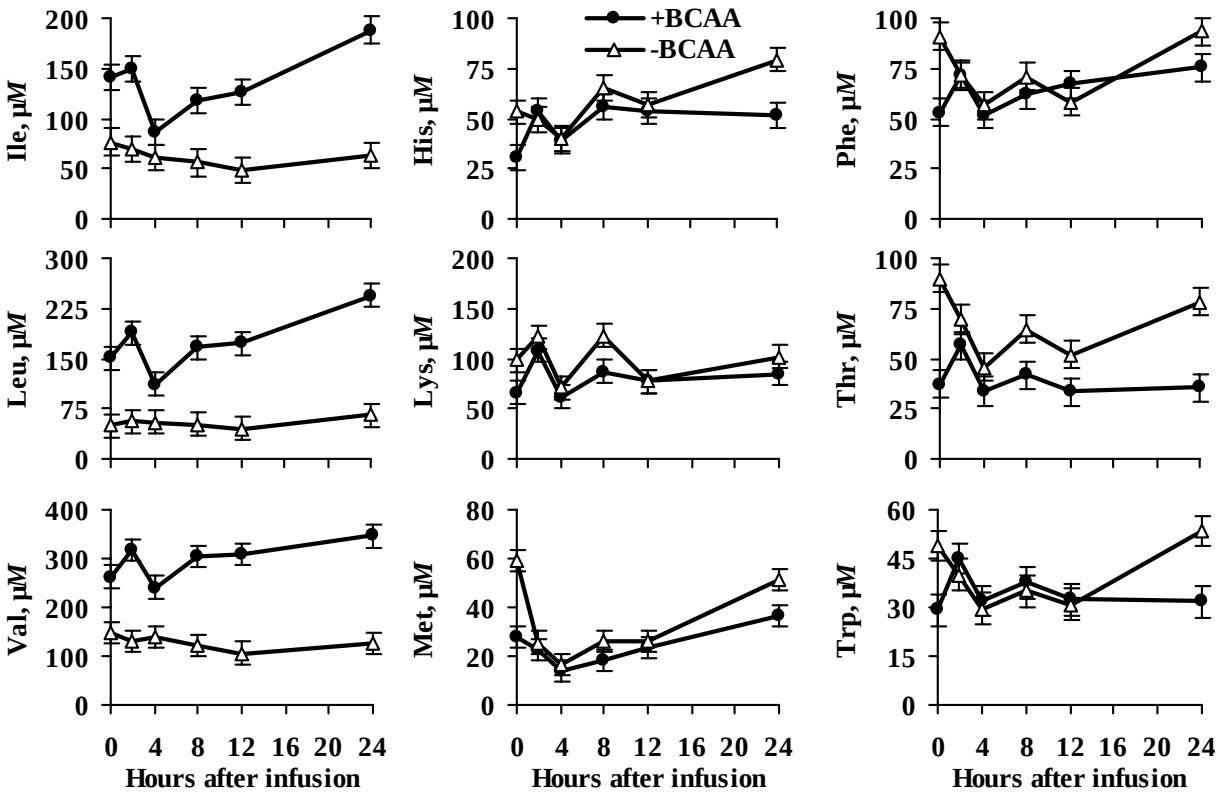


Figure 3. Effects of abomasal infusion of 0 vs 35 g/d branched-chain AA (-BCAA vs +BCAA) on plasma AA concentrations in growing steers. Effect of BCAA × h ($P < 0.05$) for Ile, Leu, Val, His, Met, Phe, Thr, and Trp.

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# REPRODUCTIVE ABNORMALITIES IN OMEGA-3 FATTY ACID DESATURASE TRANSGENIC MICE

**W.E. Pohlmeier*, K.A. Lewis, A.L. Van Eenennaam**
Department of Animal Science, University of California, Davis

**ABSTRACT:** Transgenic mice expressing the omega-3 fatty acid desaturase (*Fat-1*) gene from *Caenorhabditis elegans* under the control of the goat beta-casein promoter produce milk with elevated levels of omega-3 polyunsaturated fatty acids (PUFA). Additionally, Fat-1 transgenic mice display reproductive defects. When bred to wild-type (WT) males, Fat-1 females produce litters with an average size of 2.7 pups/litter, whereas WT females produce an average of 7.2 pups/litter. Ovulation rates do not differ between the two genotypes, however when uterine tracts were flushed at 3.5 days post coitus, Fat-1 females were observed to have significantly fewer ($p<0.05$) developing embryos. It was observed that implanted fetuses in pregnant Fat-1 females were also more frequently reabsorbed ($p<0.05$), suggesting problems with both pre- and post-implantation embryo survival in Fat-1 females. It is known that PUFA are the substrate for prostaglandin synthesis and that the PUFA content of the diet can influence ovarian and uterine function. We hypothesized that expression of the Fat-1 transgene was affecting ovarian function in Fat-1 females. To test this hypothesis, ovaries were removed and transplanted between Fat-1 and WT mice. This allowed for evaluation of the reproductive potential of the Fat-1 ovary in a WT recipient, and vice versa. Sham surgeries where ovaries were swapped between WT females were also performed. Following ovarian transplantation, females were bred and sacrificed at the 12th day of gestation. Wild-type females bearing a Fat-1 transgenic ovary averaged only 1.1 (±1.0) normally-developing pups/litter, and displayed significantly more ($p<0.05$) fetal reabsorptions/litter (2.2±1.9) compared to Fat-1 females harboring a WT ovary (0.1±0.4) and WT sham females (0.4±0.8), suggesting a relationship between transgenic ovaries and an increased incidence of fetal reabsorption. In addition, Fat-1 mice bearing a WT ovary also had a significantly ($p<0.05$) reduced litter size (2.0±2.4) when compared to WT sham females (7.2±2.1), suggesting that non-ovarian factors also contribute to the decreased reproductive capacity of Fat-1 transgenic mice.

Keywords: PUFA, reproduction

## INTRODUCTION

Both n-6 and n-3 polyunsaturated fatty acids (**PUFA**) can affect reproduction through changes in prostaglandin and steroid synthesis and membrane properties of the gametes (Wathes et al., 2007). Dietary PUFA content has been found to affect follicle size (Bilby et al., 2006) and number (Robinson et al., 2002) in dairy cattle. And in mice, dietary n-3 PUFA supplementation have been observed to decrease *in vivo* fertilization capacity and subsequent pre-implantation embryo development (Wakefield et al., 2007). PUFA provide the precursors for prostaglandin synthesis. The primary substrate for prostaglandin synthesis is the 20-carbon arachidonic acid. This n-6 fatty acid is also a substrate for the *Caenorhabditis elegans* n-3 fatty acid desaturase (***Fat-1***) which is able to act on a range of 18- and 20-carbon n-6 substrates (Spychalla et al., 1997). Cyclooxygenase-2 (COX-2) is the rate-limiting enzyme in the conversion of PUFA into prostaglandin substrates, and COX-$2^{-/-}$ knockout mice were found to have a number of reproductive defects, including decreased ovulation, fertilization and impaired embryonic implantation (Lim et al., 1997). Reproductive defects and a correspondingly small litter size phenotype was also observed in a line of transgenic mice (**Fat-1**) which express the *Fat-1* gene under the control of the goat beta-casein promoter which is known to be strongly expressed in the lactating mammary gland (Roberts et al., 1992). It was hypothesized that expression of the n-3 fatty acid desaturase transgene was affecting ovarian function in Fat-1 females. To test this hypothesis, ovarian transplantation surgeries were performed between C57BL/6 wild-type (**WT**) and Fat-1 mice.

## MATERIALS AND METHODS

*Animals.* Experimentation on animal subjects was conducted in accordance with the regulations of the American Association for Accreditation of Laboratory Animal Care in fully accredited facilities at the University of California, Davis. Transgenic mice expressing the *Fat-1* gene (GenBank accession number L41807) under the control of the goat beta-casein promoter of the pBC1 mammary expression vector (Invitrogen, Carlsbad, CA) were generated by pronuclear microinjection (Kao et al., 2006).

*Fetal Parameters.* To assess fetal parameters at mid-gestation, Fat-1 and WT mice were bred to fertile males and sacrificed at day 12 of gestation. The day of mating was determined by the presence of a vaginal plug. Following sacrifice, the total number of normally developing pups and also fetal reabsorptions was determined.

*Ovulation.* Fat-1 and WT females were housed with fertile WT males and checked daily for the presence of a vaginal plug. To assess ovulation rates, females were sacrificed on the morning on which the vaginal plug was observed at 0.5 days post coitus (**DPC**). Following sacrifice, oviducts were isolated and placed into M2 media (Sigma; St. Louis, MO). Once in M2, the cumulus sac was

identified and ruptured. The total number of ovulated oocytes was then quantified.

*Uterine Flush.* To determine if there were any differences in embryonic development between Fat-1 and WT mice, females were bred and sacrificed 3.5 DPC. Females were placed with a fertile male and the presence of vaginal plugs was observed. After observation of a vaginal plug, females were sacrificed at 3.5 DPC and their uterine tracts were isolated by cutting on the vaginal side of the cervix and also through the oviducts. Care was taken so as to dissect away as much connective tissue and fat from the uterus as possible before transferring the uterus to a drop of M2 media. At this point, the utero-tubal junctions were cut. Following bisection of the utero-tubal junction, a 27 gauge needle, attached to syringe containing M2 media was navigated through the cervix and into the lumen of one uterine horn. M2 was gently released to flush embryos from the uterine horn. Following flushing of the first horn, the process was repeated on opposite horn. The flushed uterus was then removed and the development of flushed embryos was assessed.

*Transgene Expression Analysis.* Expression analysis of the Fat-1 transgene in uterine and ovarian tissues was assessed through reverse transcription-PCR of RNA. Extraction of RNA from tissues was performed following the single-step extraction method (Chomczynski and Sacchi, 1987). In order to perform first strand cDNA synthesis, the following reagents; 1 to 3 µg of RNA, 1 µL of 0.5 µg/µL oligo$(dT)_{18\text{-}20}$, 1 µL of 10m*M* deoxyribo-nucleotide triphosphate (dNTP) mix, and sterile water to a total volume of 14 µL; were added to a microfuge tube and heated to 65°C for 5 min. The tube was then placed on ice for 1 minute. Next, to perform first-strand cDNA synthesis, 4 µL of 5X First-Strand Buffer, 1 µL of 0.1 *M* dithiothreitol, and 1 µL of (200 u/µL) SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA) were added to each tube and heated to 50°C for 60 min, followed by a heat inactivation period of 70°C for 15 min. cDNA samples were then used as a template for PCR using a goat beta-casein exon 1 forward primer (5'TCCATTCA-GCTTCTCCTTCA3') and a Fat-1 internal reverse primer (5'TTCCATGATGGCATTGCTT3'). These primers were designed so as to span the transgenic goat beta-casein intron 1, so following proper mRNA splicing, a 634-bp fragment would be amplified compared to a 2,655-bp fragment which would be amplified from genomic DNA. As a control, forward (5'GACGGCCAGGTCATCACTAT3') and reverse (5'AGTCCGCCTAGAAGCACTTG3') primers for the cDNA of the housekeeping mouse beta-actin gene were used to amplify a 406-bp product.

*Ovarian Transplantation.* In order to ascertain whether ovary function was contributing to the observed impaired reproductive phenotype in Fat-1 female mice, we performed ovarian transplants between 6 week old Fat-1 and WT females. This transplantation procedure, as previously described (Cargill et al., 1999), allowed us to evaluate the reproductive potential of females harboring Fat-1 ovaries in a WT environment, and WT ovaries in a Fat-1 environment. Females were anesthetized and the ovaries were exposed by paralumbar incision. Next, the ovarian bursa was opened and the ovary removed by clamping on the ovarian hilum. With the hilum clamped, the ovary was removed and placed in cold saline until it was transplanted into the recipient. The ovaries stored in cold saline were placed into the ovarian bursa of recipient females and the bursa was closed with 6-0 polyester suture. A total of 20 mice, 10 WT and 10 Fat-1 females were used in this experiment. In addition, 10 WT mice underwent a sham surgery where ovaries were swapped between WT females. Following surgery, females were allowed to recover for two weeks, at which point they were bred. Females were sacrificed at day 12 of their second post-operative gestation, and fetal parameters were assessed as described previously.

*Statistical Analysis.* Differences between treatment groups were tested using Student's T-test. Differences were considered significant at $P<0.05$.

## RESULTS

*Fetal Parameters.* Fat-1 females had significantly less normally developing pups and more fetal reabsorptions at day 12 of gestation than WT controls (Fig. 1).

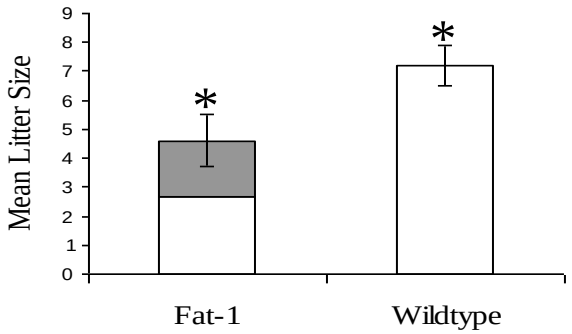


**Figure 1.** Mean litter size (±SEM) in Fat-1 and WT mice at the 12th day of gestation. White bars represent developing pups; grey shading represents fetal reabsorptions. WT females had significantly more (*$p<0.05$) normally-developed pups compared to Fat-1 females. A larger number of reabsorption sites were noted in Fat-1 females, suggesting a problem with the maintenance of pregnancy following implantation.

*Ovulation and Pre-implantation Development.* To determine whether decreased ovulation was a factor contributing to the reduced litter sizes seen with Fat-1 females, ovulation rate in both Fat-1 and WT females was assessed. The ovulation rate in Fat-1 females (6.9±0.71) did not differ ($p>0.05$) from that of WT females (6.6±0.86; Fig 2). However, when the uterine tracts of females were flushed at 3.5 DPC, Fat-1 females were observed to have significantly fewer ($p<0.05$) developing embryos (3.3±0.79) than WT females (8.3±0.27; Fig. 3) suggesting problems with pre-implantation embryo survival in the transgenic females.

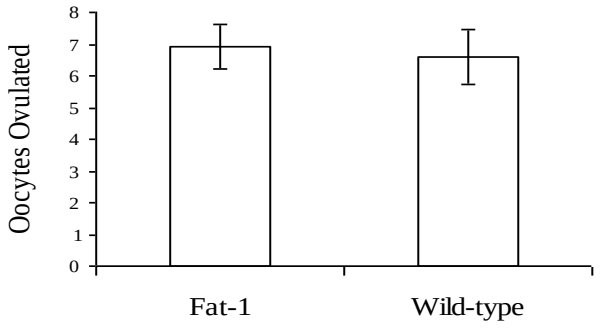


**Figure 2.** Mean number of oocytes ovulated in Fat-1 and WT females. The number of oocytes ovulated by Fat-1 females did not significantly differ from WT controls ($p$>0.05).

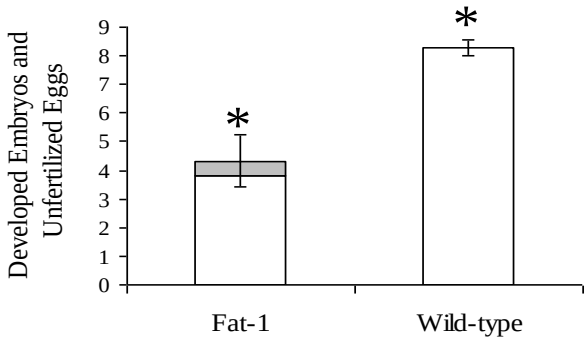


**Figure 3.** Mean number of developing embryos and unfertilized eggs at 3.5 days post coitus (DPC) in Fat-1 and WT females. White bars represent developed embryos, gray shading represents unfertilized eggs. Wild-type females had significantly more (*$p$<0.05) developing embryos than Fat-1 females.

*Ectopic Transgene Expression Analysis.* To determine whether there was evidence of ectopic expression of the *Fat-1* transgene in reproductive tissues, reverse transcriptase PCR was performed on ovarian and uterine tissues at 0.5 and 3.5 DPC. While ovarian and uterine tissues at both time points expressed the housekeeping gene beta-actin, neither of the tissues were positive for *Fat-1* expression (Fig. 4).

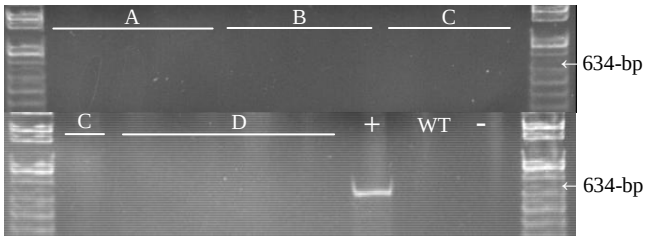


**Figure 4.** Reverse transcription-PCR analysis of ovarian and uterine tissue at 0.5 and 3.5 DPC. A) uterine tissue, 0.5 DPC; B) ovarian tissue, 0.5 DPC; C) uterine tissue, 3.5 DPC; D) ovarian tissue, 3.5 DPC; (+) positive control, Fat-1 mammary; (WT) Wild-type ovarian tissue, 0.5 DPC; (-) negative control, $H_20$. Neither ovarian nor uterine tissue showed expression of the *Fat-1* transgene at these two time points.

*Ovarian Transplantation.* To determine which reproductive tissue(s) were contributing to this reproductive phenotype, ovarian transplantation was performed between Fat-1 and WT females. As a control, sham surgeries between WT females were also performed. Following ovarian transplantation, females were sacrificed at day 12 of their second post-operative gestation. WT females bearing a Fat-1 ovary averaged only 1.1 (±1.0) normally-developing pups per litter (Fig. 5), and displayed significantly more ($p$<0.05) fetal reabsorptions (2.2±1.9) per litter compared to Fat-1 females harboring a WT ovary (0.1±0.4) and WT sham females (0.4±0.8; Fig. 6), suggesting a relationship between Fat-1 ovaries and an increased incidence of fetal reabsorption. However, Fat-1 mice bearing a WT ovary also had a significantly ($p$<0.05) reduced litter size (2.0±2.4) when compared to WT sham females (7.2±2.1; Fig. 5).

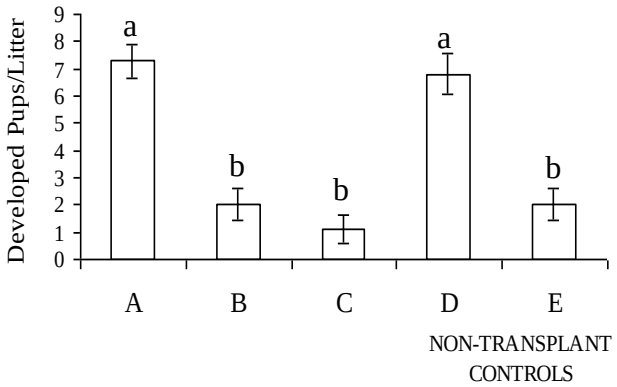


**Figure 5.** Mean number of normally-developing pups (±SEM) in various ovarian transplantation treatment groups at the 12th day of the second gestation. A) WT sham; B) Fat-1 Recipient, WT ovary; C) WT recipient, Fat-1 ovary; D) WT non-surgical control; E) Fat-1 non-surgical control. Bars with different letters are significantly different from each other ($p$<0.05).

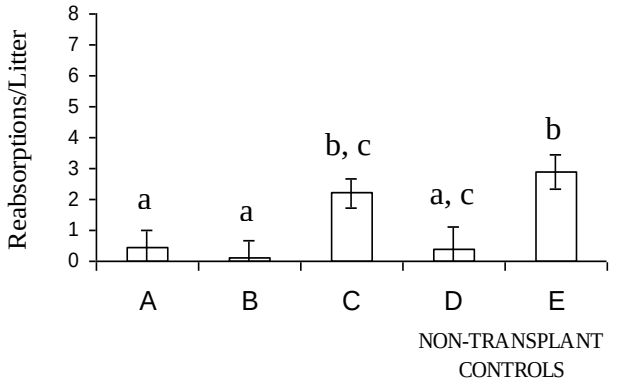


**Figure 6.** Mean number of fetal reabsorptions (±SEM) observed in the uterus of various ovarian transplantation treatment groups at the 12th day of the second gestation. A) WT sham; B) Fat-1 Recipient, WT ovary; C) WT recipient, Fat-1 ovary; D) WT non-surgical control; E) Fat-1 non-surgical control. Bars with different letters are significantly different from each other ($p$<0.05).

## DISCUSSION

Female Fat-1 transgenic mice were found to produce smaller litters (2.7±0.6) than WT females (7.2±0.7). Transgene segregation ratios in surviving pups followed Mendelian laws (data not shown), suggesting that the presence of the transgene in the embryo itself was not a factor impacting embryo survival. The establishment of pregnancy requires ovulation of a competent oocyte, fertilization, implantation, and post-implantation development of the embryo. PUFA can affect all of these events through a variety of mechanisms (Wathes et al., 2007), and are of relevance to this study because the Fat-1 transgene converts n-6 to n-3 PUFA and could therefore influence the pool of PUFA that are available for prostaglandin and steroid synthesis.

It was hypothesized that expression of the n-3 fatty acid desaturase transgene was affecting ovarian function in Fat-1 females. The presence of a Fat-1 ovary was indeed found to be associated with an increased incidence of fetal reabsorptions, regardless of maternal genotype, suggesting that there were problems with post-implantation embryonic survival of gametes derived from transgenic ovaries. RT-PCR analysis did not reveal ectopic expression of the transgene in the ovary at 0.5 and 3.5 DPC. This does not rule out the possibility that there may have been ectopic expression of the transgene in the ovary at other time points during gamete development which could affect the membrane properties of the oocytes. Alternatively, expression in other tissues may also have an impact on ovarian function through downstream effects on prostaglandin and steroid synthesis.

Although data supported the hypothesis that the presence of a Fat-1 ovary was associated with reduced litter size, it was also found that Fat-1 females harboring a WT ovary had less normally-developing pups than WT females. This suggests that the presence of the transgene in non-ovarian tissue was also a factor contributing to the smaller litter size phenotype. While ovulation rates were not found to differ between Fat-1 and WT females; the number of developed embryos in the uterus at 3.5 DPC was lower in Fat-1 females suggesting early embryo loss. Prostaglandin production in the bovine oviduct is known to increase contractility (Wijayagunawardane et al., 2001), which facilitates sperm and embryo transport. It might therefore be predicted that ectopic expression of *Fat-1* in the oviduct following ovulation could impair embryo transport by decreasing the contractility of the oviduct, however gene expression results did not show any expression of the transgene in the uterus at 0.5 and 3.5 DPC.

The data presented in this study suggest that the presence of the Fat-1 transgene has pleiotropic effects on reproductive processes in transgenic females. More detailed studies are required to elucidate how the presence of the transgene is resulting in these reproductive defects, and to ascertain whether its effect on the pool of available n-6 and n-3 PUFA is playing any role in the impaired reproductive phenotype seen in the Fat-1 mice.

## ACKNOWLEDGEMENTS

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# THE EFFECTS OF DIETARY POTASSIUM AND WATER QUALITY ON PERFORMANCE AND CARCASS CHARACTERISTICS OF FINISHING CATTLE

**J. L. Lacey†, J. J. Wagner†, T. E. Engle†, J. W. Spears*, and S. Archibeque†**
†Colorado State University, Fort Collins, CO and *North Carolina State University, Raleigh, NC

**ABSTRACT:** Four hundred and thirty-two crossbred yearling steers (339 kg ± 4.76) were used to study the effects of water quality (reverse osmosis versus well water), dietary potassium concentration (0.75 versus 1.0% of DM), and potassium source (potassium chloride versus potassium carbonate) on feedlot performance and carcass merit. Treatments included: reverse osmosis water (RO) and potassium chloride (KCl) fed to achieve a potassium (K) concentration of 0.75%, well water and KCl to achieve 0.75% K, RO water and potassium carbonate ($KCO_3$) to achieve 0.75% K, well water and $KCO_3$ to achieve 0.75% K, RO water and $KCO_3$ to achieve 1.0% K, and well water and $KCO_3$ to achieve 1.0% K. Final weight was heavier ($P < 0.04$) for RO water (585 kg) than for well water (578 kg). Average daily gain (ADG, $P < 0.04$) was affected by water treatment but not diet. Dry matter intake (DMI, $P < 0.10$) was greater for steers consuming RO water as compared with well water and was not affected by dietary treatment. Feed to gain (FG) and gain to feed (GF) were affected by diet ($P < 0.04$). The effects of water treatment and water x diet interaction on FG or GF were not significant. Recovered net energy for maintenance (NEm, $P > 0.63$) and net energy for gain (NEg, $P > 0.64$) from d1 through slaughter were not affected by water source. Recovered NEm and NEg were greater for steers fed 1% K – $KCO_3$ as compared with 0.75% K – KCl. Carcasses from steers that consumed RO water (362 kg) were heavier ($P < 0.08$) than carcasses from steers consuming well water (357 kg). The effects of diet treatment on carcass weight were not significant. Differences in dressing percentage (DP) and fat depth over the 12th rib were not significant for water quality treatment ($P > 0.37$ and $P > 0.42$ respectively) or diet treatment ($P > 1.00$ and $P > 0.86$ respectively). Yield Grade (YG) calculated from carcass measurements was not affected by diet or water treatment. Well water carcasses had higher ($P < 0.04$) marbling scores than RO water (396 versus 380 units). Steers on the well water treatment were more likely to grade USDA Choice ($P < 0.03$) versus USDA Select ($P < 0.01$), than steers on the RO water treatment (40% versus 28% Choice and 69% versus 48% Select for well water versus RO water, respectively). These data demonstrate that steers on the RO water treatments achieved better feedlot performance, but had lower grading carcasses than steers on the well water treatments. Steers fed diets with 1.0% K from $KCO_3$ were more efficient and demonstrated improved energy recovery as compared with control steers. Interactions between water source and dietary potassium treatment were not significant indicating that increasing dietary cation-anion balance (DCAB) did not alleviate poor performance associated with high sulfate water. Improved efficiency and energy recovery may be related to an observed reduction ($P < 0.12$) in the liver abscess rate for steers consuming the 1.0% K – $KCO_3$ diet. In addition, DCAB was positively related to gain efficiency and net energy recovery.

**Key Words:** Potassium chloride, Potassium carbonate, Water quality, Cation-anion balance.

## Introduction

Dietary cation-anion balance can be defined as the milliequivalents (mEq) of (Na + K + Ca + Mg) – mEq of (Cl + P + S)/100 g of diet DM (Ross et. al., 1994). Potassium (K) is generally supplemented in cattle finishing diets as potassium chloride (KCl). Addition of KCl to diets does not affect DCAB. In contrast, providing supplemental K from potassium carbonate ($KCO_3$) increases DCAB. Increasing DCAB in diets may affect performance and carcass characteristics in finishing cattle, especially during the summer when environmental temperatures are high. Raising DCAB may also be beneficial in alleviating reduced feed intake and growth observed in cattle receiving high sulfate water.

## Materials and Methods

*Animals, Facilities, and Diet.* Four hundred and thirty-two crossbred yearling steers (339 kg ± 4.76) were selected for the trial from a group of 493 steers from multiple sources. The study was conducted as a randomized block design using a 2 x 3 factorial treatment arrangement. Pens were considered the experimental unit for all cattle performance and carcass data evaluated. A total of 48 pens (8 replicates per treatment combination) were used for the study. Steers were housed in dirt surfaced pens that measured 6 meters wide by 18 meters deep. Each pen had 4 meters of bunk located on a 3 meter deep concrete feeding apron. Every two pens shared a common water fountain that was located along the fence line with the feed bunks. All diets were typically fed twice daily.

*Study Design and Treatments.* Two water sources and three potassium treatments were used in a 2 x 3 factorial configuration for the study. Water sources included: reverse osmosis water (RO) versus high sulfate well water. Potassium treatments included: 0.75% K with supplemental K from KCl, 0.75% K with supplemental K

from $KCO_3$, and 1.0% K with supplemental K from $KCO_3$. All diets were manufactured immediately prior to feeding utilizing the stationary mixer in the mill at Southeast Colorado Research Center (SECRC).

*Sample Analysis.* The finishing diets, water, and feed ingredients were sampled every week during the trial. A sub-sample of each feed commodity and diet sample was analyzed for DM at SECRC using a forced air oven set at approximately 60 ºC for 48 h. The remaining portion of each feed ingredient, water sample, and diet sample was composited by diet or feed ingredient and month and shipped to the nutrition laboratory at Cumberland Valley Analytical Services[1] for DM, NDF, and routine nutrient analyses including Cl, K, Na, and S. Water samples were for analyzed for Cl, K, Na, and S. Dietary cation-anion balance for diets and water were calculated from Cl, K, Na, and S.

*Statistics.* Feedlot performance and continuous carcass data were analyzed using mixed model procedures as described by PROC MIXED in SAS (2003). Fixed effects included in the models were water source (WAT), dietary treatment (DIET), and WAT x DIET. Replicate (REP) within treatment combination was used as a random effect in the models. Water source, DIET, and REP were considered class variables. Dietary treatment means were separated using orthogonal contrasts. Contrasts examined were KCl versus $KCO_3$ and 0.75 versus 1.0% K within the $KCO_3$ treatments. Categorical carcass data were analyzed using PROC GLIMMIX of SAS (2003) using similar models and contrasts as previously described. Water intake data were evaluated using PROC MIXED with similar models as used for the performance data. However, since every two pens shared a common water fountain (FOUN), only four replicates per treatment combination were available for the water intake analysis. Total dietary cation and anion intake from feed and water was calculated for each FOUN and expressed as mEq per 100 g DMI. PROC MIXED was used to develop prediction equations predicting performance and continuous carcass measurements from DCAB. PROC GLIMMIX was used to develop prediction equations from categorical carcass data. For the development of the prediction equations, DCAB was considered a continuous fixed effect and the class variable FOUN nested within WAT x DIET was considered a random effect.

## Results and Discussion

*Feedlot Performance.* Initial weight differences between water quality treatments were significant ($P < 0.01$) and averaged 341 kg for RO water versus 337 kg for well water. Differences for initial weight associated with diet ($P > 0.13$) or water x diet interaction ($P > 0.84$) were not significant. The effects of water quality on slaughter weight ($P < 0.04$) were significant. Final weight averaged 585 kg for the RO water treatment and 578 kg for the well water. Diet and water x diet interaction had no impact on live weight. Average daily gain (ADG) for d1-27 ($P < 0.04$), d56-83 ($P < 0.07$), d112-slaughter ($P < 0.10$), and d1-slaughter was greater for RO water as compared with well water. Average ADG from d1 through slaughter was 1.71 versus 1.76 kg per head daily for well versus RO water. The effects of diet and diet x water interaction on ADG were not significant. Dry matter intake (DMI) varied significantly among water treatments. From d56-83 ($P < 0.03$), from d84-111 ($P < 0.01$), and from d1-slaughter ($P < 0.10$) water treatment affected DMI. Dry matter intake from d1 through slaughter averaged 9.77 versus 9.97 kg per head daily for the well versus RO water treatments, respectively. Diet treatment and water x diet interaction were not significant sources of variation describing DMI. Water quality by diet interactions were important from d1-27 for feed to gain (FG, $P < 0.04$) or gain to feed (GF, $P < 0.10$) suggesting that the effect of diet on feed efficiency d1-27 depended upon water quality. Feed efficiency d1-27, as measured by FG or GF was improved for steers consuming $KCO_3$ as compared with KCl for the well water treatments but not for the RO water treatments. Feed efficiency d1-slaughter was affected by diet (FG, $P < 0.05$; GF, $P < 0.04$) and not water quality or the interaction between diet and water quality. Steers fed diets with 1.0% K – $KCO_3$ were more efficient than control steers (FG, 5.59 versus 5.76; GF 0.1792 versus 0.1738 for 1.0% K – $KCO_3$ versus control, respectively). Net energy for maintenance (NEm) and net energy for gain (NEg) recovered from the diets followed the same pattern as what was observed for feed efficiency. Energy recovery from d1-slaughter was not affected by water quality or the water x diet interaction. However, diet did have a significant impact on NEm ($P < 0.05$) and NEg ($P < 0.04$) from d1-slaughter.

*Water intake.* Overall the water intake pattern was as expected; less intake during the cooler months (May, September, and October) and more intake during the hot months (June, July, and August). The predicted values were calculated according to the water equation for feedlot steers developed by Hicks et al. (1988). Water treatment effects were significant from d1-27 ($P < 0.02$), from d28-55 ($P < 0.03$), from d56-83 ($P < 0.03$), and from d1-slaughter ($P < 0.03$) and all periods had lower water intake than predicted values. Water intake from d1-slaughter averaged 34.75 versus 37.55 l/hd/d for well versus RO water, respectively. Diet treatment and water x diet interaction were not significant sources of variation describing water intake.

*Carcass Merit.* Hot carcass weight (HCW) averaged 359 kg, and differences associated with diet or the water x diet interaction were not significant ($P > 0.52$ and $P > 0.20$, respectively). However, water treatment did have an effect on HCW ($P < 0.09$) with RO water (362 kg) having heavier carcasses than well water (357 kg). No differences for dressing percentage and fat depth over the 12th rib were found between water treatment ($P > 0.37$ and $P > 0.42$, respectively), diet treatment ($P > 1.00$ and $P > 0.86$ respectively), and water x diet interaction ($P > 0.27$ and $P > 0.86$ respectively). Statistical analysis for kidney, pelvic, and heart fat percentage was not possible because no variation existed between pens since all pens averaged

[1] Maugansville, MD.

2.0%. The water x diet interaction tended ($P < 0.10$) to be important for ribeye area (REA). Water treatment and diet treatment had no effect on REA ($P>0.14$ and $P>0.41$ respectively). No significant differences were observed for REA per 45.4 kg HCW between water sources ($P > 0.94$), diets ($P > 0.85$), and water x diet interaction ($P > 0.15$). Yield Grade (YG) calculated from carcass measurements was not affected by water treatment ($P > 0.43$), diet treatment ($P > 0.92$), or water x diet interaction ($P > 0.34$). Marbling score was significantly ($P < 0.04$) impacted by water treatment. Well water carcasses had higher marbling than RO water carcasses (396 versus 380 units). Diet treatment ($P > 0.44$) and water x diet interaction ($P > 0.21$) had no effect on marbling score. Marbling score per 0.254 cm fat depth was not affected by water quality ($P > 0.70$), diet ($P > 0.61$), or water quality x diet interaction ($P > 0.79$). The likelihood that an individual carcass graded USDA Choice or Prime ($P < 0.04$) versus USDA Select ($P < 0.01$) was affected by water treatment. Well water carcasses had a higher percentage of Choice (40 versus 28% well water versus RO water respectively) but a lower percentage USDA Select carcasses (48 versus 69% well water versus RO water respectively) than RO water carcasses. There was an interesting trend ($P < 0.12$) for reduced liver abscesses for the highest $KCO_3$ treatment. This finding is consistent with the improved feed efficiency and energy recovery findings suggesting that steers fed the high $KCO_3$ diets may experience less acidic conditions in the rumen.

*Cation-Anion Balance Analysis.* Feedlot performance and carcass data were also analyzed on the basis of the DCAB for diet plus water. Total mEq intake from feed was calculated by multiplying diet mEq (3.68, 7.39, and 10.17 per 100 g DM for the control, 0.75% K – $KCO_3$, and 1.0% K – $KCO_3$ diets, respectively) by DMI for the two pens associated with each water fountain. Next total mEq intake from water was calculated by multiplying water mEq (-0.57 and -2.46 per 100 ml for the RO and well water, respectively) by water intake for each water fountain. Total mEq intake was calculated by adding mEq from feed to mEq from water. Total mEq intake was then divided by the total g DMI for the two pens associated with each water fountain and this result was then multiplied by 100 to express the value as mEq per 100 g DMI. Average mEq intake per 100 g DMI was 7.02 and ranged from 3.58 mEq for steers that averaged 9.66 kg DMI from the control diet and 39.66 liters well water intake to 10.15 mEq for steers that averaged 10.11 kg DMI from the 1.0 % K – $KCO_3$ diet and 36.64 liters RO water intake. Final weight ($P > 0.53$), ADG ($P > 0.46$), DMI ($P > 0.40$), and water intake ($P > 0.44$) were not predicted by mEq per 100 g DMI. Feed efficiency was improved as mEq per 100 g DMI increased. Feed dry matter required per kg gain was reduced ($P < 0.07$) by 0.01 kg for each mEq increase per 100 g DMI (Figure 3). Feedlot gain per kg DMI was increased ($P < 0.06$) by 0.03 kg for each mEq increase per 100 g DMI (Figure 4). Net energy for maintenance and NEg recovered from the diet were increased ($P < 0.02$) by 0.51 and 0.26 mcal per 45.4 kg DM, respectively, for each mEq increase per 100 g DMI. There was a tendency ($P < 0.15$) for mEq per 100 g DMI to predict liver abscesses. However, total mEq intake from feed and water, expressed per 100 g DMI, was not an effective predictor of carcass merit.

## Implications

Water quality had a significant effect on performance. Water intake, ADG, DMI, final weight, and HCW were improved for steers consuming RO water as compared with well water. These results are consistent with the findings of Loneragan et al. (2001). It is interesting to note that in contrast to Loneragan et al. (2001), marbling score and the likelihood of carcasses grading USDA Choice and Prime versus USDA Select were improved for steers consuming well water as compared with RO water. Dietary K treatment had no effect on ADG, DMI, slaughter weight, or HCW. However, feed efficiency and NE recovery were improved for steers consuming the 1.0% K – $KCO_3$ diet as compared with steers consuming 0.75% K diets. Improved feed efficiency may have been the result of reduced acid load in the rumen as suggested by reduced incidence of liver abscesses for the 1.0% K – $KCO_3$. Additional research examining changes in rumen pH associated with DCAB is warranted.

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Table 1. Dry Matter composition of the finishing diets for the water quality and dietary potassium study.

| | 0.75% K – KCl | | | 0.75% K – $KCO_3$ | | | 1.0% K – $KCO_3$ | | |
|---|---|---|---|---|---|---|---|---|---|
| Item[a] | 314 | 315 | 316 | 324 | 325 | 326 | 334 | 335 | 336 |
| Corn Silage | -- | -- | 9.30 | -- | -- | 9.30 | -- | -- | 9.30 |
| Wheat Silage | 4.77 | 6.91 | -- | 4.76 | 6.91 | -- | 4.77 | 6.92 | -- |
| Steam Flaked Corn | 80.04 | 80.40 | 77.92 | 79.94 | 80.43 | 77.95 | 79.40 | 79.89 | 77.40 |
| Alfalfa Hay | 2.39 | -- | -- | 2.40 | -- | -- | 2.40 | -- | -- |
| CCDS[b] | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Yellow Grease | 3.50 | 3.50 | 3.50 | 3.50 | 3.50 | 3.50 | 3.50 | 3.50 | 3.50 |
| Soybean Meal | 2.44 | 2.30 | 2.41 | 2.43 | 2.30 | 2.40 | 2.52 | 2.40 | 2.50 |
| Supplement[c] | 3.98 | 3.87 | 3.87 | 3.95 | 3.84 | 3.85 | 4.35 | 4.26 | 4.30 |
| Theoretical Nutrients | | | | | | | | | |
| DM, % | 74.55 | 72.04 | 71.24 | 74.54 | 72.04 | 71.23 | 74.62 | 72.11 | 71.31 |
| Crude Protein | 13.50 | 13.50 | 13.50 | 13.50 | 13.50 | 13.50 | 13.50 | 13.50 | 13.50 |
| NEm, mcal/45.4kg | 102.78 | 102.99 | 103.69 | 102.81 | 103.02 | 103.72 | 102.33 | 102.53 | 103.24 |
| NEg, mcal/45.4 kg | 70.86 | 71.00 | 71.37 | 70.88 | 71.01 | 71.39 | 70.55 | 70.67 | 71.05 |
| Ether extract | 8.07 | 8.10 | 8.13 | 8.08 | 8.10 | 8.13 | 8.06 | 8.08 | 8.11 |
| Calcium | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 |
| Phosphorus | 0.35 | 0.33 | 0.32 | 0.35 | 0.33 | 0.32 | 0.35 | 0.33 | 0.32 |
| Potassium | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 1.00 | 1.00 | 1.00 |

[a]Percentage of dry matter.
[b]Condensed corn distiller's solubles.
[c]Contained calcium carbonate, urea, salt, trace mineral premix, Min Ad, mineral oil, vitamin A and E, Rumensin, Tylan, and either potassium chloride or potassium carbonate.



Table 2. Least square means showing the effects of water quality and diet on feedlot performance for the water quality and dietary potassium study.

| | RO Water | | | Well Water | | | | Prob. > F | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item[a] | 0.75 KCl | 0.75 $KCO_3$ | 1.0 $KCO_3$ | 0.75 KCl | 0.75 $KCO_3$ | 1.0 $KCO_3$ | SEM | Water | Diet | WxD |
| Initial weight[b] | 340.00 | 340.47 | 341.87 | 334.57 | 336.75 | 337.95 | 4.77 | 0.0004 | 0.1350 | 0.8414 |
| Final weight | 583.52 | 583.25 | 588.38 | 578.62 | 576.89 | 579.02 | 3.97 | 0.0413 | 0.6391 | 0.8477 |
| ADG, d1-finish | 1.75 | 1.75 | 1.78 | 1.71 | 1.70 | 1.72 | 0.03 | 0.0413 | 0.6391 | 0.8477 |
| DMI, d1-finish | 10.02 | 10.02 | 9.87 | 9.91 | 9.69 | 9.70 | 0.15 | 0.1014 | 0.4539 | 0.7275 |
| FG, d1-finish | 5.73 | 5.74 | 5.54 | 5.79 | 5.69 | 5.64 | 0.67 | 0.4772 | 0.0412 | 0.5264 |
| GF, d1-finish | 0.1747 | 0.1745 | 0.1809 | 0.1729 | 0.1757 | 0.1774 | 0.0021 | 0.4475 | 0.0382 | 0.5369 |
| NEm, d1-finish | 97.09 | 97.00 | 101.01 | 96.27 | 98.19 | 99.10 | 1.31 | 0.6374 | 0.0358 | 0.4834 |
| NEg, d1-finish | 63.93 | 63.89 | 65.87 | 63.48 | 64.49 | 64.94 | 0.66 | 0.6354 | 0.0391 | 0.4984 |

[a]ADG = average daily gain, kg/hd/d; DMI = dry matter intake, kg/hd/d; KCl = Potassium chloride; $KCO_3$ = Potassium carbonate; 0.75 = 0.75% K; 1.0 = 1.0% K; WxD = Water x diet interaction, FG = feed DM/gain; GF = gain/ feed DM; NEm = net energy for maintenance, mcal/45.4 kg dry matter; NEg = net energy for gain, mcal/45.4 kg dry matter.
[b]Live weight data expressed as kg/hd.

Table 3. Least square means describing the effect of water quality and diet on carcass merit for the water quality and dietary potassium study.

| | RO Water | | | Well Water | | | | Prob. > F | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item[a] | 0.75 KCl | 0.75 $KCO_3$ | 1.0 $KCO_3$ | 0.75 KCl | 0.75 $KCO_3$ | 1.0 $KCO_3$ | SEM | Water | Diet | WxD |
| Hot carcass weight, kg | 358.03 | 359.45 | 367.11 | 357.65 | 357.03 | 355.49 | 3.30 | 0.0813 | 0.5226 | 0.2045 |
| Dressing % | 61.24 | 61.46 | 62.10 | 62.33 | 62.19 | 61.49 | 0.54 | 0.3697 | 0.9970 | 0.2706 |
| Fat depth, cm | 1.17 | 1.17 | 1.17 | 1.19 | 1.17 | 1.22 | 0.05 | 0.4178 | 0.8582 | 0.8591 |
| Ribeye area[b] | 83.35 | 82.13 | 85.16 | 81.35 | 83.42 | 82.19 | 1.03 | 0.1440 | 0.4129 | 0.0964 |
| Muscling[c] | 10.58 | 10.38 | 10.52 | 10.32 | 10.65 | 10.52 | 0.13 | 0.9364 | 0.8545 | 0.1546 |
| Calc YG | 2.91 | 2.99 | 2.90 | 3.04 | 2.90 | 3.00 | 0.08 | 0.4346 | 0.9220 | 0.3455 |
| Marbling score[d] | 378.49 | 390.76 | 369.44 | 407.17 | 388.19 | 392.64 | 9.41 | 0.0383 | 0.4419 | 0.2198 |
| Marbling/0.254 cm fat | 89.04 | 95.33 | 90.01 | 94.06 | 94.19 | 90.49 | 4.72 | 0.7087 | 0.6197 | 0.7965 |
| Likelihood for each QG category | | | | | | | | | | |
| Choice | 26.56 | 36.98 | 21.53 | 44.10 | 39.24 | 36.46 | 6.44 | 0.0334 | 0.3585 | 0.4541 |
| Select | 70.66 | 60.24 | 75.69 | 47.57 | 53.47 | 62.15 | 6.30 | 0.0075 | 0.1388 | 0.4365 |
| Standard | 2.78 | 2.78 | 2.78 | 8.33 | 7.29 | 1.39 | 2.34 | 0.1382 | 0.2898 | 0.2898 |
| Likelihood for each YG category. | | | | | | | | | | |
| YG1 & 2 | 46.70 | 46.01 | 58.68 | 40.45 | 48.96 | 43.23 | 6.42 | 0.2395 | 0.5211 | 0.3664 |
| YG3 | 50.52 | 48.44 | 35.76 | 49.65 | 46.70 | 48.09 | 6.30 | 0.5318 | 0.4219 | 0.4634 |
| YG4 & 5 | 2.78 | 5.56 | 5.56 | 9.90 | 4.34 | 8.68 | 2.58 | 0.1605 | 0.6977 | 0.2819 |
| Liver Abscess[e] | 18.06 | 11.11 | 10.59 | 17.19 | 21.88 | 5.90 | 4.76 | 0.6571 | 0.1111 | 0.2502 |

[a]Calc YG = Yield Grade calculated from carcass measurements; QG = Quality Grade; YG = Yield Grade; KCl = Potassium chloride; KCO3 = Potassium carbonate; 0.75 = 0.75% K; 1.0 = 1.0% K; WxD = Water quality x diet interaction.

[b]Square centimeters.

[c]$Cm^2$ ribeye area per 45.4 kg hot carcass weight.

[d]Marbling score units, 200= $Traces^{00}$, 300 = $Slight^{00}$, 400 = $Small^{00}$.

[e]Likelihood that an individual liver showed signs of abscesses.

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# Gestational Nutrition Affects Growth and Adipose Tissue Deposition in Steers

**K. R. Underwood[1], J. F.Tong[1], J. M. Kimzey[1], P. L. Price[1], E. E. Grings[2], B. W. Hess[1], W. J. Means[1], and M. Du[1]**
University of Wyoming, Laramie, WY 82071[1]; USDA-ARS, Miles City, MT 59301[2]

**ABSTRACT:** Nutrient restriction of the dam during gestation can have detrimental influences on the progeny. In semi-arid climates nutrient restriction during mid to late gestation occurs frequently due to reduced forage production because of drought conditions. Mid to late gestation is crucial for adipocyte differentiation and therefore nutrition during this time is expected to affect adipose tissue development and resulting carcass composition of steers. Thus, the objective of this study was to examine if cow nutrition by pasture management during mid to late gestation, would affect growth and carcass composition of steer offspring. Fifteen crossbred beef cows were randomly placed on improved pasture (**IP**, n = 8) or native range (**NR**, n = 7) from 120 to 150 through 180 to 210 d of gestation. Then, both groups were placed together and allowed to calve. Steers were weaned at 190 d of age and entered the feedlot at 315 d of age. Steers were fed in a single pen and then slaughtered at 15 mo of age. *Longissimus* muscle and adipose tissue samples were collected. Carcass characteristics were measured at 48 h postmortem. Subcutaneous adipose tissue was fixed, sectioned, stained and used for analyses of cell number and cell diameter. Weight entering the feedlot did not differ (P = 0.71) between treatments. At slaughter, steers born to mothers grazed on IP had heavier live body weight ($P$ = 0.04) and hot carcass weight ($P$ = 0.01) than NR steers. *Longissimus* muscle area, *semitendinosus* weight, marbling score, kidney, pelvic and heart fat, and yield grade were similar ($P$ > 0.12) between NR and IP steers. Twelfth rib fat thickness ($P$ = 0.05) and adjusted 12$^{th}$ rib fat thickness ($P$ = 0.02) were greater for IP steers than for NR steers. Adipose tissue cell number per field tended ($P$ = 0.09) to be greater for IP steers than for NR steers. These data show improving pasture quality available to cows during mid to late gestation affects overall growth and adipose tissue deposition in steers.

**Key words**: beef cattle, fat deposition, growth.

## Introduction

The fetal origins hypothesis states that a stress during gestation will cause fetal adaptations which can affect the animal later in life (Barker, 1995). Gestational nutrition is related to altered adiposity (Bispham et al., 2003) and changes in muscle development (Nordby et al., 1987; Zhu et al. 2004).

Arid environments, with extreme variation in precipitation, yield dynamic rangelands that vary in forage production and quality (Grings et al., 2005; DelCurto et al., 2000). Much of the western US is affected by these conditions during various parts of the year. Vavra and Raliegh (1976) demonstrated that as summer progressed, forage quality decreased with increased plant maturity. Thus, gestating beef cows grazing western rangelands can experience extended periods of low quality forage during late summer into fall, especially in drought conditions.

Carcasses from beef cattle have considerable variability in many routinely evaluated attributes (McKenna et al., 2002). Additionally, beef cuts have shown variability in quality attributes (Brooks et al., 2000). The factors associated with such variability are not fully understood. The objective of this study was to test if nutrition by pasture management during mid to late gestation will affect live performance and carcass traits of steers.

## Materials and Methods

### *Animals*

All animal procedures were approved by the USDA-ARS, Fort Keogh Livestock and Range Research Laboratory (LARRL) and the University of Wyoming Animal Care and Use Committees. A crossbred beef cattle herd located at LARRL in Miles City, Montana were bred over a 32 d breeding period to begin calving at the end of January. Cows were then managed on native range during early to mid gestation. At d 120 to 150 of gestation, cows were allotted randomly to 1 of 2 dietary treatments for 60 d, either native range (**NR,** n = 40) or improved pasture (**IP,** n = 40) consisting of irrigated pastures with increased forage production. During the 60 d grazing period IP varied from 11.1% CP of OM from esophageal extrusa early to 6.0% CP of OM from esophageal extrusa at the end of the grazing period. Whereas, the NR ranged from 6.5% CP of OM from esophageal extrusa during early grazing to 5.4 % CP of OM from esophageal extrusa at the end of the grazing period. Throughout this time NR cows gained 43 kg while IP cows gained 60 kg of BW showing a disparity in nutritional status of the dams on IP and NR. After the 60 d period all cows were moved to native range pastures and managed together to meet requirements of beef cows during late gestation. Cows calved and calves were weaned at 191 ± 2.3 d of age. Steer calves were then back-grounded until 315 ± 2.3 d of age when they were transported to a University of Wyoming research center near Lingle, Wyoming.

Steers from cows gestating on improved pasture (n = 8) and steers from cows gestating on native range (n = 7) were weighed upon entering the feedlot. Steers were placed in a single pen and fed the diets in Table 1 with the ration being changed every wk until steers were on the finishing ration. Steers were weighed 1 wk after adjustment to the finishing diet and then again after 70 d and at the end of the feeding period (115 d).

***Slaughter***

Steers were transported to Laramie, Wyoming and slaughtered at the University of Wyoming Meat Laboratory as previously described by Underwood et al. (2008) on 2 separate d in 1 wk. Steers were allowed free access to water with a 24 hr feed withdrawal. *Longissimus* muscle samples were collected within 10 min postmortem, snap frozen in liquid nitrogen, and stored at -80°C for biological analysis. The KPH was removed and weighed at slaughter.

Table 1. Diets provided to steers that were gestated in cows grazing either native range or improved pasture during mid to late gestation

| Ration | Receiving | Step1 | Step2 | Finishing |
|---|---|---|---|---|
| Ingredient | Ration Composition, % DM | | | |
| Whole corn | 23.5 | 43.3 | 60.2 | 74.9 |
| Corn silage | 53.7 | 35.6 | 20.2 | 6.9 |
| Alfalfa hay | 13.3 | 12.3 | 11.4 | 10.6 |
| Soybean meal | 6.7 | 6.2 | 5.8 | 5.4 |
| Urea | 1.0 | 1.0 | 0.9 | 0.8 |
| Limestone | 0.9 | 0.8 | 0.8 | 0.7 |
| Salt | 0.8 | 0.7 | 0.7 | 0.6 |
| Mineral supplement | 0.2 | 0.1 | 0.1 | 0.1 |
| Analyzed Composition | ----------% DM---------- | | | |
| CP | 13.2 | 12.9 | 12.6 | 12.4 |
| NDF | 29.1 | 25.4 | 25.4 | 19.6 |
| ADF | 15.6 | 12.7 | 10.3 | 8.2 |

***Carcass characteristics***

*Longissimus* muscle area, 12th rib fat thickness, marbling score, and maturity were determined after a 48-h chill at 2 to 4°C as previously described by Underwood et al. (2008).

***Fabrication***

Carcasses were fabricated at d 14 postmortem and the whole *semitendinosus* muscle was dissected and weighed as an estimate of muscle growth (Underwood et al., 2008).

***Subcutaneous Adipose Tissue Analysis***

A subcutaneous adipose tissue sample (0.5 cm × 0.5 cm) was removed at 48 h postmortem at the 13th rib. Adipose tissue was frozen at -80°C. Samples were fixed according to Hulver et al. (2003). Samples were then mounted in OCT compound (Sakura Finetech, Torrance, CA) and sectioned at 16 µm thickness. Sections were stained with Harris Modified Hematoxylin (Fisher Scientific, Fair Lawn, NJ) for 2 min followed by a 5 min wash with running tap water. Sections were counterstained with 1% Eosin Y followed by 2 min wash with deionized water. Cover slips were mounted over the sections using glycerol. Sections were analyzed for cell diameter using light microscopy with images analyzed using Image J Software (NIH, Bethesda, MD). Cell diameter was measured by averaging the widest diameter and the narrowest diameter of each cell.

***Shear Force***

Samples for Warner-Bratzler shear force analysis were removed at fabrication (14 d postmortem) and frozen at -20°C for later analysis. Warner-Bratzler shear force analysis was performed as previously described by Underwood et al. (2008).

***Identification of myofiber isoforms***

Myofiber isoforms were identified according to Underwood et al. (2007). Briefly, purified myofibrillar proteins were re-suspended in 200 µl water and 300 µl of standard 2 x sample loading buffer and then boiled for 5 min. After centrifugation at 12,000 g for 5 min, the supernatant was used for electrophoresis. Stacking gels (4%) and gradient separation gels (5 to 20%) were used with the upper running buffer and lower running buffer as previously described (Underwood et al., 2007). Gels were run at 4°C in a Bio-Rad minigel system (Bio-Rad Inc, Hercules, CA), at constant 71 V for 30 h. After electrophoresis, gels were stained with Coomassie blue, and scanned with a densitometer to determine the amount of Type I and Type II myosin isoforms. Data was reported as Type I /Type II myosin isoform ratio.

***Statistical Analysis***

Animal performance, carcass measurements, and myofiber analysis were analyzed as a completely randomized design using the PROC GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Individual animal was considered as the experimental unit. Data are presented as least squares means ± SEM. Statistical significance was considered when $P < 0.05$ and trends were considered when $P < 0.10$.

**Results and Discussion**

Performance data of steers gestated by cows on IP and NR are reported in Table 2. Steers from both treatments entered the feedlot at a similar ($P = 0.71$) BW. Steers gestated by cows on NR had lower ADG ($P = 0.05$), lighter total BW gain ($P = 0.05$), and tended to have lighter BW ($P = 0.07$) after the 115 d feeding period than steers gestated on IP. This is in agreement with Nordby et al. (1987) and Beerman (1983) who reported lambs and rat pups born to mothers on low planes nutrition during gestation were slower growing, had lower ADG, and lighter BW. Steers from IP had heavier BW at slaughter ($P = 0.04$) and heavier HCW (P = 0.01). This is inconsistent with our previous data that showed steers gestated by mothers on a low plane of nutrition were similar to controls in BW at slaughter (Underwood, 2007). In our previous study, the low plane of nutrition was applied much earlier during gestation, which may account for differences in animal performance.

Carcass characteristics of steers from NR and IP are presented in Table 2. Steers from NR and IP had similar LM area ($P = 0.26$) and *semitendinosus* weights ($P = 0.27$). This is similar to our previous data showing no differences in muscle growth of steers gestated by mothers on a low plane of nutrition during early to mid-gestation

(Underwood, 2007). However, Nordby et al. (1987) showed decreased *semitendinosus* weights of lambs on a low plane of nutrition during gestation. Zhu et al. (2004) has reported a decreased number of secondary myotubes in skeletal muscle of fetal sheep on a low plane of nutrition during early to mid-gestation. These discrepancies may be due to different time, duration, and severity of gestational plane of nutrition.

Fat thickness and adjusted fat thickness at the 12th rib was greater ($P = 0.05$) for IP carcasses when compared to NR carcasses. This is consistent with our previous data in which steers gestated by mothers on a low plane of nutrition tended to have increased 12th rib fat thickness and a greater amount of fat as a percentage of the 9-10-11 rib section (Underwood, 2007). These results are in contrast to previous studies in sheep, however, that reported no difference in external fat thickness (Nordby et al., 1987). The KPH as a percentage of HCW were similar ($P = 0.32$) between treatments. However, recent studies showed an increased adiposity of fetuses that were on a low plane of nutrition during gestation (Bispham et al., 2003). Marbling score was similar ($P = 0.12$) between treatments, which supports previous findings of steers gestated on a low plane of nutrition (Underwood, 2007). These data indicate gestational plane of nutrition may alter subcutaneous adipose tissue deposition preferentially over visceral and intramuscular adipose depots.

Due to the difference in 12th rib fat thickness, we investigated if gestational plane of nutrition in cattle would alter adipocyte number and size using fixed sections stained with Harris Hematoxylin and Eosin Y. Subcutaneous adipose tissue sections showed a tendency ($P = 0.09$) for IP steers to have a greater number of cells per field of view using light microscopy (Figure 1). We then examined the mean adipocyte diameter and found no differences ($P = 0.41$) between treatments (Figure 2). These results indicate increased fat thickness of these animals may be due to increased number of adipocytes, possibly affected by gestational nutrition.

The Warner-Bratzler shear force of steers from NR and IP is shown in Figure 3. The IP steers had lower ($P = 0.01$) shear force and thus presumably more tender meat. Previous reports showed no difference in Warner-Bratzler shear force of steers gestated on a low plane of nutrition (Underwood, 2007). It is possible that gestational nutritional status may affect collagen development or the calpain/calpastatin system which are regulators of meat tenderness.

The myosin isoform results are reported in Figure 4 as a Type I/Type II ratio. Myosin heavy chain analysis showed treatments were similar ($P = 0.46$) in Type I/Type II ratio. We were able to identify Type I, Type IIA, and Type IIX myosin heavy chain isoforms; but, were unable to detect Type IIB myosin heavy chain isoform. This is consistent with other reports in beef cattle (Underwood et al., 2007) and goats (Arguello et al., 2001) in which researchers were unable to detect the Type IIB myosin heavy chain isoform in skeletal muscle. Thus, the Type IIB myosin heavy chain isoform is most likely not present in beef and goat skeletal muscle.

## Conclusions

Gestational nutritional status can alter animal performance during the finishing period, subcutaneous adipose tissue deposition, HCW, and Warner-Bratzler shear force in steers finished to slaughter weights.

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Table 2. Growth and carcass characteristics of steers from cows grazing either native range (n = 7) or improved pasture (n = 8) during mid to late gestation

| | Treatment | | |
|---|---|---|---|
| Item | Native range | Improved pasture | *P*-value |
| Initial BW, kg | 355.1 ± 4.7 | 357.5 ± 4.4 | 0.71 |
| Final BW, kg | 538.0 ± 8.3 | 560.2 ± 7.7 | 0.07 |
| ADG, kg/d | 1.50 ± 0.07 | 1.66 ± 0.06 | 0.05 |
| Total BW gain, kg | 180.2 ± 8.0 | 200.4 ± 7.5 | 0.05 |
| Live BW at slaughter, kg | 520.6 ± 7.7 | 543.9 ± 7.1 | 0.04 |
| HCW, kg | 329.5 ± 4.8 | 348.2 ± 4.5 | 0.01 |
| 12th rib fat thickness, cm | 1.11 ± 0.15 | 1.51 ± 0.14 | 0.05 |
| Adjusted 12th rib fat thickness, cm | 1.24 ± 0.12 | 1.64 ± 0.11 | 0.02 |
| KPH, % HCW | 3.96 ± 0.25 | 3.59 ± 0.24 | 0.32 |
| LM area, $cm^2$ | 75.4 ± 2.2 | 78.7 ± 2.0 | 0.26 |
| Yield grade | 3.54 ± 0.18 | 3.84 ± 0.17 | 0.23 |
| Marbling score[a] | 420 ± 16 | 455 ± 15 | 0.12 |
| Semitendinosus, kg | 1.98 ± 0.03 | 2.03 ± 0.01 | 0.27 |

[a] 400 = small.

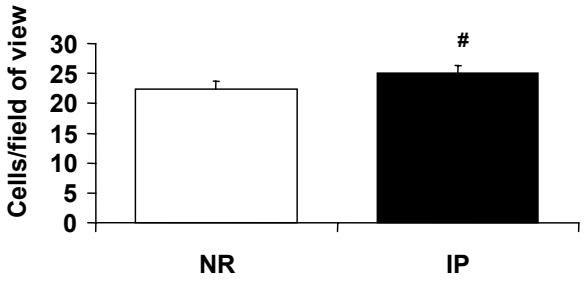


Figure 1. Mean number of adipocytes from the 13th rib subcutaneous adipose tissue per field of steers gestated on native range (n = 7) and improved pasture (n = 8). #$P < 0.10$.

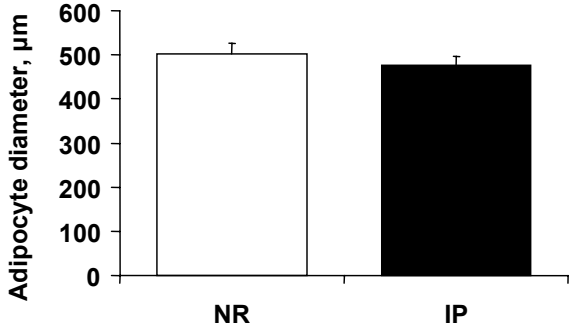


Figure 2. Mean adipocyte diameter from the 13th rib subcutaneous adipose tissue of steers gestated on native range (n = 7) and improved pasture (n = 8).

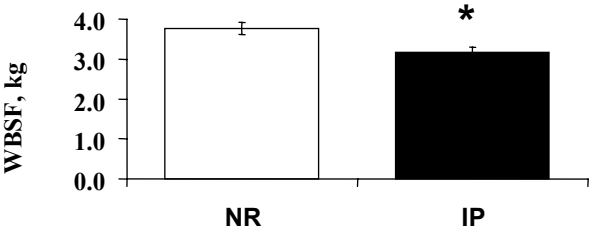


Figure 3. Warner-Bratzler shear force of LM from steers gestated on native range (n = 7) and improved pasture (n = 8). *$P < 0.05$.

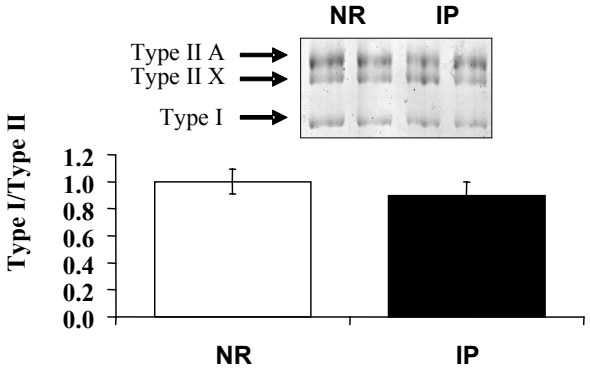


Figure 4. Myosin isoform typing of steers gestated on native range (n = 7) and improved pasture (n = 8).

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# EFFECT OF DURATION OF INTRAVAGINAL PROGESTERONE INSERT (CIDR) TREATMENT ON PREGNANCY RATE PER AI IN DAIRY HEIFERS USING A TIMED-AI PROTOCOL

**C.R. Johnson[1], A. Ahmadzadeh[2], K.S. Jensen[3], S. Etter[4], R. Wilson[5], N. Dalton[6], and J.C. Dalton[1]**
[1]Caldwell Research and Extension Center, Caldwell, ID, [2]University of Idaho, Moscow,
[3]University of Idaho Extension, Marsing, [4]University of Idaho Extension, Caldwell,
[5]University of Idaho Extension, Emmett, and [6]Intermountain Pet Hospital, Meridian, ID

**ABSTRACT:** The objective of this experiment was to determine the effect of reducing the length of time of intravaginal progesterone insert (CIDR) exposure in a timed-AI (TAI) protocol (CIDR-$PGF_{2\alpha}$-GnRH and TAI) on pregnancy rate per AI in dairy heifers. Holstein heifers (n = 241) were assigned to one of two treatments. Heifers in Treatment 1 (7-d CIDR; n = 120; 398.6 ± 1.2 kg BW [mean ± SEM]) received a CIDR insert (d -7) for 7 d and $PGF_{2\alpha}$ (25 mg) at CIDR removal (d 0). Heifers in Treatment 2 (5-d CIDR; n = 121; 401.2 ± 1.3 kg BW) received a CIDR insert (d -5) for 5 d and $PGF_{2\alpha}$ at CIDR removal (d 0). All heifers received GnRH (100 μg) and TAI 53 to 55 h after CIDR removal. Three professional technicians performed AI. Blood samples were taken on d -14, CIDR insertion (d -7 or d -5), and on the day of TAI for progesterone ($P_4$) analyses. Pregnancy status was diagnosed by palpation of uterine contents 35 d after TAI. Eight heifers were removed from the study after losing a CIDR insert. Five heifers were removed for inaccurate records (date of AI did not match date of TAI). Treatment had no effect on pregnancy rate per AI, 41.9% vs. 42.3% for 7-d CIDR (n = 117) and 5-d CIDR (n = 111) treatments, respectively. There was an effect of AI technician ($P = 0.02$). There was no effect of BW or BW by treatment interaction on pregnancy rate per AI. Based on $P_4$ concentrations (> 1 ng/mL) in blood samples collected on d -14 and at CIDR insertion (d -7 or d -5), 97.9% of the heifers were cycling. On the day of TAI, $P_4$ concentrations in a subset of heifers (n = 208) were less than 1 ng/mL in 96.6% of the heifers. Reducing the duration of CIDR treatment (5-d vs. 7-d) in a CIDR-based TAI protocol did not affect pregnancy rate per AI in dairy heifers.

Key Words: synchronization, CIDR, dairy heifers

## Introduction

Timed AI (**TAI**) protocols were developed to alleviate the difficulties associated with estrous detection and to increase the AI submission rate. However, TAI protocols have generally failed to yield acceptable results in heifers (Pursley et al., 1997; Martinez et al., 2002).

It is not clear why previous TAI protocols have not produced acceptable results in heifers. One reason may be the differences in follicular dynamics in heifers compared to cows (Day, 2004; Rivera et al., 2005). Heifers tend to have a shorter duration of dominance of the preovulatory follicle than cows (by 1.2 d; Wolfenson et al., 2004) with evidence of decreased fertility when the duration of dominance exceeds 8 days (Austin et al., 1999). Consequently, long progesterone (**$P_4$**) exposure may affect follicular turnover and delay or inhibit heifers from exhibiting estrus at the desired time for TAI (Day et al., 2004), resulting in reduced fertility. It was hypothesized (Day et al., 2004) that induction of a new follicular wave with GnRH in the presence of short $P_4$ exposure, prevents the development of a persistent follicle, thus increasing conception to AI. A modified intravaginal $P_4$ insert (**CIDR**)-based protocol was used in beef heifers (British crossbreds; n = 82) to reduce exogenous $P_4$ exposure from 7 days to 5 days to improve conception (Gunn et al., 2007). The conception rates were 39.0% and 65.9% for 7-d (n = 41) and 5-d (n = 41) CIDR treatments respectively.

The objective of this experiment was to determine the effect of reducing the length of time of CIDR exposure in a TAI synchronization protocol (CIDR-$PGF_{2\alpha}$-GnRH and TAI) on pregnancy rate per AI in dairy heifers. It was hypothesized that a reduction in the length of time of CIDR exposure would increase pregnancy rates to AI.

## Materials and Methods

This study was approved by the University of Idaho Animal Care and Use Committee. In October 2007, 241 Holstein heifers in one pen on a heifer raising facility in Southern Idaho were used for this study. Heifers were randomly assigned to one of two treatments (Figure 1). Heifers in Treatment 1 (**7-d CIDR**; n = 120; 398.6 ± 1.2 kg BW [mean ± SEM]) received an intravaginal progesterone insert (EAZI-BREED CIDR, Pfizer Animal Health, New York, NY) on study d -7 for 7 d and $PGF_{2\alpha}$ (25 mg i.m.; Lutalyse, Pfizer Animal Health, New York, NY) at CIDR removal. Heifers in Treatment 2 (**5-d CIDR**; n = 121; 401.2 ± 1.3 kg BW) received a CIDR insert on study d -5 for 5 d and $PGF_{2\alpha}$ at CIDR removal (d 0). All heifers received GnRH (100 μg i.m.; Cystorelin, Merial Limited, Iselin, NJ) and TAI 53 to 55 h after CIDR removal (d 3). To reduce the possibility of CIDR removal by pen mates, CIDR tails were clipped to 7.6 to 10.2 cm of length. Three professional technicians performed AI using frozen thawed semen from one bull. Pregnancy status was diagnosed by palpation of uterine contents 35 d after TAI. Pregnancy rates per AI were determined by dividing the number of heifers diagnosed as pregnant on d 35 by the total number of heifers in each treatment.

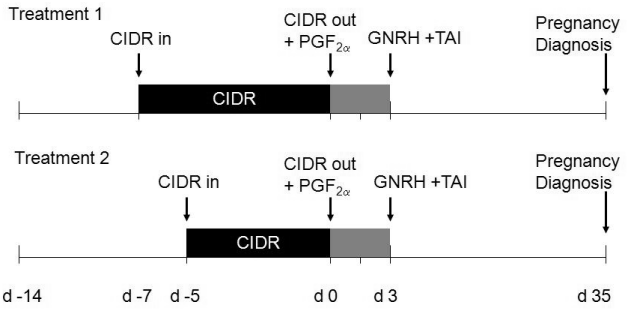


**Figure 1.** Diagram of Treatment 1(7-d CIDR) and Treatment 2 (5-d CIDR). Timed –AI (TAI) and GnRH administration occurred at 53 to 55 h after CIDR removal.

Blood samples were taken on a subset of heifers via coccygeal venipuncture into MONOJECT (Kendall Tyco Healthcare, Mansfield, MA) heparinized blood collection tubes on d -14, before CIDR insertion on d -7 or d -5, and on the day of TAI for $P_4$ analyses. Samples were placed on ice until centrifuged at 2500 × *g* for 12 minutes. Plasma was stored at -60°C until radioimmunoassay (DSL-3400 Progesterone RIA kit, Diagnostic Systems Laboratories, Inc., Webster, TX). Inter-assay variation was 9.2% and intra-assay variation was 2.13%. collected on day -14, before CIDR insertion (d -7 or d -5), and

≥ 1 ng/ml) or low (< 1 ng/ml) $P_4$ concentration. If any of the first two blood samples contained plasma $P_4$ levels ≥ 1 ng/ml, heifers were classified as being cyclic before treatment initiation. The last blood samples were collected to determine if heifers responded to $PGF_{2\alpha}$ with luteolysis and were thus synchronized. If samples contained plasma $P_4$ concentrations of < 1 ng/mL, it was considered that luteolysis had occurred and heifers were synchronized.

Data were analyzed using logistic regression in SAS (SAS Inst. Inc., Cary, NC). The full statistical model included the effects of treatment, AI technician, treatment by AI technician, BW, BW by treatment, and BW by AI technician. To examine the effect of $P_4$ on pregnancy rate per AI for the subset of heifers, logistic regression procedure was performed. The model included the effect of treatment, $P_4$ value (high or low) on d -14, $P_4$ value before CIDR insertion on d -7 or d -5, and their interaction with treatment.

## Results and Discussion

Thirteen heifers were removed from the study. Heifers (n = 8) were removed from the study after losing a CIDR insert. Five heifers were removed for inaccurate records because recorded AI dates at the heifer facility did not correspond with the study date for TAI.

Treatment had no effect on pregnancy rate per AI (Table 1), 41.9% vs. 42.3% for Treatment 1 (7-d CIDR; n = 117) and Treatment 2 (5-d CIDR; n = 111), respectively (Table 1). Although a similar study found differences in conception per AI (39.0% and 65.9% for 7-d and 5-d CIDR exposure respectively; Gunn et al., 2007), the heifers used in that study were beef heifers (British crossbreds). Gunn et al. (2007) hypothesized that use of the 5–d CIDR protocol in beef heifers increased the incidence of ovulation to the GnRH injection, resulting in improved conception per AI. Nevertheless, we did not observe a difference between the 5-d CIDR and 7-d CIDR treatments in the current study. It is plausible that the 5-d CIDR protocol did not improve the incidence of ovulation to GnRH in dairy heifers. Further research will be necessary to provide evidence for the mechanism(s) that may contribute to increased fertility following short $P_4$ exposure.

**Table 1.** Pregnancy rate per AI, by treatment, of heifers diagnosed pregnant on d 35 after Timed-AI (TAI).

| Treatment[1] | Pregnancy Rate per AI |
|---|---|
| 7-d CIDR ( n = 117) | 41.9% |
| 5-d CIDR (n = 111) | 42.3% |
| Overall (n = 228) | 42.11% |

[1] 7-d CIDR: 7 d CIDR exposure (d -7) + 25 mg $PGF_{2\alpha}$ (d 0) + 100 µg GnRH (53 to 55 h after CIDR removal) + TAI; 5-d CIDR: 5d CIDR exposure (d -5) + 25 mg $PGF_{2\alpha}$ (d 0) + 100 µg GnRH (53 to 55 h after CIDR removal) + TAI.

The overall pregnancy rate per AI was lower than expected (42.11%; Table 2). One factor that may partially explain the overall reduced fertility may be the time of TAI. Although Gunn et al. (2007) used only one technician, the time of insemination was 56 to 58 h after $PGF_{2\alpha}$ administration and CIDR removal rather than 53 to 55 h used in the present study. Moreover, many heifers were observed in estrus on the day of TAI in the current study. Thus the time of insemination might not have been optimal, resulting in an overall reduced pregnancy rate per AI.

There was no treatment by AI technician interaction. However, there was an effect of AI technician ($P = 0.02$) on pregnancy rates per AI. Pregnancy rates per AI were 43.6% (n = 94), 30.3% (n = 76), and 55.2% (n = 58) for Technicians 1, 2, and 3 respectively (Figure 2). Because heifers were randomly assigned to each AI technician and frozen thawed semen from one bull was used, another factor must account for these differences. Significant differences between AI technicians have been reported previously (Senger et al., 1984; Dalton et al. 2004). The differences observed in the present study may be related to semen handling and site of semen deposition.

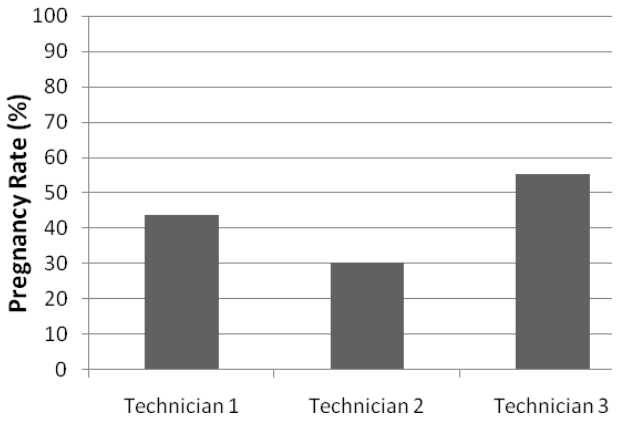


**Figure 2.** Pregnancy rate per AI for three professional technicians.

There was no effect of BW (Table 2) or BW by treatment interaction on pregnancy rate per AI. At the facility where the experiment was conducted, heifers are moved into breeding pens based on body weight rather than age.

**Table 2.** Mean[1] body weight (BW) on d -15 for heifers that completed the study.

| Treatment[2] | BW (kg) |
|---|---|
| 7-d CIDR ( n = 117) | 398.7 ± 1.2 |
| 5-d CIDR (n = 111) | 401.2 ± 1.4 |
| Overall (n = 228) | 399.9 ± 0.9 |

[1]Mean ± SEM

[2] 7-d CIDR: 7 d CIDR exposure (d -7) + 25 mg $PGF_{2\alpha}$ (d 0) + 100 µg GnRH (53 to 55 h after CIDR removal) + TAI; 5-d CIDR: 5d CIDR exposure (d -5) + 25 mg $PGF_{2\alpha}$ (d 0) + 100 µg GnRH (53 to 55 h after CIDR removal) + TAI.

Blood $P_4$ concentrations (high or low) on d -14, or before CIDR insertion (d -7 or -5) had no effect on pregnancy rate per AI. Further, there was no $P_4$ value by treatment interaction effect on the probability of pregnancy rate per AI.

Based on $P_4$ concentrations (> 1 ng/mL) in blood samples collected on day -14 and before CIDR insertion (d -7 or d -5), 97.9% of a subset of heifers (n = 142) were cycling. Because of this high percentage, it is not likely that pregnancy rates were affected by prepubertal heifers. On the day of TAI, $P_4$ concentrations in a subset of heifers (n = 208) were less than 1 ng/mL in 96.6% of the heifers indicating that heifers responded to $PGF_{2\alpha}$ with luteolysis and were thus synchronized. The mean plasma $P_4$ levels before CIDR insertion on d -7 or d-5 and on the day of TAI are shown in Table 3.

**Table 3.** Mean[1] plasma progesterone ($P_4$) levels before CIDR insertion (d -7 or d -5) and on the day of timed-AI (TAI; d 3).

| Treatment[2] | $P_4$ ng/mL Day of CIDR Insertion | $P_4$ ng/mL Day of TAI |
|---|---|---|
| 7-d CIDR | 4.31 ± 0.40 (n = 108) | 0.33 ± 0.07 (n = 105) |
| 5-d CIDR | 6.26 ± 0.52 (n = 96) | 0.46 ± 0.07 (n = 103) |
| Overall | 5.34 ± 0.34 (n = 204) | 0.40 ± 0.05 (n = 208) |

[1]Mean ± SEM

[2] 7-d CIDR: 7 d CIDR exposure (d -7) + 25 mg $PGF_{2\alpha}$ (d 0) + 100 µg GnRH (53 to 55 h after CIDR removal) + TAI; 5-d CIDR: 5d CIDR exposure (d -5) + 25 mg $PGF_{2\alpha}$ (d 0) + 100 µg GnRH (53 to 55 h after CIDR removal) + TAI.

## Implications

Reducing the duration of CIDR treatment (5-d vs. 7-d) in a CIDR-based TAI protocol did not affect pregnancy rate per AI in dairy heifers. In order to further improve pregnancy rates in dairy heifers and obtain the benefits of using TAI, further research must be done.

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# EFFECTS OF RACTOPAMINE HYDROCHLORIDE AND FEEDING PERIOD ON CARCASS QUALITY AND SENSORY CHARACTERISTICS IN MARKET DAIRY COWS[1]

**J.D. Allen[2], J.K. Ahola[3], M. Chahine[4], J.I. Szasz[2], and C.W. Hunt[2]**

[2]Department of Animal and Veterinary Sciences, University of Idaho, Moscow 83843; [3]Department of Animal and Veterinary Sciences, Caldwell Research and Extension Center, University of Idaho, Caldwell 83605; [4]Department of Animal and Veterinary Sciences, Twin Falls Research and Extension Center, University of Idaho, Twin Falls 83301

**ABSTRACT**: Market Holstein cows (n = 26; 659 ± 25 kg initial BW) in 3 replicates were allocated to 1 of 3 dietary treatments to determine the effects of ractopamine-HCl in combination with a short feeding period on carcass quality and sensory characteristics. Dietary treatments were non-fed (**CON**), fed for 90 d (**NoR**), and fed for 90 d with the inclusion of ractopamine (312 mg·cow$^{-1}$·d$^{-1}$) for the final 32 d (**RAC**). Cows assigned to CON treatment were harvested at arrival, whereas NoR and RAC treatments were individually fed a high concentrate diet for 90 d prior to harvest. Fed cows had greater ($P$ = 0.001) final BW (760.0 vs. 644.4 kg), HCW (415.4 vs. 325.6 kg), and marbling score (Modest$^{30}$ vs. Slight$^{69}$; $P$ = 0.03) compared to CON cows, respectively. Fed market cows also tended to have more desirable fat color [2.2 vs. 3.7 (1=white, 9=yellow); $P$ = 0.08] and quality grade [4.6 vs. 3.2 (3=average Utility; 4=high Utility); $P$ = 0.09] vs. CON cows. A taste panel evaluation involving 12 trained panelists revealed fed cows had more desirable tenderness ($P$ = 0.04), juiciness ($P$ = 0.02) and overall acceptability ($P$ = 0.02) and tended to have improved flavor intensity ($P$ = 0.10) in comparison to CON cows. No difference in off-flavor was observed between fed and CON cows ($P$ = 0.53). No differences were observed between NoR and RAC groups for all quality and sensory traits studied ($P$ > 0.10). Our findings suggest feeding market Holstein cows a high concentrate diet for 90 d improved carcass quality and meat palatability; however, no effect of supplementing ractopamine was observed.

**Key words**: carcass, cull, dairy, ractopamine, quality

## Introduction

Market dairy cows add a significant amount of beef to the United States beef industry each year (NCBA, 1999). However, the quality of their carcasses has been scrutinized, with various carcass defects being reported (NCBA, 2007). Quality defects can result in a reduction in carcass weight, meat quality and consumer satisfaction (NCBA, 1999). Dairy producers realize only about 4% of total income from market dairy animal sales; therefore, variables such as conformational issues, poor milk production and health status are used to select unprofitable cows and remove them from the herd (USDA, 1996). These culling variables may affect the quality of market dairy carcasses and quantity of marketable beef.

Beyond the use of proper culling procedures, research has shown that the administration of a short feeding period prior to harvest can help improve carcass and meat quality on market beef and dairy cows (Schnell et al. 1997; Sawyer et al., 2006). A high concentrate diet can also modify external fat color from a greasy yellow to white, which has an influence on consumer buying habits (Schnell et al., 1997).

To maximize growth and economic return, the use of ractopamine HCl, has been included into finishing swine and cattle feeding programs. Ractopamine alters the lean to fat ratio as well as improves muscle protein accretion, HCW and the gain to feed ratio (Mersmann, 1998; Walker et al., 2006). However, ractopamine has also been shown to increase muscle fiber diameter and proportion of type IIB muscle fibers, which are potential factors for decreased tenderness and palatability (Aalhus et al., 1992; Gonzalez et al., 2007). Studies have reported a decrease in or absence of type IIB fibers in older cattle and humans (Short et al., 2005; Gonzalez et al., 2007), indicating that the effect of ractopamine may be minimal on older livestock. Since ractopamine (Optaflexx, Elanco Animal Health, Greenfield, IN) was only recently approved for use in beef production (2004), little research has been performed to examine the effect of ractopamine supplementation on market beef and dairy cows. Therefore, the objectives of this project was to determine the effects of two management strategies – a short feeding period and inclusion of ractopamine – on carcass quality and sensory characteristics in market dairy cows.

## Materials and Methods

All procedures involving the use of animals were approved by the University of Idaho Institutional Animal Care and Use Committee. Twenty-seven cows (n = 27) – 9 from each of 3 different Pacific Northwest commercial dairies – were used in 3 feeding replicates. Initial BW and body condition scores (**BCS**; 1 to 5 scale; 1 = emaciated, 5 = obese; Wildman, 1982) were recorded for all cows. Cows were randomly assigned to one of 3 treatments: non-fed control (**CON**), feeding for 90 d only (**NoR**), and feeding for 90 d with inclusion of ractopamine (312 mg·cow$^{-1}$·d$^{-1}$; **RAC**). Cows assigned to the CON group were immediately harvested to characterize initial body composition and

[1]The authors wish to acknowledge support for this research to the Idaho Beef Council and the $1-per-head beef check-off.

carcass traits of all cows and to mirror the current industry practice.

Cows in the NoR and RAC groups were placed in individual pens and fed a finishing ration twice daily for 90 d (Table 1). Feed intakes were recorded daily. Cows in the RAC group were fed a ground corn supplement (227 g, top-dressed) containing 312 mg of Optaflexx at each nightly feeding beginning 32 d prior to harvest. In contrast, cows in the NoR group received only the ground corn supplement (227 g). Final BW and BCS were recorded on d 90.

Twenty-six cows (n = 26) were harvested at the University of Idaho abattoir. Carcass traits were measured by a trained evaluator after a 24-hr chill. Quality grades (**QG**) were derived from industry standards. Both strip loins were removed from each carcass and steaks (cut 2.54 cm thick) were aged for 14 d, vacuum packaged and stored at -20$^{o}$C for subsequent analysis.

To obtain Warner-Bratzler shear force (**WBS**) values, cores (1.27 cm in diameter) were cut parallel to the muscle fiber orientation of cooked steaks. A Texture Analyzer (TA-XT2, Texture Technologies Corp., Scarsdale, NY) equipped with a Warner-Bratzler shear blade was used to obtain WBS values (kg/cm$^2$).

A 12-person trained sensory panel evaluated palatability (tenderness, juiciness, flavor, off-flavor, and overall acceptability) differences among steaks. Steaks were thawed overnight and broiled on DeLonghi Alfredo grills (Model BG-16: DeLonghi America Inc., Carlstadt, NJ) to an internal temperature of 71$^{o}$C. Steaks were separated from external fat and cut into 2.54 x 1.27 x 1.27 cm cubes and served warm to each panelist. Panelists evaluated 8 to 9 samples per session for tenderness (0 = extremely tough to 10 = extremely tender), juiciness (0 = dry to 10 = juicy), flavor intensity (0 = bland to 10 = intense), off-flavor (0 = none detected to 10 = pronounced) and overall acceptability (0 = not acceptable to 10 = highly acceptable).

Statistical analyses were conducted using SAS (Version 9.1, SAS Inst., Inc., Cary, NC). The experiment was analayzed as a completely randomized design. One cow in the NoR group was removed from the trial prior to inclusion of ractopamine because of poor health status from the initiation of the feeding period and failure to maintain adequate DMI. The GLM procedure was invoked for all measurements. Means and partitioning were generated using the LSMEANS and PDIFF options of SAS. Comparisons between 2 different treatments were also generated using contrast statements (CON vs. all fed cows, and RAC vs. NoR). Effects of initial BW and BCS as well as age and pregnancy status were first analyzed to determine any covariate effects. Effect of treatment on all measurements was then determined between treatment groups as well as between non-fed and feeding groups.

## Results and Discussion

Body weight, meat quality parameters, and sensory panel results are shown in Table 2. Initial BCS and BW were not different among the three treatments ($P > 0.30$). However, feeding significantly improved final BCS, BW as well as HCW ($P \leq 0.001$) when compared to CON cattle. This is consistent with previous feeding trials (Matulis et al., 1987; Schnell et al., 1997). Apple et al. (1999) reported a positive influence of BCS on carcass quality and quantity, indicating a disappearance of quality defects in market cow carcasses as cow condition improves. The current study observed no difference in final BW, BCS and HCW between NoR and RAC groups. Ractopamine's effect on BW and HCW of feedlot cattle is mixed (Gruber et al., 2007; Quinn et al., 2008).

Marbling, fat color and quality grade were unaffected by the addition of ractopamine ($P > 0.80$). Others have reported mixed results pertaining to its effect on marbling in younger cattle (Winterholler et al., 2007; Gruber et al., 2007). Quality grades were unaffected with ractopamine supplementation in heifers (Sissom et al., 2007; Quinn et al., 2008).

Fat color and quality grade tended to be improved with feeding ($P = 0.09$). Schnell et al. (1997) also reported an improvement in fat color with feeding. Matulis et al. (1987) reported improved quality grade in fed mature dairy cows. Feeding in the current study improved marbling score over non-fed CON cattle ($P = 0.03$). Matulis et al. (1987) reported improved marbling scores in market dairy cows after a 28 d feeding period.

Warner-Bratzler shear force measurement was not affected by either feeding or ractopamine supplementation ($P > 0.20$), although CON cows had the highest numerical mean WBS value. This result is inconsistent with other market cow research. Feeding market beef and dairy cows for at least 54 d was reported to improve shear force (Matulis et al., 1987; Boleman et al., 1996). Classification of an "intermediate" meat (between "tender" and "tough") has been suggested to be between 3.92 and 4.5 kg/cm$^2$ (Miller et al., 2001). Accordingly, beef from the 17 fed market dairy cows falls within a tenderness range in which consumers find "acceptable", whereas the 9 non-fed cows were outside the acceptable range.

No differences in any of the taste panel measurements were observed between NoR and RAC groups ($P \geq 0.28$). Off-flavor was also not affected by feeding ($P = 0.53$), and flavor intensity tended ($P = 0.10$) to be improved with feeding. Steaks from fed cows had greater sensory tenderness, juiciness, and overall acceptability when compared to CON cows ($P < 0.04$). Literature comparing fed and non-fed mature dairy cattle sensory traits is limited. However, in a survey including market dairy beef leaving the packing plant, Stelzleni et al. (2007) reported no difference between beef from fed and non-fed market dairy cattle. Others have reported inconsistent differences in juiciness and tenderness among non-fed beef cows and beef cows fed for increasing feeding periods (Boleman et al., 1996; Schnell et al., 1997). Schnell et al (1997) also observed no difference in flavor intensity, whereas Boleman et al. (1996) observed an improvement in flavor intensity and a decrease in off-flavor intensity of fed cattle. Although reports are inconclusive, there is no evidence of decreased palatability in mature cows after a short feeding period.

## Implications

Feeding market dairy cows for 90 days can improve carcass quality and several parameters of meat palatability. Also, in this study the inclusion of ractopamine with feeding did not improve quality traits above regular feeding. However, the number of cows involved in this study was limited. Therefore, research using a larger number of cow observations is necessary to evaluate ractopamine's effect.

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**Table 1.** Dietary ingredients and composition of the finishing diet, DM basis

| Item | |
|---|---|
| Ingredient, % | |
| Alfalfa hay | 8.00 |
| Timothy hay | 5.99 |
| Barley, rolled | 35.51 |
| Corn, rolled | 47.50 |
| Soybean Meal (46% CP) | 1.49 |
| Urea | 0.51 |
| Limestone | 0.20 |
| Trace mineral mix | 0.31 |
| Rumensin premix | 0.50 |
| Fine ground corn[1] | ------ |
| | |
| Chemical composition | |
| DM, % | 84.32 |
| Crude protein, % | 10.63 |
| $NE_g$, Mcal·kg$^{-1}$ | 0.21 |
| TDN, % | 64.88 |
| Fat (ether extract), % | 2.83 |
| Calcium, % | 0.25 |
| Phosphorus, % | 0.37 |

[1]Fine ground corn (227 g) was used as a carrier to deliver ractopamine at a rate of 312 mg·cow$^{-1}$·d$^{-1}$ beginning 32 d prior to harvest in RAC cows, while only 227 g of fine ground corn was provided to NoR cows.

**Table 2.** Least squares means and SEM for market dairy cow live condition and meat quality parameters corresponding to the main effects of treatment

| | Treatment | | | | P < | |
|---|---|---|---|---|---|---|
| Trait: | CON[1] | NoR | RAC | SEM | CON vs Fed[2] | NoR vs RAC |
| Initial BCS[3] | 2.8 | 3.1 | 2.9 | 0.20 | 0.37 | 0.61 |
| Initial BW, kg | 644.4 | 662.8 | 670.2 | 25.30 | 0.62 | 0.84 |
| Final BCS | 2.8 | 4.1 | 4.2 | 0.20 | 0.001 | 0.69 |
| Final BW, kg | 644.4 | 759.9 | 759.9 | 26.03 | 0.001 | 1.00 |
| HCW, kg | 325.6 | 416.3 | 414.6 | 15.50 | 0.0001 | 0.94 |
| Marbling score[4] | 369 | 539 | 522 | 60.0 | 0.03 | 0.85 |
| Fat Color[5] | 3.7 | 2.3 | 2.1 | 0.70 | 0.09 | 0.89 |
| Quality grade[6] | 3.2 | 4.5 | 4.7 | 0.63 | 0.09 | 0.86 |
| WBS[7], kg/cm$^2$ | 4.9 | 4.3 | 4.5 | 0.35 | 0.23 | 0.70 |
| | | | | | | |
| Sensory Panel Traits | | | | | | |
| Tenderness[8] | 5.7 | 7.6 | 7.0 | 0.60 | 0.04 | 0.47 |
| Juiciness | 5.7 | 7.0 | 7.2 | 0.50 | 0.02 | 0.83 |
| Flavor intensity | 5.9 | 6.8 | 6.5 | 0.33 | 0.10 | 0.54 |
| Off-flavor | 1.7 | 1.3 | 1.6 | 0.30 | 0.53 | 0.49 |
| Acceptability | 5.2 | 7.0 | 6.3 | 0.50 | 0.02 | 0.28 |

[1]CON = non-fed control group; NoR = fed 90 d; RAC = fed 90 d with inclusion of ractopamine (312 mg·cow$^{-1}$·d$^{-1}$) for the final 32 d.
[2]Comparison of non-fed group (CON) to both feeding groups (NoR and RAC).
[3]Body condition score between 1 (emaciated) to 5 (obese).
[4]400 = Slight$^0$; 500 = Small$^0$; etc.
[5]Fat color scale: 1 (white) to 9 (greasy yellow).
[6]1 = Cutter; 3 = Average Utility; 4 = High Utility; 7 = High Commercial; etc.
[7]Warner-Bratzler shear force.
[8]Scale: Tenderness (0 = extremely tough, 10 = extremely tender); Juiciness (0 = extremely dry, 10 = extremely juicy); Flavor Intensity (0 = no flavor, 10 = intense flavor); Off-Flavor (0 = no off-flavor, 10 = intense off-flavor); Acceptability (0 = not acceptable, 10 = very acceptable).

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# EFFECT OF RU486 ON DEVELOPMENT OF TESTICULAR STEROIDOGENESIS AND RAM SEXUAL BEHAVIOR

**P. Singh, K. J. Austin, V. A. Uthlaut, B. M. Alexander**
**Department of Animal Science, University of Wyoming, Laramie, Wyoming**

**ABSTRACT:** Progesterone influences the development and expression of male sexual behavior in rodents and may be important for the expression of sexual behavior in rams. Masculinization and/or defeminization of the central nervous system in sheep occur between d 60 and 70 of pregnancy. A second phase of testosterone-responsive sexual development occurs at 6 to 8 weeks of age in ram lambs. To determine if progesterone influences adult sexual behavior during this developmental period, twin born male lambs (n = 10) were used in this study. One of each twin was treated with 10 mg of the progesterone receptor antagonist mifepristone (**RU486**; n = 5) and his co-sibling was treated with an equal volume of vehicle (n = 5) twice daily from 4 to 8 wk of age. Sexual behavior and serum concentrations of testosterone were evaluated at 9 mo of age. Lamb BW were similar (P = 0.4) at the end of RU486 treatment (8 wk of age), and did not differ (P = 0.15) during behavior testing at 9 mo of age. Change of weight, however, tended (P = 0.08) to be greater in RU486 treated ram lambs. Testes were measured by a scrotal tape at the end of the treatment period and during behavior testing at 9 mo of age. Although testicular circumferences ($P \geq 0.4$) and BW (P = 0.15) did not differ, serum concentrations of testosterone were less (P = 0.06) at 9 mo of age in rams treated with RU486. Sexual behavior was evaluated for 30 min at three different times by placing the rams with two estrous ewes. Behavior was classified as investigatory (ano-genital sniffs, flehmen, fore-leg kick, and nudge) or consummatory (mount attempt, mount, and ejaculation) behavior. Expression of investigatory behavior was decreased (P = 0.03) at the first exposure to estrous ewes in RU486 treated rams, but not ($P \geq 0.4$) in subsequent tests. Consummatory behavior was similar ($P \geq 0.24$) among treatment groups at all observations. Blocking the progesterone receptor at 6 – 8 weeks of age influences steroid production in the yearling ram, but a robust influence on the expression of sexual behavior remains to be determined.

## Introduction

Typical breeding practices for food-animal species utilize limited numbers of males to inseminate large numbers of females. Therefore, it is critical that libido (sexual interest or motivation), mating competence (ability to inseminate females) and fertility (semen quality) of males is adequate to insure reproductive success. Libido in rams is highly variable and is influenced by developmental (Roselli et al., 2003) and environmental (Price, 1987) factors.

Progesterone is named for its progestational role in maintaining pregnancy in mammals, and is traditionally regarded as a "female hormone." The facilitory and inhibitory effects progesterone exerts on female reproductive behavior are well documented (Blaustein and Erskine, 2002). Progesterone is a precursor for both androgen and estrogen synthesis. In the male, androgens are necessary for the development of secondary sex characteristics and testosterone is considered the primary male sex hormone. However the role of testosterone in the expression of male-typical behavior has been overstated since there is little correlation between plasma testosterone concentrations and male behavior (reviewed in: Andersen and Tufik, 2006). Testosterone is aromatized to estradiol 17β in specific hypothalamic nuclei and is considered the centrally active hormone in the male (reviewed in Resko et al., 1999). Progesterone receptors are also present in behaviorally relevant nuclei of the male brain, and progesterone receptor knock-out male mice exhibit sexual-behavior deficits (Phelps et al., 1998).

The physiological significance of progesterone in the male, outside of its role as a precursor for androgen production, is not well understood. The progesterone receptor is upregulated in the hypothalamus of male fetuses during brain sexual differentiation (Roselli, et. al., 2006) and may play a role in the development of central pathways necessary for the expression of adult sexual behavior in rams. A second phase of testosterone-responsive sexual development occurs in male sheep during 6 to 8 weeks of age (Orgeur and Signoret, 1984). Although expression of progesterone receptor in the sheep brain has not been evaluated at this developmental period, progesterone acting through its receptor may affect the expression of sexual behavior either directly or indirectly by altering testosterone production of the testes. The objective of the current study was to evaluate the role of progesterone, acting at its receptor, in post-natal development of ram sexual behavior.

## Materials and Methods

Animal care and use was approved by the University of Wyoming internal animal care and use committee. Twin born male lambs (n = 10) 4 wk of age were used for this study. One sibling of each pair was treated with 10 mg of the progesterone receptor antagonist mifepristone (**RU486**; n = 5) with his co-sibling treated with an equal volume of vehicle (n = 5) twice daily from 4 to 8 wk of age. At the end of the treatment period lambs were weaned and fed a forage-based diet which supported moderate growth for 7 mo. Body weights and scrotal circumference were

collected weekly during the treatment period and at 9 mo of age.

Rams at 9 mo of age were individually exposed to ewes in estrus on three occasions separated by approximately 14 days. Rams were confined in a pen (2.4 m x 4.7 m) with two ewes in estrus. Behavior was monitored by digital camera for 30 minutes during each exposure period. Behavior was quantified by manually viewing the digital recording. Behaviors were classified as investigatory (ano-genital sniffs, flehmen, fore-leg kick and nudge) or consummatory (mount attempt, mount and ejaculation).

Ovariectomized ewes were used as teaser ewes by exposing them to progesterone for at least 14 d by intravaginal progesterone CIDR. Following CIDR removal, ewes were treated daily with 50 µg of estradiol (IM). Estrus behavior was evident by 48 hr following initial estradiol treatment. Receptive behavior was maintained for five days with daily estradiol treatments. Estrous behavior was confirmed using intact rams with known breeding capacity.

A single blood sample was collected by jugular venipuncture at 9 mo of age for analysis of serum concentrations of testosterone. Blood samples were allowed to clot overnight at 4° C. Serum was separated by centrifugation at 1500 *g* for 20 min, and stored at -20° C. Concentrations of serum testosterone were determined in a single radioimmunoassay. Antibody coated tubes and radiolabeled testosterone were purchased from Diagnostic Products Corporation (Los Angeles, CA). Standards were prepared by serial dilutions of a stock solution in charcoal treated whether serum. Assay tubes were incubated at room temperature for 4 hours. Intra-assay coefficient of variation was <10%.

Behavior was summarized as investigatory or consummatory and analyzed using GLM methods of SAS (Ver. 9.1, Cary, NC). Effects of treatment were tested as the main effect for behavior with time and treatment by time interactions tested as subplot effects. Animal within treatment was used as the error term for treatment effects. Treatment effects of BW, scrotal circumference and serum concentrations of testosterone were analyzed by analysis of variance using GLM procedures of SAS (Ver. 9.1, SAS Inst. Inc., Cary, NC).

## Results

Lamb weights and testicular circumference did not differ when treatments were initiated at 4 wk of age, nor did they differ (P > 0.4) at 8 wk of age following RU486 treatment (21.3 ± 1.7 kg; 14.6 ± 0.8 cm). Rams weighed 66.4 ± 3.2 kg at 9 mo of age with an average testicular circumference of 33.2 ± 0.4 cm. Neither weight nor testicular circumference varied (P > 0.15) among treatment

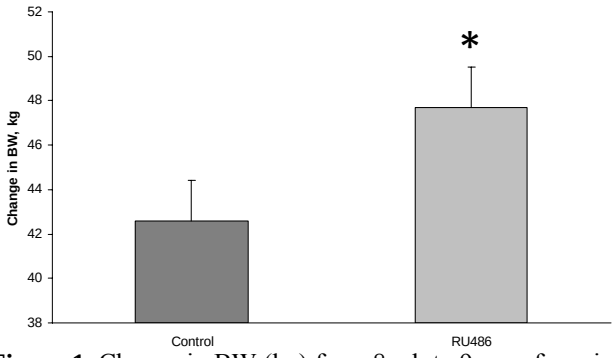


**Figure 1.** Change in BW (kg) from 8 wk to 9 mo of age in control and ram lambs treated with RU486 from 4 to 8 wk oof age *(P = 0.08)

groups. Although BW was not different at the time of RU486 treatment or at 9 mo of age, change in weight tended to be greater (P = 0.08) in rams treated with RU486 as lambs (Fig. 1).

Although BW and testicular circumference did not differ among treatment groups, serum concentrations of testosterone were greater (P = 0.06) in control rams than rams treated with RU486 as lambs (Fig. 2).

Expression of investigatory behavior was decreased (P = 0.03) in RU486 treated rams compared to control rams at the first exposure to estrous ewes, but not (P ≥ 0.4) in subsequent tests (Table 1). Consummatory behavior was similar (P ≥ 0.2) among treatment groups at all observations (Table 1).

## Discussion

Differences in mating behavior exist among individuals of all species studied (Meisel and Sach, 1994). Mating performance of rams is important for the profitability of the sheep industry. Stellflug et al. (2006) indicated twice as many poor-performing rams were needed to obtain breeding results equal to a single high-sexually performing ram. With nearly 30% of rams classified as non-performers (Fitzgerald and Perkins, 1991), the importance of ram sexual behavior is well recognized.

Testicular androgen production increases in male sheep fetuses between d 35 and 70 of pregnancy (Pomerantz and Nalbandov, 1975). Masculinization of external genitalia occurs early in that developmental period with masculinization and/or defeminization of the central nervous system occurring later in the period of sexual differentiation. Expression of progesterone receptor mRNA, but not androgen or estrogen receptor, was greater

**Table 1.** Sexual behavior expressed in three separate tests at 9 mo of age during exposure to estrous ewes in control and rams treated with RU486 from 4 to 8 wk of age.

| TRT | Test | Invest[a] | SE | Consum[b] | SE |
|---|---|---|---|---|---|
| **Con** | **1** | **24.0** | **4.9** | **7.8** | **3.1** |
| **Con** | **2** | **16.2** | **4.9** | **4.4** | **3.1** |
| **Con** | **3** | **16.6** | **4.9** | **5.2** | **3.1** |
| **RU486** | **1** | **9.8** | **4.9** | **1.8** | **3.1** |
| **RU486** | **2** | **11.4** | **4.9** | **7.4** | **3.1** |
| **RU486** | **3** | **10.7** | **4.9** | **3.4** | **3.1** |

[a]Investigatory Behavior

[b]Consummatory Behavior

in the hypothalami of male than female sheep fetuses at 64 d of gestation (Roselli et al., 2006) suggesting that expression of the progesterone receptor may be important in sexual differentiation of the male brain.

A second phase of testosterone-responsive sexual differentiation occurs in rams between 6 and 8 wk of age. This period coincides with the expression of intense sexual play by the male lamb (Orgeur and Signoret, 1984). Although progesterone receptors have not been quantified in the hypothalamus of lambs at this developmental period, progesterone acting through its receptor may affect the expression of play behavior and alter expression of adult sexual behavior. Progesterone receptor knock-out mice show greater androgen-receptor immunoreactivity in the medial preoptic area than WT males (Schneider et al., 2005). Therefore, altering activity of the progesterone receptor may affect biological change in the male through altered expression of the androgen receptor.

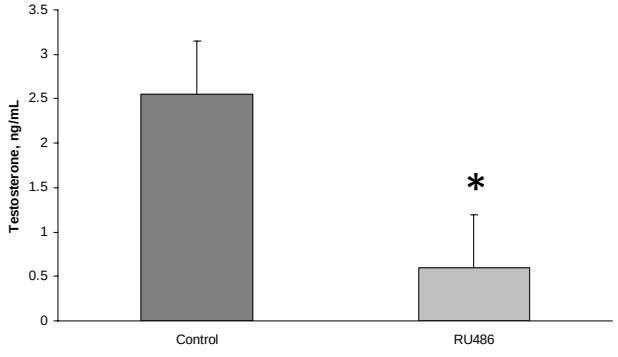


**Figure 2.** Serum concentration of testosterone (ng/mL) in control and ram lambs treated with RU486 from 4 to 8 wk of age *(P = 0.06).

In the present study, testosterone production was reduced in RU486 treated male lambs. Although minimal amounts of testosterone are required to exhibit mounting behavior in sexually experienced males (Andersen and Tufik, 2006), it is possible that greater amounts of testosterone may boost sexual activity in sexually naïve males. Reduced production of testosterone in RU486 treated rams may have delayed sexual investigation of estrous ewes during the initial test. Increased growth in RU486 treated ram may manifest through changes in expression of the androgen receptor caused by alterations in the progesterone receptor (Schneider et al., 2005).

## Conclusion

In conclusion blocking the progesterone receptor at 6 – 8 wk of age enhanced growth, and decreased testosterone production, but a robust influence on the expression of sexual behavior remains to be determined.

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# FEEDING GRAIN DECREASES TRAINING EFFECTIVENESS IN 2-YEAR-OLD QUARTER HORSES

**W. R. Black,* M. C. Greenwood, J. L. Weeding, S.J.J. Moreaux, L. C. Gagnon, and J.G.P. Bowman**
Montana State University, Bozeman 59717

**ABSTRACT:** Two replicated experiments (Exp. 1: May 14 to June 8; Exp.2: June 25 to July 20) evaluated effects of feeding grain to 2-yr-old Quarter horses on behavior and physiological parameters during early stages of training. In each experiment, 6 different horses were allotted by sex and weight to 2 diets; hay only or hay plus 2.3 kg/d grain. Horses were group-housed with ad libitum access to grass/alfalfa hay and water, and were individually fed 1.15 kg grain or 40 g salt (placebo) at 0800 and 1600 for 7 d prior to and during training. The trainer was blind to diet assignments. Horses were trained 5 d/wk for 3 wk and scored (1 to 5) by the trainer daily on obedience (willingness to ride with a loose rein and little leg pressure), life (willingness to move at any desired speed), and direction (suppleness in the poll and loin), while an observer scored fearfulness. A heart monitor recorded minimum, maximum, and mean heart rate daily during training. Categorical data were transformed by subtracting the daily median as each horse's score was relative to the other horses on that day. Data were analyzed as repeated measures (Proc Mixed of SAS) with horse as the experimental unit. In Exp. 1 grain did not affect ($P = 0.83$) obedience, while horses fed grain in Exp. 2 were less obedient during training ($P = 0.02$) than those not receiving grain. Horses fed grain showed greater ($P = 0.05$) fearfulness than horses fed hay alone. Life:direction (ideal is 1.0, > 1.0 indicates high self-preservation) was higher ($P = 0.04$) in horses fed grain than in those fed hay alone (1.29 vs. 1.08, respectively). Maximum heart rate was not affected ($P = 0.21$) by grain, while mean heart rate was higher ($P = 0.03$) for horses fed grain than hay alone (126 vs. 119 beats/min, respectively). Horses fed grain during training exhibited more self-preservation behavior, increased mean heart rate, and an unbalanced life to direction ratio, which could inhibit training effectiveness.

**Key words:** behavior, horses, training effectiveness

## Introduction

Horses, being ridden by less experienced riders, need to be calm and easy to handle, characteristics that may be enhanced by more effective early training (Lansade et al., 2005). Behavior problems in horses often arise as a result of self-preservation. When the level of fear rises, then the horse's reasoning ability starts shutting down and defensive reactions start to surface (Black, 2005). Increased dietary energy was shown to increase the level of self-preservation in horses, resulting in less learning during training, and feeding grain has been suggested to cause excitable behavior (Greiwe et al., 1989). Horses fed grain had higher spontaneous activity, and greater reactivity to stimuli than horses fed energy in the form of fat (Holland et al., 1996), while learning performance was higher in calm horses (Kusunose and Yamanobe, 2002). Nervous horses, identified by having an increased heart rate (McCann et al., 1988), were found to be less trainable, as indicated by the negative correlation between higher levels of emotionality and the number of trials to criterion in a learning study (Heird et al., 1981). It is common in today's equine industry to feed young and growing horses grain once or twice a day (Steelman et al., 2006) with the potential of reducing training effectiveness.

The purpose of this study was to determine the effect that feeding grain to 2-yr-old Quarter horses had on measures of training effectiveness (obedience, self-preservation behavior, heart rate, and time to achieve training satisfaction).

## Materials and Methods

### *Animals, Design and Treatments*

Procedures were approved by the Montana State University Institutional Animal Care and Use Committee. A total of 12 Quarter horses (24 to 28-mo-old; 4 geldings, 8 fillies; 417 ±27.5 kg initial BW) were group-housed in two 30.5 x 45.7 m pens (6 horses per pen). In each of 2 experiments, 6 different horses were allotted by sex and weight to 2 treatments (3 horses•treatment$^{-1}$•experiment$^{-1}$). All horses had ad libitum access to grass/alfalfa hay and water.

Two replicated experiments (Exp. 1: May 14 to June 8; Exp.2: June 25 to July 20) were conducted testing the effect of 2 dietary treatments, hay only or hay plus 2.3 kg/d grain (commercial mixture of corn, oats, barley, and molasses; as-fed basis), on training effectiveness. Horses were placed in individual 3 x 3 m pens and fed 1.15 kg grain or 40 g salt (placebo) at 0800 and 1600 for 7 d prior to and during training. After the grain was consumed at each feeding, the horses were turned back out into the large pen. Each experiment consisted of 26 d, with 7 d for diet adaptation followed by 19 d of data collection. Within the data collection period, horses were ridden and trained 5 d/wk for 3 wk for a total of 15 d training. The trainer was blind to diet assignments. Both experiments utilized the same procedures**.**

### *Training Effectiveness*

Effective training was defined as when the horse had: 1) a solid foundation of maneuvers, 2) a balanced life to direction ratio, 3) willing submission, and 4) low levels of

self-preservation. During each training session, the following components were measured:

***Solid Foundation of Maneuvers.*** A solid foundation of maneuvers consisted of 3 stages: stopping forward motion pivoting around inside front foot, lateral movement of shoulders and hindquarters together, and stopping forward motion pivoting around the inside hind foot. Foundation of maneuvers was defined as the horse's willingness to move the front feet and the hind feet in any direction to accomplish any job. On d 15 to 19, and d 22 to 26, the trainer scored each horse on a scale from 1 to 5 (1 = very unwilling; 5 = very willing) on willingness to move the front feet and the hind feet.

***Life and Direction.*** Life was defined as the willingness of the horse to move with any speed at any time; get-up-and-go, or liveliness. The trainer scored life on d 15 to 19, and d 22 to 26 on a scale of 1 to 5 (1 = drive spurs into belly to get movement, constant pressure; 5 = very free, fan legs or light pressure with calves).

Direction was defined as when the slack was taken out of the rein, the horse put the slack back in the rein for horizontal and vertical flexion, with suppleness through the poll and loin. The trainer scored direction on d 15 to 19, and d 22 to 26 on a scale from 1 to 5 (1 = fighting; when slack was taken out of the rein the horse flipped or shook his head; 5 = total agreement; when the slack was taken out of the rein the horse immediately put the slack back in the rein seeking relief).

The life to direction ratio was calculated by dividing the daily score for life by the daily score for direction. A balanced life to direction ratio was defined as being able to willingly bring the life up and direct it, and was considered numerically close to 1.0. A ratio greater than 1.0 indicated that the life was greater than the ability to direct it, and signified lack of control of the horse.

***Willing Submission.*** Willing submission was measured by observations of obedience, and time to achieve training satisfaction. Obedience was defined as after an initial cue, the horse performed the task on a loose rein, with no leg pressure; it was the horse's idea. Obedience was scored on d 11 to 12, d 15 to 19, and d 22 to 26 by the trainer on a scale from 1 to 5 (1 = lack of obedience; 5 = completely obedient).

During each training session, d 8 to 12, d 15 to 19, and d 22 to 26, the trainer recorded the amount of time it took to reach training satisfaction for that day. Training satisfaction was defined as the horse had improved from the day before in building a solid foundation of maneuvers, balancing the life to direction ratio, and attaining willing submission. Time spent training on the ground, and time to saddle the horse before riding were also recorded.

***Self-Preservation.*** Self-preservation was measured through heart rate (HR), locomotor activity, fearfulness, reaction to social separation, and response to a novel stimulus, in this case a flag. Heart rate was recorded during each training session with a Polar S810i model HR monitor (Polar Electro Oy, Kempele, Finland) that consisted of 2 electrodes, a built-in transmitter, and a wrist watch receiver (Visser et al., 2002). The electrode belt was specially made to fit horses. The data received were stored and later downloaded via a Polar InfraRed Interface to a computer, using Polar Equine Software 4.0. Data were recorded as average HR every 5 sec during the training session as well as the mean and maximum HR. A pedometer was used to measure locomotor activity during training. An observer scored the horses during training on d 8 to 12, d 15 to 19, and d 22 to 26 from 1 to 5 on fearfulness (1 = no signs; 5 = high levels, bucking), and on d 8 scored the horses on response to a flag (1 = calm; 5 = highly excitable). Reaction to social separation was scored by the trainer on d 16, 17, and 19, which were the first solo rides outside the arena, away from other horses. Horses that lack confidence in the rider will seek comfort and companionship with other horses by constant vocalizations. Horses were scored on a scale from 1 to 5 (1 = constant whinnying; desperately looking for other horses; 5 = no vocalization, confident in rider, not concerned with other horses).

### *Statistical Analyses*

Categorical data were transformed by calculating each daily score as a deviation from the daily median of that parameter as each horse's score was relative to the other horses on that day. All data were analyzed using repeated measures analysis with the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Covariance structure was modeled for each parameter. The model included effects of experiment, treatment, day, and all possible interactions. Data are presented as least squares means with differences considered significant at $P < 0.10$.

## Results and Discussion

***Solid Foundation of Maneuvers.*** The ability to move the horse's front feet or hind feet in the foundation of maneuvers was not affected ($P > 0.17$) by diet. When a horse's self-preservation is high they become stiff through the poll and loin, which can cause poor direction. However, although the poll and loin may be stiff, the horse's feet can still be directed in the foundation of maneuvers; forward, backward, and laterally.

***Life and Direction.*** Life was greater ($P = 0.09$) for horses fed grain compared with horses fed only hay. Diet or experiment did not affect ($P > 0.93$) direction. However, the life to direction ratio was more ($P = 0.07$) unbalanced (defined as > 1.0) in grained horses than in horses fed hay alone (average 1.28 vs. 1.08, respectively). An unbalanced ratio indicates poor control in relation to life and can cause numerous problems during training. The further a horse's life to direction ratio exceeds 1.0, the more self-preservation increases, and behavior problems may surface. To prevent behavior problems in young horses and possible injuries for unconfident riders, the ideal situation would be to have a balanced life to direction ratio. Our study suggests that feeding grain to horses resulted in a more unbalanced life to direction ratio compared to horses fed hay only.

***Willing Submission.*** Experiment x treatment interactions were observed for obedience ($P = 0.02$), total time to achieve training satisfaction ($P = 0.005$), and ground time ($P = 0.02$). In Exp. 1, grain did not affect ($P > 0.10$) obedience, while horses fed grain in Exp. 2 were less

obedient during training ($P = 0.02$) than those not receiving grain. Total time to achieve training satisfaction was increased ($P = 0.005$) by 20%, and time spent training on the ground before riding was increased ($P = 0.02$) by 40% by feeding grain in Exp. 2, but were not different ($P > 0.10$) due to diet in Exp. 1. The time it took to saddle the horses was increased ($P = 0.07$) by 42% in Exp. 2 compared with Exp. 1, indicating more time was required before the horses' self-preservation levels were decreased enough to proceed with mounted training.

The experiment x treatment interactions may have been due to an increased BCS in horses in Exp. 2 compared with horses in Exp. 1. When the horses were initially allotted to their assigned treatments, the groups weighed the same. However, by the time Exp. 2 was conducted, the second group of horses, who had been eating hay ad libitum and were not being ridden, weighed 19 kg more ($P < 0.001$) than the horses in Exp 1, approximately equivalent to 1 BCS (NRC, 2007). Average daily gain during Exp. 1 was not different ($P > 0.10$) between diets (average 0.58 kg), whereas ADG during Exp. 2 was less ($P = 0.004$) for horses fed hay alone compared with those fed grain (-0.08 vs. 0.08 kg, respectively), and less ($P < 0.001$) than ADG in Exp. 1. This suggests that horses in Exp. 2 worked harder or expended more energy than horses in Exp. 1, resulting in a longer time to achieve training satisfaction. McCall (1989) found that horses with a higher body condition score were distracted more easily during discrimination testing than horses with a lower BCS. As long as horses are maintained in moderate body condition, these results suggest that trainers under time constraints could increase their training effectiveness during the early stages of training by not feeding excess dietary energy.

***Self-Preservation.*** Minimum HR during training was greater in grained horses ($P = 0.003$) during Exp. 2, suggesting a higher level of self-preservation compared with horses fed only hay. No difference ($P > 0.10$) in minimum HR was seen due to diet in Exp. 1. Horses in Exp. 2 had a higher ($P = 0.01$) mean HR during training than horses in Exp. 1 (average 127 vs. 117 beat/min, respectively), another indication that horses in Exp. 2 worked harder than those in Exp. 1. Maximum HR during training was not affected ($P > 0.21$) by diet or experiment (average 208 beats/min). Mean HR has been shown to be highly correlated with behavioral estimates of self-preservation in horses (McCall et al., 2006). Self-preservation is one of the horse's primary driving factors and may be the greatest limiting factor when training horses (Murphy and Arkins, 2007).

Fearfulness levels were higher in grained horses ($P = 0.05$) than in horses fed only hay. Horses fed grain had higher incidences of bucking and running compared to horses fed hay alone. Fiske and Potter (1979) reported high levels of fearfulness reduced learning ability in yearling horses. High levels of self-preservation not only decrease training effectiveness, but may lead to injury of the rider or the horse (Warren-Smith et al., 2005).

The locomotor activity during a training session was 20% greater ($P = 0.008$) for horses fed grain compared to those fed only hay in Exp. 2, while no difference ($P > 0.10$) in steps per session was seen due to diet in Exp. 1. Increased step count suggests a greater level of nervousness during training and nervous horses have been shown to be less trainable (Heird et al., 1981).

There was an experiment x treatment interaction ($P = 0.09$) for reaction to social separation. Horses fed grain in Exp. 2 showed more signs of whinnying and desperately wanting to return to the other horses, indicating a lack of confidence in the rider compared with horses fed hay alone. Horses are driven by comfort and companionship (McGreevey, 2007); if this cannot be found with the rider horses become unconfident and insecure. Horses without security in the rider may be more difficult to train because they are not focused on the rider's cues; instead focusing on seeking comfort and companionship with other horses.

An experiment x treatment interaction was observed for the response to a flag ($P = 0.07$). Horses fed grain in Exp. 2 demonstrated more self-preservation behavior during exposure to a flag on the first day of training compared with horses consuming hay only, while no difference was observed due to diet in Exp. 1. Holland et al. (1996) found horses fed energy in the form of fat had lower reactivity in a startle test, measured by abruptly opening a brightly colored umbrella, compared with horses fed grain.

Willing submission is a key factor in starting young horses. Horses that do not submit willingly may become resentful to commands and dangerous to their riders. Horses in training programs are often ridden for a defined period of time. These results suggest that training satisfaction may be reached sooner if horses are maintained in moderate body condition, and not fed excess dietary energy.

## Implications

These findings imply that feeding grain during the early stages of training decreased training effectiveness in 2-yr-old Quarter horses by increasing self-preservation behavior, decreasing willing submission behavior, and causing an unbalanced life to direction ratio.

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Table 1. Effects of feeding grain on measures of self-preservation behavior, willing submission behavior, and training effectiveness in 2-year-old Quarter horses

| | Treatments[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Exp. 1 | | Exp. 2 | | | *P*-values | | |
| Item | No grain | Grain | No grain | Grain | SEM | Exp | Trt | Exp x Trt |
| n | 3 | 3 | 3 | 3 | | | | |
| Initial wt, kg | 405.8 | 407.5 | 425.5 | 426.1 | 3.87 | <0.001 | 0.76 | 0.88 |
| ADG, kg | 0.58$^{c}$ | 0.57$^{c}$ | -0.08$^{a}$ | 0.08$^{b}$ | 0.028 | <0.001 | 0.01 | 0.004 |
| Foundation of maneuvers | | | | | | | | |
| Front feet[2] | -0.49 | 0.06 | -0.06 | -0.03 | 0.196 | 0.40 | 0.17 | 0.22 |
| Hind feet[2] | -0.09 | 0.13 | -0.13 | -0.03 | 0.249 | 0.72 | 0.54 | 0.82 |
| Life and direction indices | | | | | | | | |
| Life[2] | -0.19 | 0.08 | -0.43 | 0.23 | 0.269 | 0.88 | 0.09 | 0.48 |
| Direction[2] | -0.05 | 0.02 | 0.04 | -0.08 | 0.286 | 1.00 | 0.93 | 0.75 |
| Life:direction | 1.15 | 1.31 | 1.01 | 1.25 | 0.108 | 0.40 | 0.07 | 0.73 |
| Willing submission indices | | | | | | | | |
| Obedience[2] | -0.24$^{a}$ | 0.17$^{ab}$ | 0.41$^{b}$ | -0.24$^{a}$ | 0.22 | 0.59 | 0.59 | 0.02 |
| Total time, min | 34.3$^{ab}$ | 31.4$^{a}$ | 31.1$^{a}$ | 37.4$^{b}$ | 1.60 | 0.41 | 0.27 | 0.005 |
| Ground time, min | 11.7$^{a}$ | 10.8$^{a}$ | 11.2$^{a}$ | 15.7$^{b}$ | 1.15 | 0.09 | 0.13 | 0.02 |
| Time to saddle, min | 5.0 | 5.5 | 6.5 | 8.4 | 1.06 | 0.07 | 0.31 | 0.53 |
| Self-preservation indices | | | | | | | | |
| Minimum HR, bpm | 53.8$^{a}$ | 54.5$^{a}$ | 54.4$^{a}$ | 69.5$^{b}$ | 1.65 | 0.002 | 0.002 | 0.003 |
| Mean HR, bpm | 115.5 | 118.8 | 121.2 | 132.6 | 3.11 | 0.01 | 0.02 | 0.19 |
| Maximum HR, bpm | 203.9 | 208.5 | 204.9 | 213.8 | 4.94 | 0.54 | 0.21 | 0.68 |
| Activity, steps | 3,239$^{bc}$ | 2,895$^{ab}$ | 2,831$^{a}$ | 3,391$^{c}$ | 166.5 | 0.80 | 0.52 | 0.008 |
| Fearfulness[2] | 0.09 | 0.36 | -0.01 | 0.34 | 0.137 | 0.69 | 0.05 | 0.75 |
| Social separation[2] | -0.14$^{ab}$ | 0.31$^{ab}$ | 1.03$^{b}$ | -0.36$^{a}$ | 0.470 | 0.61 | 0.34 | 0.09 |
| Response to flag[2] | 0.17$^{ab}$ | -0.83$^{a}$ | -0.50$^{a}$ | 1.17$^{b}$ | 0.646 | 0.33 | 0.62 | 0.07 |

[1] Two experiments with horses having ad libitum access to hay and supplemented with 0 kg/d (No grain) vs. 2.3 kg/d (Grain) commercial grain mix. Six different horses were used in each experiment.

[2] Scored as 1 to 5; units are deviations from daily median for that parameter.

[a-c] Within a row, means without a common superscript letter differ ($P < 0.10$).

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# RELATIONSHIP BETWEEN RESIDUAL FEED INTAKE AND GROWTH PERFORMANCE, EPD PROFILES, AND VALUE INDICES OF SPRING-BORN ANGUS BULLS[1]

**J. M. Cardin[2], S. P. Doyle[2], J. A. Harrington[2], K. L. Cooprider[2], C. R. Johnson[2], and L. Rechel[3]**
[2]College of Agriculture, California State University, Chico 95929
[3]Snyder Livestock Company, Inc., Yerington, NV 89447

**ABSTRACT**: The objectives of this study were to: 1) determine the phenotypic relationship between residual feed intake and growth performance and 2) characterize low, moderate and high residual feed intake (RFI) beef cattle for phenotypic growth performance, growth and ultrasound carcass EPDs, and value indices. Ninety-one spring-born Angus bulls were consigned to a 112-d central bull test in Yerington, NV. Individual feed intake data and BW gain were collected over a period of 62 d using the GrowSafe automated feeding system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). RFI was calculated for each bull; values were used to classify bulls into efficient (< -0.5 SD; n = 22; RFI = -1.35 kg/d), marginal (± 0.5 SD; n = 40; RFI = -0.05 kg/d) and inefficient (> 0.5 SD; n = 29; RFI = 1.09 k/d) groups (Basarab et al., 2003). Means among RFI groups were analyzed using ANOVA (Statistix8, 2003). Phenotypic correlations among RFI, feed conversion ratio (FCR; kg feed:kg of gain), average daily gain (ADG), and end weight (EW) were determined using Pearson Correlation (Statistix8, 2003). There were no differences ($P > 0.05$) among RFI group means for birth weight, weaning weight, yearling weight, and milk EPDs. Furthermore, there were no apparent differences ($P > 0.05$) among RFI group means for ultrasound carcass EPDs or value indices. Inefficient animals exhibited greater FCR compared to marginal animals (7.43 kg and 6.98 kg, respectively; $P < 0.05$); efficient animals exhibited the lowest FCR of the three (6.16 kg; $P < 0.05$). Correlations of RFI with ADG and EW were not significant ($P > 0.05$); the correlation of RFI with FCR was significant ($r = 0.60$; $P < 0.05$), supporting results of the RFI groups analysis. Results suggest phenotypic selection on improved RFI may improve feed efficiency without adversely affecting growth performance.

Key Words: Beef Cattle, Residual Feed Intake, Feed Efficiency

## Introduction

Feed inputs make up a significant proportion of commercial beef production costs. It is estimated that as much as 60-65% of the total production expenses represent feed inputs. With rising feed costs and shrinking profit margins, increasing feed efficiency through reducing feed inputs is imperative.

There are many proposed feed efficiency traits (Archer et al., 1999). Feed conversion ratio (kg input/kg output or the inverse) is commonly used in evaluating production efficiency; however, FCR appears to be negatively correlated with mature size (Koots et al, 1994; Archer et al., 1999). Thus, selection for improved FCR may lead to increases in cowherd mature size. A more appropriate feed efficiency measure would be one that selection pressure can be applied, possesses favorable genetic relationships with reproduction, growth, and carcass traits, and reflects efficiency throughout the production segments of the beef industry.

An alternate measure of feed efficiency is residual feed intake (RFI; Koch et al., 1963) and is the difference between an animal's actual intake and its predicted intake, accounting for average daily gain (ADG) and body weight maintenance (BW). Others have included body composition in the linear regression equation in addition to ADG and BW (Basarab et al., 2003; Ahola et al., 2007). As data collection becomes economically feasible due to advances in feeding technology, RFI is receiving greater attention as the preferred feed efficiency measure due to its favorable or negligible phenotypic and genetic relationships with feed intake, ADG, FCR, and body weight (Jensen et al., 1992; Arthur et al, 2001a,b; Hoque et al., 2006; Tedeschi et al., 2006; Ahola et al., 2007). Furthermore, RFI appears to be favorably related to methane production, adding a favorable environmental component to beef cattle selection (Nkrumah et al., 2006).

Understanding the relationships among RFI and commonly measured production traits in addition to the characterization of efficient cattle are key to a successful livestock selection program. Thus, the objectives of this study were to: 1) determine the phenotypic relationship between residual feed intake and growth performance and 2) characterize low, moderate and high residual feed intake (RFI) beef cattle for phenotypic growth performance, growth and ultrasound carcass EPDs, and value indices.

## Materials and Methods

Ninety-one spring-born Angus bulls were consigned to a 112-d central bull test in Yerington, NV. Bulls were fed two rations while on test, a grower and a finisher (Table 1), following a 28-d adjustment period at the start and 7-d adjustment period during the transition. Average start and finish weights (average of two consecutive days) were collected along with 28-d interval weights. Bulls were given ad libitum access to feed and water throughout the trial period. Ultrasound data were collected by a certified ultrasound technician using an Aloka 500 real-time

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ultrasound unit equipped with a 3.5-MHz transducer. EPDs and indices values were obtained from the American Angus Association (St. Joseph, MO). The RFI evaluation was initiated during the 112-d test period (initial weight = 436.73 kg). Individual dry matter feed intake (DMI) data and BW gain were collected over a period of 62 d using the GrowSafe automated feeding system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada).

RFI was calculated for each bull as the difference between actual DMI and predicted DMI. Predicted DMI was determined as the linear regression (Statistix8, 2003) of DMI on mid-test metabolic body weight ($BW^{.75}$) and ADG (Koch et al., 1963). RFI values were used to classify bulls into efficient (< -0.5 SD; n = 22; RFI = -1.35 kg/d), marginal (± 0.5 SD; n = 40; RFI = -0.05 kg/d) and inefficient (> 0.5 SD; n = 29; RFI = 1.09 k/d) groups (Basarab et al., 2003).

Means among RFI groups were analyzed using ANOVA (Statistix8, 2003); all pairwise comparisons were made using Tukey HSD. Phenotypic correlations among RFI, feed conversion ratio (FCR; kg feed:kg of gain), average daily gain (ADG), gain:feed ratio (GFR), feed intake (FI), initial weight (IW) and end weight (EW) were determined using Pearson Correlation (Statistix8, 2003).

## Results and Discussion

Classifying bulls by RFI resulted in expected differences in FCR and daily feed intake. Inefficient animals exhibited greater FCR compared to marginal animals (7.43 kg and 6.98 kg, respectively; $P < 0.05$); efficient animals exhibited the lowest FCR of the three (6.16 kg; $P < 0.05$). Efficient bulls appeared to eat less but gain the same on average compared to their marginal and inefficient counterparts (Table 2). Similar results were reported by Basarab et al. (2003), Ahola et al. (2007), and Nkrumah et al. (2007).

Mean EPD and indices profiles across RFI groups are included in Table 3. There were no differences ($P > 0.05$) among RFI group means for birth weight, weaning weight, yearling weight, and milk EPDs. Furthermore, there were no apparent differences ($P > 0.05$) among RFI group means for ultrasound carcass EPDs or value indices. One notable trend is that of ultrasound rib fat EPD among RFI groups ($P = 0.06$); inefficient bulls tended to have higher ultrasound rib EPD. While not specific to rib fat EPD, Carstens et al. (2002) and Nkrumah et al. (2007) reported high RFI steers had greater amounts of ultrasound back fat compared to low RFI steers. Little to no information is available regarding the characterization of efficient animals according to individual EPD profiles. Results appear to support the availability of efficient bulls fitting various selection objectives. With no current national genetic evaluation available for RFI, selection on phenotypic RFI data should be weighed within contemporary group.

Phenotypic correlations of RFI with FCR and FI (Table 4) supported findings of the RFI groups analysis and were in agreement with previously studies. The correlations of RFI with FCR and FI were significant ($r = 0.60$ and $r = 0.72$, respectively; $P < 0.05$). Tedeschi et al. (2006) reported phenotypic correlations of RFI with FCR and FI of 0.72 and 0.74, respectively, in group-fed cattle while Herd and Bishop (2000) reported similar correlations of 0.61 and 0.70 in Hereford cattle. Furthermore, Hoque et al. (2006) reported phenotypic correlations for RFI with FCR and with FI in Wagyu bulls to be 0.76 and 0.72, respectively. Reported phenotypic correlations included 0.53 and 0.57 for RFI and FCR and 0.72 and 0.60 for RFI and FI in Angus and Charolais cattle, respectively (Arthur et al., 2001a,b). Current findings and previous reports suggest a significant phenotypic relationship between RFI and both FCR and FI; thus, phenotypic selection for improved RFI would appear to result in improved feed conversion and lowered feed intakes, reducing feed inputs.

Correlations of RFI with ADG and test weights (IW and EW) were not significant (Table 4; $P > 0.05$). Similar results were reported by Jensen et al. (1992), Herd and Bishop (2000), Arthur et al. (2001a,b), Hoque et al. (2006), and Tedeschi et al. (2006). Phenotypic correlations of RFI with ADG and body weight were negligible. Specifically, the correlation between RFI and ADG ranged from -0.06 to 0.01 and were not significantly different than zero. Results suggest the phenotypic selection for improved RFI appears to result in more efficient animals that consume less but gain the same as their less efficient counterparts without significantly increasing body weight.

## Implications

Results suggest phenotypic selection on improved RFI may improve feed efficiency without adversely affecting growth performance and ultrasound genetic prediction of marbling and REA. The use of RFI as a selection tool for bull buyers can have an impact on profitability for commercial cattlemen. Okine et al. (2004) reported that selection for 5% increase in RFI has a larger economic impact than 5% increase in ADG. Given continued economic and production pressures, future research efforts should include economic analysis of the impact of selecting bulls for improved RFI on progeny performance and carcass efficiency of production. Improving efficiency of production is critical to producer profitability given current economic signals.

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Table 1. Test diet composition (DM basis).

| Chemical Composition | Grower | Finisher |
|---|---|---|
| DM, % | 88.00 | 88.00 |
| CP, % | 12.00 | 10.76 |
| Crude Fiber, % | 13.96 | 5.53 |
| Crude Fat, % | 3.73 | 4.03 |
| NEm, Mcal/kg | 1.45 | 1.81 |
| NEg, Mcal/kg | 0.86 | 1.15 |
| Calcium, % | 0.75 | 0.45 |
| Magnesium, % | 0.25 | 0.19 |
| Phosphorus, % | 0.37 | 0.67 |
| Potassium, % | 1.11 | 0.68 |

Table 2. Mean (SE) phenotypic performance for bulls classified as efficient (< -0.5 SD; n = 22; RFI = -1.35 kg/d), marginal (± 0.5 SD; n = 40; RFI = -0.05 kg/d) and inefficient (> 0.5 SD; n = 29; RFI = 1.09 k/d) based on individual residual intake values.

| | RFI Group | | | |
|---|---|---|---|---|
| Trait | Efficient | Marginal | Inefficient | *P*-value |
| Initial Wt, kg | 429.29 (8.66) | 440.97 (6.43) | 436.52 (7.55) | 0.56 |
| End Wt, kg | 557.28 (9.36) | 566.93 (6.94) | 563.52 (8.15) | 0.71 |
| Average Daily Gain, kg/d | 2.06 (0.06) | 2.03 (.04) | 2.05 (.05) | 0.91 |
| Feed Conversion Ratio, kg feed/kg gain | 6.16 (0.14)[a] | 6.98 (0.11)[b] | 7.43 (0.12)[c] | 0.00 |
| Gain:Feed Ratio, kg gain/kg feed | 0.16 (0.003)[a] | 0.15 (0.002)[b] | 0.14 (0.003)[c] | 0.00 |
| Feed Intake, kg/d | 12.61 (0.23)[a] | 13.98 (0.17)[b] | 15.11 (0.20)[c] | 0.00 |
| Residual Feed Intake, kg | -1.35 (0.10)[a] | -0.05 (0.07)[b] | 1.09 (0.09)[c] | 0.00 |

[a,b,c]RFI groups within row without common superscripts differ (P < 0.05).

Table 3. Mean (SE) American Angus Association EPDs and indices values for bulls classified as efficient (< -0.5 SD; n = 22; RFI = -1.35 kg/d), marginal (± 0.5 SD; n = 40; RFI = -0.05 kg/d) and inefficient (> 0.5 SD; n = 29; RFI = 1.09 k/d) based on individual residual feed intake values.

| | RFI Group | | | |
|---|---|---|---|---|
| EPD | Efficient | Marginal | Inefficient | *P*-value |
| Birth Weight | 2.57 (0.33) | 2.71 (0.24) | 2.54 (0.28) | 0.88 |
| Weaning Weight | 43.11 (1.47) | 43.21 (1.04) | 40.25 (1.21) | 0.15 |
| Yearling Weight | 79.88 (2.81) | 79.91 (1.93) | 77.04 (2.20) | 0.58 |
| Milk | 21.90 (1.05) | 20.47 (0.74) | 21.61 (0.87) | 0.45 |
| Ultrasound PIMF | 0.16 (0.04) | 0.13 (0.03) | 0.15 (0.03) | 0.23 |
| Ultrasound REA | 0.26 (0.04) | 0.22 (0.03) | 0.21 (0.03) | 0.83 |
| Ultrasound Rib Fat | 0.005 (0.003) | 0.004 (0.002) | 0.011 (0.002) | 0.06 |
| EN Index[a] | 3.42 (1.38) | 4.33 (0.94) | 4.36 (1.08) | 0.84 |
| BEEF Index[b] | 35.05 (2.13) | 34.08 (1.49) | 33.24 (1.69) | 0.80 |
| FEEDLOT Index[c] | 24.05 (2.21) | 23.36 (1.54) | 21.64 (1.76) | 0.65 |
| GRID Index[d] | 15.18 (1.48) | 14.53 (1.03) | 14.81 (1.17) | 0.93 |

[a]Cow Energy Value ($ savings/cow).

[b]Expected average difference - progeny performance for postweaning and carcass value ($/head).

[c]Expected average difference - progeny performance for postweaning merit ($/head)

[d]Expected average difference - progeny performance for carcass grid merit ($/head).

Table 4. Pearson correlations of residual feed intake (RFI) with performance traits measured on bulls on test.

| Trait | RFI, kg/d | *P*-value |
|---|---|---|
| Initial Wt, kg | 0.00 | 1.00 |
| End Wt, kg | 0.00 | 1.00 |
| Average Daily Gain, kg/d | 0.00 | 0.99 |
| Feed Conversion Ratio, kg feed/kg gain | 0.60 | 0.00 |
| Gain:Feed Ratio, kg gain/kg feed | -0.63 | 0.00 |
| Feed Intake, kg/d | 0.72 | 0.00 |

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# ALTERED GROWTH CURVE CHARACTERISTICS AND PREGNANCY RATE LEVELS IN 34 YEARS OF BRANGUS CATTLE PRODUCTION IN THE CHIHUAHUAN DESERT

**P. Luna-Nevarez[1], D. VanLeeuwen[1], D. W. Bailey[1], R. M. Enns[2], S. Soto-Navarro[1], G. A. Silver[1], M. G. Thomas[1].**
[1]New Mexico State University, Las Cruces, New Mexico 88003
[2]Colorado State University, Fort Collins, Colorado 80523

**ABSTRACT:** Phenotypic trends in growth and reproductive traits from 1972 to 2006 were evaluated in 525 Brangus cows grazing Chihuahuan desert rangeland. Sire selection involved AI and bulls selected from a within-herd post-weaning gain test. Mixed effect models appropriate for predicting continuous and bivariate traits were used to analyze phenotypic trends (i.e., linear, quadratic or cubic trend advancing with years) in autumn cow weight and pregnancy rate. Model included year as a fixed effect, cow nested within sire as a random effect, and body condition score and age as covariates. Autumn cow weight and pregnancy rate across the 34 years averaged 499.6 ± 0.1 kg and 86.8 ± 1.0 %, respectively. Autumn cow weight gradually increased until 1997 (509.5 kg) and then gradually decreased 0.6 kg/yr. Pregnancy rate decreased gradually until 1995 (78.4%) and then slightly increased 0.002%/yr. A quadratic effect ($P < 0.01$) appeared to be the most appropriate descriptor of these relationships across years. Growth curve parameters of cows born between 1972 and 1996, which had data through 6 years of age, were estimated using a nonlinear logistic function and the formula: ($W_t = A / (1+b_0e^{-kt})$) which included weight ($W_t$), asymptotic weight (A), age (t), maturing index (k), and scaling parameter ($b_0$). Mean values for asymptotic weight and age were 530.9 ± 5.2 kg and 3.74 ± 0.1 yr of age, respectively. A mixed model was used to evaluate trends in these data with year fitted as a fixed effect and sire as random effect. A quadratic effect best described the trends across years for these growth curve parameters. Asymptotic weight gradually increased until 1990 ($P < 0.01$), then it slightly decreased 2.2% through 1996. Asymptotic age ($P < 0.10$) increased gradually until 1983, which was the maximum point calculated by the first derivative. Slope from the second derivative suggested asymptotic age decreased 27% through 1996. Analyses of 34 years of body weight and pregnancy rate data in a herd of desert Brangus cattle suggest opposing trends of cow-size and fertility with a transition towards earlier maturity in latter years.

**Key Words**: asymptotic weight, bovine, Brangus, fertility.

## Introduction

Since the late 1970's, cattle inventory in the United States declined while annual quantity of beef produced increased, which suggest cow-size has increased coincident with larger beef carcasses (Hughes, 2001). This trend may not be advantageous for beef cows grazing semi-arid rangeland as optimal biological efficiency was observed in cows that were moderate in both weight and stature (Kattnig et al., 1993). Winder et al. (2000) observed similar relationships in a study that evaluated heat tolerant composite breeds in the Chihuahuan desert.

Because of the relationship of body size to efficiency, variables associated with mature weight and (or) age were developed. Asymptotic weight is an example of such variables. This weight is defined as predicted mature weight relative to the growth curve based on at least 6 years of data (Kaps et al., 1999; Arango and Van Vleck, 2002). These types of variables are important considerations in study of cow-size as growth curve parameters may greatly differ among early and late maturing beef cows (DeNise and Brinks, 1985).

A Chihuahuan Desert Brangus breeding program was initiated in the late 1960's (Winder et al., 1992). The program involved post-weaning gain testing of its replacement bulls and heifers. In order to learn about the effects of this selection and management on the suitability of cows for this environment, the initial objective of this study was to determine phenotypic trends in growth and reproductive traits in Brangus cows grazing Chihuahuan desert rangeland from 1972 to 2006. Evaluation of changes in growth curve parameters across these years was a second objective.

## Materials and Methods

### *Description of Cattle*

Brangus cows grazed Chihuahuan Desert rangeland as a spring-calving and autumn-weaning herd. Data used in this study was collected from 525 cows from 1972 to 2006. Natural service breeding was from May 1 to August 1 each year. In some years, matings were preceded by AI and estrous synchronization. Pregnancy was determined after each breeding season by rectal palpation. Body weight and condition score (range: 1 = emaciated and 9 = obese) were also collected at this time. Weaned bull and heifer calves were moved to the New Mexico State University Campus Farm each autumn for evaluation in post-weaning gain test. These tests were completed when the animals were ~365 days of age. Data were used to select herd replacements in consideration to maintain familial diversity (i.e., limited inbreeding). Prior to 1997, cows were culled from the herd the second time they failed to become pregnant after a breeding season. After 1997, any female determined not pregnant at the time of autumn pregnancy palpation was culled. Nutritional management included protein supplementation (~30% CP) during peak lactation (March 1–May 1), followed by low protein-high energy

supplement after May 1 until the beginning of the summer monsoon rain, which initiated forage growth. Annual growing season precipitation from 1972 to 2006 was 105 ± 5 mm/year.

### *Statistics*

***Phenotypic Trend Analyses.*** The MIXED and GLIMMIX procedures of SAS (SAS Inst. Inc., Cary, NC) were used to analyze phenotypic trends (i.e., linear, quadratic or cubic trend advancing with years) in autumn cow weight and pregnancy rate, respectively. The model included body condition score and age as covariates and cow nested within sire as a random term. Extreme (i.e., maximum or minimum) values were estimated through first derivative of a quadratic function according to formula:

$$f'(x) = 2ax + b, \quad \text{where } x = -b/2a \text{ for } f'(x) = 0$$

where $x$ was the predictor variable, $a$ was the parameter value of the quadratic term, and $b$ was the parameter value of the linear term.

***Growth Curve Parameter Estimation and Trend.*** The NLIN procedure of SAS (SAS Inst. Inc., Cary, NC) was used to estimate growth curve parameters through a non-linear logistic function based on the formula:

$$(W_t = A / (1+b_0 e^{-kt}))$$

where $W_t$ was the body weight at given time (t) in years, A was the parameter that predicted asymptotic weight, $b_0$ was the scaling parameter relating weight at t = 0 (i.e., at birth) to mature size, e was the base of the natural logarithm, and k was the curve parameter representing the ratio of maximum growth rate to mature size (Saxton, 2004). This model was fitted to the age-weight profile of each cow and included data from cows born between 1972 and 1996. These cows had data through at least 6 years of age.

Age at 99% of mature weight ($t_{0.99A}$), which will be defined in this document as asymptotic age, was calculated from the following equation, which used the terms of the previous formula:

$$\text{Age99\%}(t_{0.99A}) = 1/k[\ln b - \ln[(0.99)^{-1} - 1]]$$

Growth curve parameters were computed for each cow. A mixed model was then used to evaluate trends (i.e., linear, quadratic or cubic trend advancing with years) in asymptotic weight and age derived from these curves. The model included year fitted as a fixed effect and sire as a random effect. First derivative of quadratic function was used to estimate maximum values for the variables asymptotic weight and age.

## Results

Autumn cow weight across the 34 years averaged 499.6 ± 0.1 kg, while the pregnancy rate averaged 86.8 ± 1.0 %. Autumn cow weight gradually increased until 1997 to reach a maximum weight of 509.5 kg, and then gradually decreased 0.6 kg/yr. Pregnancy rate decreased gradually until 1995 to reach a minimum of 78.4%, and then slightly increased 0.002%/yr. A quadratic effect ($P < 0.01$) appeared to be the most appropriate descriptor of both autumn cow weight and pregnancy rate across years (Figure 1).

Mean values for asymptotic weight and age were 530.9 ± 5.2 kg and 3.74 ± 0.1 yr of age, respectively. Phenotypic trends for asymptotic weight and asymptotic age are shown in Figure 2. Asymptotic weight gradually increased until 1990 ($P < 0.01$) to reach a maximum of 572.4 kg, then it slightly decreased 2.2% through 1996. Asymptotic age ($P < 0.10$) increased gradually until 1983 to reach a maximum of 4.0 yr, which was calculated using the first derivative. Asymptotic age then decreased 27% from 1983 to 1996. A quadratic effect appeared to best describe the trend across years for asymptotic weight ($P < 0.01$) and asymptotic age ($P < 0.10$).

## Discussion

Opposing trends in autumn cow weight and pregnancy rate suggest that as Brangus cows increased in size, they became less fertile in this Chihuahuan Desert production system. Even though these trends were gradual, results concur with the report of Kattnig et al. (1993) studying cattle grazing these rangelands. These results also support the concepts presented by Lopez de la Torre et al. (1992), which suggest increased maintenance requirements and reduced productivity in large-sized cows. It should be noted that the inflection points on these curves appear to have been influenced by the management decision in 1997 to cull all females that failed to become pregnant after each breeding season.

The observation of steeper declining slope of asymptotic age relative to decreasing slope of asymptotic weight suggest a reduced time to reach maturity. These results were probably influenced by selection of replacement bulls and heifers using post-weaning gain test data. Previous studies suggested that the shape of a growth curve was largely determined by the negative relationship among cow-size and early maturity with higher efficiency observed in fast-maturing animals (Lopez de la Torre et al., 1992). Correlation among asymptotic weight and mature index (i.e., inverse of asymptotic age) are strongly negative (DeNise and Brinks, 1985; Kratochvilova et al., 2002). Even with documentation of these relationships, the relationships of asymptotic weight and age warrant further study as MacNeil et al. (2000) only reported marginal effects of selection for higher yearling weight on maturity rate.

In summary, studies of long-term trends of cow size and maturity rate in beef production systems in harsh environments, such as deserts, suggest opposing relationship among cow-size and fertility trait levels. The growth curves of Brangus cows managed in this Chihuahuan Desert production system appear to be moving towards earlier maturity; however, additional studies are needed to decipher the benefits and detriments of these changes.

**Acknowledgements**

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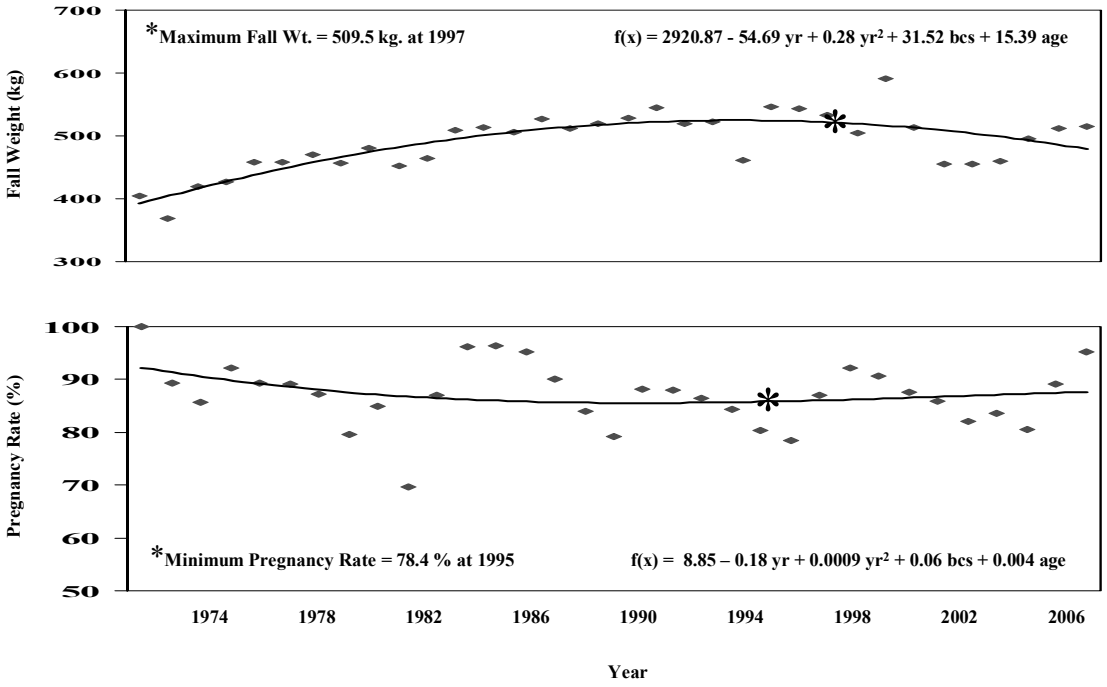


**Figure 1. Phenotypic trends for Brangus autumn cow weight and pregnancy rate. Pooled SE of autumn weight was ± 8.8 kg.**

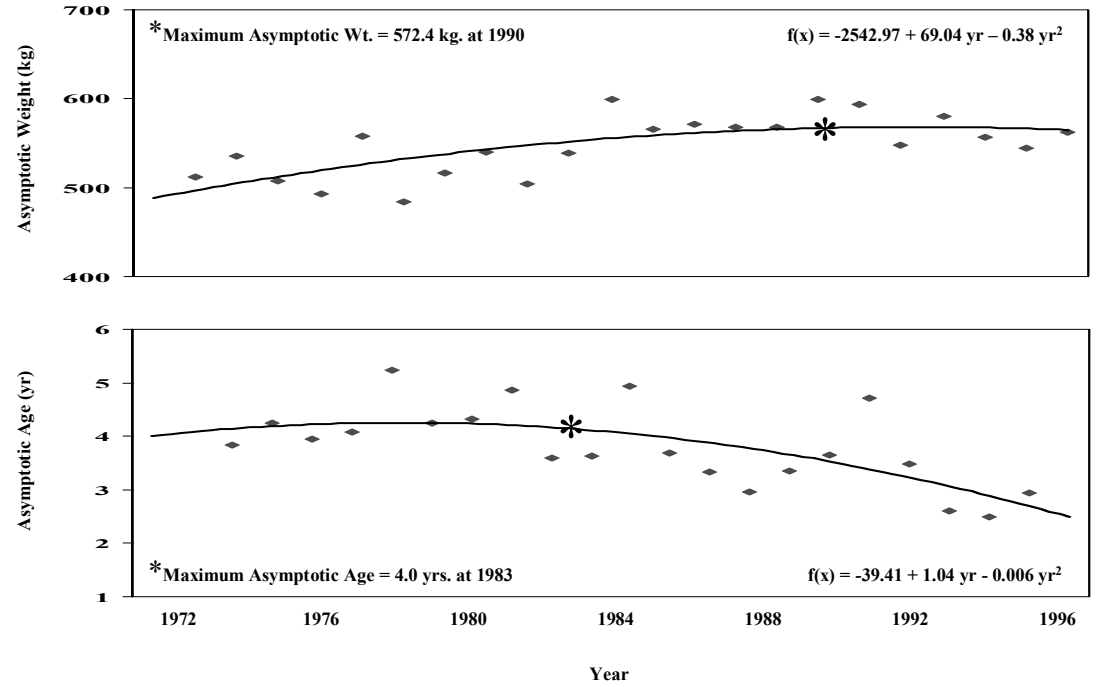


**Figure 2. Phenotypic trends for Brangus asymptotic weight and asymptotic age. Pooled SE were ± 19.0 kg and 0.4 years, respectively.**

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# EFFECT OF PREVIOUS EXPERIENCE ON GRAZING DISTRIBUTION AND DIET QUALITY OF BRANGUS COWS IN THE CHIHUAHUAN DESERT

**D.W. Bailey*[1], M.G. Thomas[1], J.W. Walker[2], B.K. Witmore[1], D. Tolleson[3], and C.C. Bailey[1]**

New Mexico State University, Las Cruces, New Mexico, USA[1], Texas A&M University, San Angelo, Texas, USA[2], GAN Lab, Texas A&M University, College Station, Texas, USA[3]

**ABSTRACT:** The ability to adapt to different grazing environments is critical when cattle are moved. A study was conducted to evaluate the impact of previous experience on grazing distribution and diet quality of Brangus cattle in desert conditions. Cows originating from a subtropical environment in Leona, Texas were brought to the Chihuahuan Desert (**naïve**) and evaluated against native cows that were moved to Leona, Texas during the preceding 3 years (**moved**) and native cows that remained in the desert (**native**). Cows from the 3 groups (3 to 10 years of age) were tracked with global positioning system (GPS) collars in 3 extensive pastures (> 1000 ha) for three 8- to 10-d periods in a Latin square design during winter, early summer and late summer. These cows never grazed in the 3 experimental pastures before the study, but native and moved cows had grazed in adjacent pastures. When cows were rotated among pastures, fecal material was collected and analyzed with near infrared spectroscopy (NIRS) to estimate diet quality. Across winter, spring and summer evaluations, native cows (1629 ± 191 m) tended to be farther ($P = 0.09$) from water than naïve cows (788 ± 191 m). Body weight and condition scores were similar among groups during evaluation periods. Nonetheless, during winter, native (6.6 ± 0.3%) and moved (7.7 ± 0.3%) cows selected a diet with greater ($P < 0.05$) CP than naïve cows (4.5 ± 0.3%). In spring, moved cows (8.5 ± 0.3%) tended to have the highest ($P < 0.10$) diet CP and native (7.1 ± 0.3%) and naïve (6.0 ± 0.3%) cows had the lowest CP. In contrast, naïve cows had higher ($P < 0.05$) diet CP than native cows in summer, whereas moved cows were intermediate. Diet CP levels during this time period for the groups were 15.5, 13.4, and 12.4 ± 0.4 %, respectively. Native Brangus cows that had been moved to a subtropical environment for 3 years behaved similar to cows that remained in the desert; however, naïve Brangus cows from a subtropical environment spent more time near water and appeared to have less ability to select higher quality diets during drought conditions in the Chihuahuan Desert.

**Key Words**: Behavior, Brangus, Diet quality, Distribution.

## INTRODUCTION

Livestock are often moved from one location to another because of drought or other management considerations. In some cases, animals are moved to areas with forages and environmental conditions that differ greatly from conditions they are accustomed. To perform optimally on rangelands, livestock must be familiar with the plants, topographical features and water locations in their environments (Launchbaugh and Howery, 2005). Moving livestock to unfamiliar areas and conditions often results in animals spending more time grazing but with lower total intake rates (Provenza, 2003). The first objective of this study was to compare grazing distribution patterns and quality of selected diets of Brangus cows that were familiar to the Chihuahuan Desert to Brangus cows that were naïve to desert conditions and were accustomed to a subtropical environment. The second objective was to determine if Brangus cows that were moved to a subtropical environment for 3 years would have similar movement patterns and diet quality compared to Brangus cows that remained in the Chihuahuan Desert their entire lives.

## MATERIALS AND METHODS

*Study Site and Animals*. Procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. The study was conducted at the Chihuahuan Desert Rangeland Research Center (CDRRC) located 35 km north of Las Cruces, NM. Dominant perennial grasses include dropseeds (*Sporobulus* spp), threeawns (Aristida spp.) and black grama (*Bouteloua eriopoda* (Torr.) Torr.). Honey mesquite (*Prosopis glandulosa* Torr.), four-wing saltbush (*Atriplex canescens* (Pursh) Nutt.) and creosote bush (*Larrea tridentata* (DC.) Coville) are commonly found shrubs. The 74-year average annual precipitation is 234 mm. From November 2005 to June 2006, conditions were unusually dry with only 22 mm of precipitation. However during July to September 2006, rainfall was uncommonly high with 355 mm of precipitation. The study was conducted in three pastures (1002, 1959 and 3994 ha), which had relatively gentle terrain and only one water source.

The study compared three groups of mature Brangus cows (3 to 10 years of age) with similar pedigrees. One group (**naïve**) had not been to the Chihuahuan Desert before the study and had lived in a subtropical environment (1040 mm average precipitation) near Leona, TX. A second group (**native**) had been raised at the CDRRC and remained there before and during the study. The third group (**moved**) was moved from the CDRRC to Leona, TX in 2002 because of limited forage availability resulting from drought. These cows remained in Leona, TX for 3 years and were returned to the CDRRC immediately before the study. All cows calved in late February or March 2006 and nursed calves from calving until the calves were weaned at the end of the study in early September 2006. Prior to the study, all

cows had not grazed in the three study pastures at the CDRRC. The moved and native groups grazed pastures adjacent to study pastures. Each group consisted of 7 cows.

*Design and Protocol*. The three groups of cows were compared and evaluated during three different sessions (28 to 30 d).The first session (winter) began 9 January 2006, before calving. The second session (early summer) began 10 May 2006, during early lactation. The third session (late summer) began 8 August 2006, during late lactation. Each group of cows grazed in each of the three study pastures for 8- to 10-d periods during a session (3 periods per session) in a Latin-square design. During the interim between the January-February session and the May-June session, groups were kept in separate pens and fed hay. During the interim between the May-June session and the August-September session, groups remained separated from and grazed separate pastures at the CDRRC that were adjacent to the study pastures.

*GPS Tracking*. One randomly selected cow from each group was tracked with global positioning system (GPS) collars. During the January-February session cows were tracked with WTIGPS 500b collars (Wildlife Track Inc., Caldwell, ID) that recorded the position every hour. During the May-June and August-September sessions, cows were tracked with Lotek GPS 3300 collars (Lotek Wireless, Newmarket, Ontario) at 10-min intervals. Observers located and recorded positions of all cows 2 or 3 times each week to determine if positions of collared cows were similar to other cows in the group.

Distance from water was calculated for each recorded position and averaged together for each period. For each period, the maximum distance from water and area enclosed by the minimum convex polygon encompassing all recorded positions of a collared cow was calculated.

*Diet Quality Sampling*. Fecal samples were collected from each cow at the end of each period. Fecal samples were frozen, dried, and ground in a Wiley mill to pass a 1 mm screen. Fecal samples were ground a second time in a cyclone mill to pass through a 1-mm screen, dried in a forced-air oven (50°C for 12 h), and conditioned for 24 h in an environment with constant temperature and humidity (21°C, 65%). Approximately 4 g of ground, conditioned samples were packed in quarter-cup sample cells with a near-infrared, transparent, quartz cover glass. Cells were scanned 32 times using a scanning reflectance monochromator (model 6500, NIR Systems Inc., Silver Springs, MD). Reflected energy (log [1/R], where R = reflectance) was measured and averaged over the 32 scans and recorded at 2-nm intervals from 1,100 to 2,500 nm. Diet DOM and CP of cows during a period were determined using the equations originally developed by Lyons and Stuth (1992) and later expanded by Stuth (unpublished).

*Statistical Analyses*. Average distance from water, maximum distance from water and minimum area used for each session were analyzed separately using a model that included experience, pasture and period ($n$ = 9 and error $df$ = 2). Single degree of freedom orthogonal contrasts were used to compare naïve cows to cows from the CDRRC (native and moved pooled) and to compare native cows to cows that did not remain of the CDRRC (moved and naïve pooled). A repeated measures model was used to analyze data from all three sessions. The model included experience, session, pasture, period, and session by experience interaction as fixed effects. The repeated term used pasture by experience as the subject and covariance between repeated records was modeled using compound symmetry (Littell et al., 1996).

Each session was analyzed separately for diet quality (DOM and CP). Values for each cow were analyzed using a mixed model (Littell et al., 1996) where pasture and period were fixed effects and the interaction between treatment, pasture and period was considered random and used as the error term (error $df$ = 2).

## RESULTS

*Forage Characteristics*. Perennial grasses at the beginning of the study were of low quality (Table 1). During the last session (late summer), forage quality was greater than during the first two sessions. Although the standing crop of perennial grasses was not greater during the last session, annual forbs and grasses were available during the last session. There was virtually no annual vegetation available during the winter and early summer sessions.

Table 1. Quantity and quality of perennial grasses in the study pastures at the beginning and end of the study.

| | Pasture | | |
|---|---|---|---|
| Attribute[1] | 3 | 12 | 13 |
| Beginning, winter session | | | |
| CP, % | 6.3 | 5.3 | 5.0 |
| ADF, % | 43.6 | 42.7 | 44.5 |
| NDF, % | 66.1 | 66.4 | 67.9 |
| Perennial grass standing crop, kg/ha | 82 | 110 | 90 |
| End, late summer session | | | |
| CP, % | 9.3 | 15.0 | 12.9 |
| ADF, % | 42.6 | 37.9 | 49.2 |
| NDF, % | 66.2 | 62.9 | 62.5 |
| Perennial grass standing crop, kg/ha | 105 | 50 | 11 |
| Annuals, kg/ha [2] | 186 | 89 | 46 |

[1] Crude protein, ADF and NDF are expressed on a DM basis.

[2] Annual forbs and grasses were not in sufficient abundance to be measured at the beginning of the study.

*Body Weight and Condition.* Experience did not affect cow weight or body condition ($P > 0.20$).

*Visual Observations*. Observers always found cows in one group, usually less than 40 m apart.

*Winter Distribution Patterns*. When averaged over the entire winter session, native cows native 635 ± 105 m farther from water ($P$ = 0.03) than the other two groups (pooled), and naïve cows were 479 ± 105 m closer to water ($P$ = 0.04) than cows that were raised at the CDRRC

(Figure 1). When the naïve cows were first released during the winter session, they did not travel more than 300 m for 8 consecutive days even though the pasture was 3994 ha in size. The maximum distance traveled from water by naïve cows during the winter session was 2070 ± 214 m less (P = 0.01) than the other two groups that were raised at the CDRRC. The naïve cows also grazed 335 ± 83 ha less area than the other groups ($P = 0.056$).

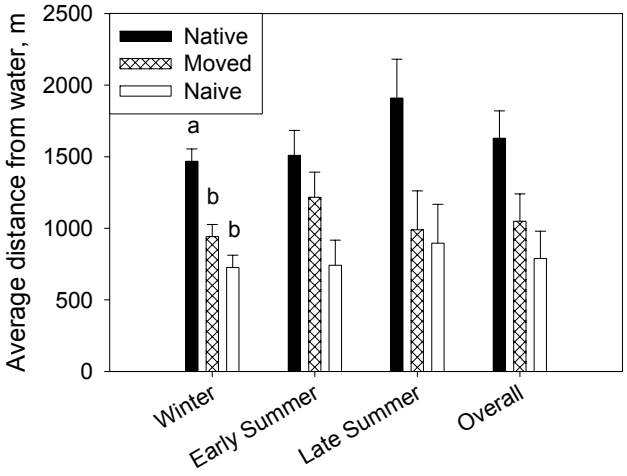


Figure 1. Average distance from water of GPS collared cows during 8- to 10-d periods within a session. Bars with different letters differ ($P < 0.05$).

*Early Summer Distribution Patterns*. The naïve cows tended to remain closer to water ($P = 0.10$) over the early summer session than cows raised at the CDRRC (native and moved groups pooled). The difference in distance from water between the naïve cows and cows raised at the CDRRC was 621 ± 214 m. The maximum distance traveled to water was similar among the native, moved and naïve groups of Brangus cows ($P = 0.92$), and all groups used similar areas within the pastures ($P = 0.44$).

*Late Summer Distribution*. The native cows tended to remain farther from water ($P = 0.10$) during the late summer session than cows that did not spend their entire life at the CDRRC (moved and naïve cows pooled). The average distance from water differed between the native cows and the other two groups of cows by 967 ± 332 m. The maximum distance traveled from water was similar ($P = 0.19$) for all cows, and the area used did not differ ($P = 0.60$) between the native, moved and naïve groups (Figures 2 and 3).

*Overall Distribution*. When data from all three sessions were combined, the native cows were 710 ± 234 m farther from water ($P = 0.04$) than cows that did not spend their entire life in the Chihuahuan Desert (Figure 1). Naïve cows also tended to remain closer to water than cows that were raised at the CDRRC ($P = 0.08$; difference = 551 ± 234 m). Similarly, the maximum distance traveled from water by naïve cows tended to be less ($P = 0.08$) than for cows raised in the Chihuahuan Desert (Figure 2). The difference between the naïve cows and the other two groups (pooled) was 741 ± 316 m. Cows raised in the Chihuahuan Desert used 166 ± 60 ha more area ($P = 0.05$) than naïve cows over the entire study (Figure 3).

*Winter Diet Quality*. Naïve cows selected a diet with lower CP ($P = 0.02$) than the other cows during the winter session (Figure 4). However, diet DOM was similar ($P = 0.63$) for all three groups (Figure 5).

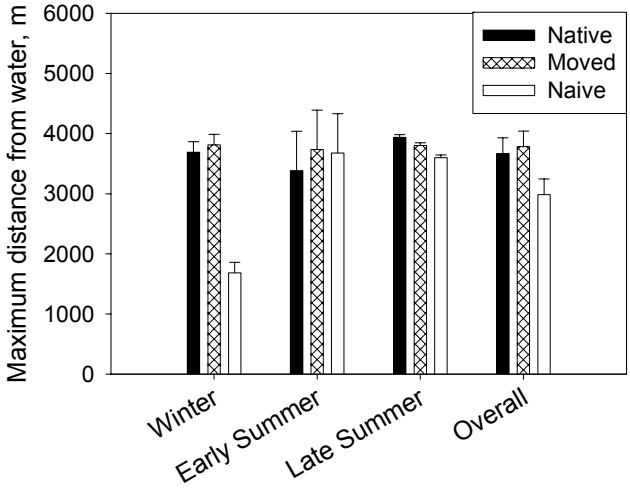


Figure 2. Maximum distance traveled from water by GPS collared cows during 8- to 10-d periods during the sessions and pooled across sessions.

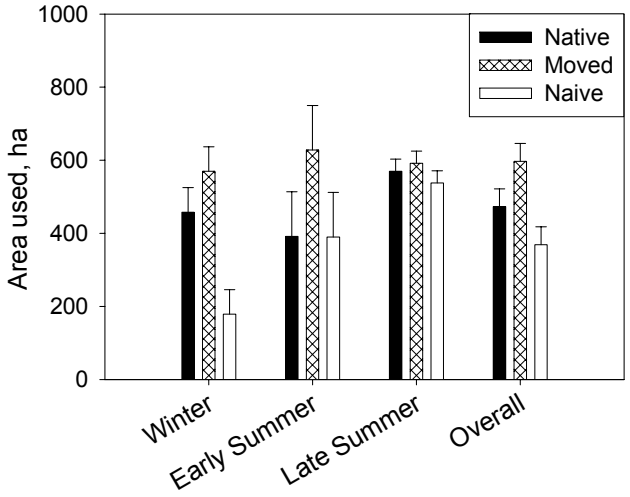


Figure 3. Average area used (minimum convex polygon) by GPS collared cows during 8- to 10-d periods during sessions and pooled across sessions.

*Early Summer Diet Quality*. In the early summer session, naïve cows selected a diet that was 1.78 ± 0.35 percentage points lower in CP ($P = 0.04$) than the cows raised in the Chihuahuan Desert (moved and native pooled). Diet DOM was similar ($P = 0.73$) for all cows.

*Late Summer Diet Quality*. During late summer, native cows tended to select a diet that was lower ($P = 0.07$) in CP than cows that did not spend their entire life in the Chihuahuan Desert. For this contrast, the difference was 1.59 ± 0.44 percentage points. Diet CP tended to be higher ($P = 0.07$) for naïve cow than for cows raised at the CDRRC. The difference between diet CP of naïve cows and the other groups was 1.61 ± 0.45 percentage points. Native cows selected a diet that was 3.39 ± 0.84 percentage points lower in DOM ($P = 0.056$) than the other two groups

(pooled). Diet DOM for naïve cows was 3.44 ± 0.85 percentage points greater ($P = 0.056$) than for cows raised in the Chihuahuan Desert.

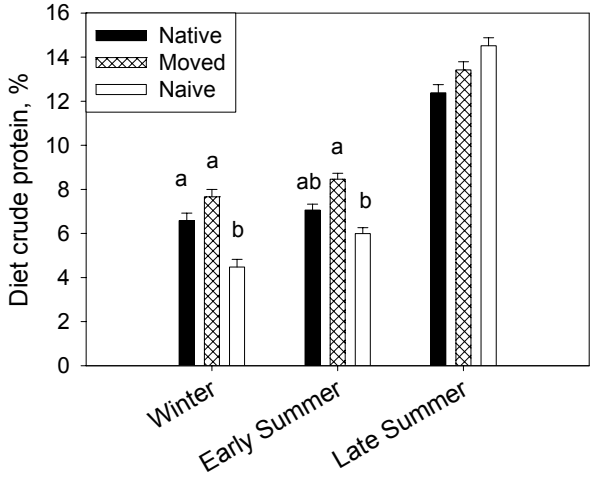


Figure 4. Diet crude protein of cows during the winter, early summer and late summer sessions. Bars within a session with different letters differ ($P < 0.05$)

## DISCUSSION

Naïve cows initially appeared reluctant to travel away from water, but soon learned, at least periodically, to travel long distances (> 3 km) from water. Cows that had been moved away from the Chihuahuan Desert to a tropical environment for 3 years readily traveled from water and used a large area throughout the study. However, native cows that remained in desert conditions spent the greatest time on average at farther distances from water compared to cows that had spent time in subtropical conditions. Similar to the hypothesis presented by Launchbaugh and Howery (2005), experience and learning early in life appears to play a role in the willingness of Brangus cows to consistently travel away from water, however, recent experience (within the last 3 years) also may play a role.

All Brangus cows regularly traveled over 3.2 km from water even during the summer when temperatures were over 35º C. Holechek (1988) suggested that areas between 1.6 and 3.2 km from water be stocked with half of the animals recommend for areas less than 1.6 km from water and that areas over 3.2 km from water should be considered ungrazable. Results from this study suggest that Brangus cows are well adapted to desert conditions and can readily travel through relatively gentle terrain and use areas greater than 1.6 km from water and ever farther than 3.2 km from water during winter and summer seasons.

Cows that were raised in the Chihuahuan Desert selected higher quality diets than naïve cows during drought conditions. Their experience in desert conditions may have helped cows locate forages with more CP. Recent experience of native cows (within the last 3 years) did not appear to provide any additional benefit in selecting a higher quality diet compared to cows that were moved away for 3 years. After unusually heavy monsoon rains in late summer, naïve cows selected the highest quality diets. Perhaps, the naïve cows' experience with the more mesic conditions of the subtropics allowed them to readily choose plants with high forage quality when such plants were available. In contrast, cows from the desert may have chosen plants that were more nutritious in dry conditions but were not experienced in selecting the most nutritious plants when overall forage quality was high.

## IMPLICATIONS

Brangus cattle are well adapted to desert conditions, and even naïve Brangus cows raised in radically different environmental conditions can readily learn to forage in extensive Chihuahuan Desert pastures. However, cows exposed to desert conditions throughout life may continue to have an advantage over naïve animals. Ranchers using desert rangelands should stock at levels that will allow them to retain at least a core herd of cows adapted to their environmental conditions under drought conditions so that they can produce their own replacements. If replacements must be purchased, animals should be selected from herds that were developed with forages and environmental conditions similar to their location.

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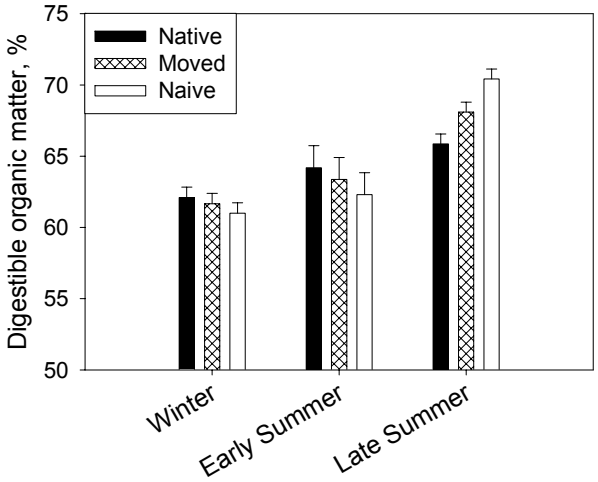


Figure 5. Diet DOM of cows during winter, early summer and late summer sessions.

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# EVALUATION OF COLUMBIA, USMARC-COMPOSITE, SUFFOLK, AND TEXEL RAMS AS TERMINAL SIRES: EFFECTS OF RAM BREED ON EWE PRODUCTIVITY AND $F_1$ LAMB SURVIVAL AND GROWTH[1]

**T. D. Leeds[*], M. R. Mousel[*], D. R. Notter[†], K. A. Leymaster[‡], and G. S. Lewis[*]**
[*]USDA, ARS, U.S. Sheep Experiment Station, Dubois, ID 83423
[†]Virginia Polytechnic Institute and State University, Blacksburg 24061
[‡]USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933

**ABSTRACT:** Objectives were to estimate effects of ram breed on ewe fertility, prolificacy, and $F_1$ lamb survival and growth. Columbia, USMARC-Composite, Suffolk, and Texel rams (n = 15, 15, 15, and 13, respectively) were sampled from US flocks. Data were from 665 exposures to Rambouillet ewes (3 to 7 yr of age) and 1,243 $F_1$ lambs over 2 yr. Ewes lambed in March or April and were herded with litters on sagebrush steppe and subalpine ranges. Lambs were weaned at 130 d of age (SD = 5.8 d). Ewe fertility (lambed or did not lamb), ewe prolificacy (number born; number born alive), lamb birth and weaning weights, and lamb survival from birth to weaning (age at death or censoring) were recorded. Fertility data were analyzed using a categorical analysis with effects of sire breed, year, and dam age. Prolificacy data were described using a mixed linear model that included fixed sire breed, year, and dam age effects and sire within breed and year as a random effect. Litter size and gender were added to the model to describe lamb weight data, and weaning age was included as a covariate for weaning weight. Survival data were analyzed using a Weibull survival model that included effects of sire breed, year, dam age, litter size, gender, and linear and quadratic effects of birth weight. Sire breed did not influence ewe fertility, prolificacy, or lamb survival ($P > 0.10$), but was important for weight traits ($P < 0.05$). No sire breed effect was detected ($P > 0.10$) for lamb survival when birth weight effects were removed from the model. Suffolk-sired lambs (5.26 kg) were 0.24 kg heavier at birth than USMARC-Composite- or Texel-sired lambs ($P < 0.05$), but not different from Columbia-sired lambs (5.13 kg). Suffolk-sired lambs (39.4 kg) were heaviest at weaning ($P < 0.05$). Texel-sired lambs (36.7 kg) were lighter at weaning than Columbia-sired lambs (37.8 kg; $P < 0.05$), but not different from USMARC-Composite-sired lambs (37.4 kg). These data provide breed comparisons in an extensive range environment that can be used to develop breeding objectives for terminal sires.

**Key Words:** Sheep Breeds, Survival, Growth

## Introduction

Breed diversity is an asset for the US sheep industry. Systematic use of breed diversity in terminal crossbreeding systems can improve the efficiency of commercial lamb production (Leymaster, 2002). Breeds must be comprehensively evaluated, in different environments and production systems, to determine their appropriate use. Traits of importance for breeds used as terminal sires include ram longevity, fertility, and prolificacy (i.e., number of lambs born per ewe lambing), as well as survival, rate and efficiency of growth, and composition of terminal $F_1$ progeny.

A comprehensive evaluation of Columbia, Suffolk, USMARC-Composite (Leymaster, 1991), and Texel breeds as terminal sires in an extensive rangeland production system was initiated at the US Sheep Experiment Station near Dubois, ID. This report presents data on these breeds, through weaning of $F_1$ lambs, for the first 2 yr of the study. Our objective was to evaluate breed effects on ewe fertility, ewe prolificacy, and $F_1$ lamb survival and growth. Dam age, production year, litter size, gender, and birth weight effects on lamb survival were also estimated.

## Materials and Methods

*General Experimental Design.* The US Sheep Experiment Station Institutional Animal Care and Use Committee (Dubois, ID) reviewed and approved all husbandry practices used in this study. Columbia, Suffolk, and Texel rams (n = 15, 15, and 13, respectively) were sampled from US flocks (8 to 11 per breed) between 2005 and 2006; USMARC-Composite rams (n = 15) were sampled from the US Meat Animal Research Center population during the same 2-yr period. Eight rams per breed were each mated in single-sire pens to approximately 10 US Sheep Experiment Station Rambouillet ewes (3 to 7 yr of age) in 2005; 9 rams per breed were used in a similar mating scenario in 2006. Two rams each of Columbia, USMARC-Composite, and Suffolk, and 4 Texel rams, were used in both years. Each year, the mating season was approximately 21 d beginning mid-October. After mating, ewes were herded on shrub-dominated winter range until late January, then maintained in a feedlot and fed to meet or exceed NRC requirements (NRC, 1985).

Ewes lambed in March or early April in outdoor lots and were moved with their litter to individual, indoor claiming pens for approximately 48 h. Ram lambs were castrated (elastrator bands) within 24 h after birth. During the initial claiming period, live lambs were removed from litters based on subjective evaluation of the ewe's ability to rear the litter (e.g., milking ability), and subjective evaluation of the lamb's viability (e.g., birth weight, abomasal fill, and vigor). Approximately 14.3 and 2.2% of live lambs were orphaned or cross-fostered, respectively, as

[1] The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the USDA or the ARS of any product or service to the exclusion of others that may be suitable.

Table 1. Summary data and unadjusted sire breed means for ewe and lamb data used in analyses.

| Item | Sire breed | | | |
|---|---|---|---|---|
| | Columbia | USMARC[1] | Suffolk | Texel[2] |
| Sires, n | 15 | 15 | 15 | 10 |
| Ewes | | | | |
| Mated, n | 162 | 165 | 164 | 145 |
| Lambed, n | 155 | 158 | 159 | 136 |
| Fertility, % | 95.7 | 95.8 | 97.0 | 93.8 |
| TNB[3], n | 2.01 | 2.06 | 2.06 | 2.04 |
| NBA[4], n | 1.88 | 1.96 | 1.92 | 1.85 |
| Lambs | | | | |
| Birth wt, kg | 5.30 | 5.13 | 5.37 | 5.13 |
| Stillborn, % | 6.7 | 5.2 | 7.0 | 9.4 |
| Orphaned[5], % | 14.1 | 16.2 | 15.4 | 10.8 |
| Fostered[5], % | 1.4 | 2.3 | 2.6 | 2.8 |
| Deaths[5], % | 5.5 | 5.5 | 3.6 | 7.2 |
| Weaned[5], % | 79.4 | 76.7 | 79.0 | 79.7 |
| Weaned wt, kg | 35.8 | 35.2 | 37.6 | 34.5 |

[1] USMARC = USMARC-Composite.
[2] Thirteen Texel sires were used in the experiment, but 3 were found to be sterile in retrospect. Data from 29 ewes mated to the sterile rams were excluded from the analyses.
[3] TNB = total number of lambs born per ewe lambing.
[4] NBA = number of lambs born alive per ewe lambing.
[5] Percentage based on lambs born alive.

a result of this litter reduction practice. Only 6 litters, all born in 2007, were allowed to be reared as triplets.

After the claiming period, ewes and litters were commingled in outdoor lots, and ewes were fed to meet or exceed NRC requirements (NRC, 1985). Ewes and litters were herded on sagebrush steppe range beginning late April and subalpine range beginning early July. Lambs were weaned in early August at 130 d of age (SD = 5.8 d).

*Description of Traits.* Data recorded as traits of the ewe included age (yr), fertility (binary; lambed or did not lamb), and prolificacy of ewes that lambed (total number of lambs born; number of lambs born alive). Data recorded as traits of the lamb included gender (male or female), birth weight, survival from birth to weaning (age at death or censoring, d), weaning weight, and age at weaning (d). No differentiation was made for cause of death (e.g, disease, predation, and starvation).

*Data Editing and Statistical Analyses.* All rams were libido tested before commencement of each mating season. However, 3 Texel rams failed to sire a litter in 2006, and subsequent semen testing confirmed that these rams were sterile. Our objective was to evaluate sire breeds using only fertile rams, therefore ewe data (n = 29) from these sterile rams were excluded from the analyses. Table 1 contains a summary of the data used in the analyses. All analyses were performed using SAS (SAS Inst., Cary, NC).

Fertility data were described with a logistic regression model using the CATMOD procedure. Effects modeled included sire breed, ewe age, and production year. Probability of lambing success was estimated for sire breed as the antilogit of the linear function, $e^{\alpha + \beta i}/(1 + e^{\alpha + \beta i})$, where $\alpha$ is baseline log odds and $\beta_i$ is differential change in log odds for the *i*th sire breed (Stokes et al., 1995).

Prolificacy data were described with a mixed linear model using the MIXED procedure. The model included fixed sire breed, ewe age, and production year effects and mating sire nested within breed and year as a random effect. With this model, the estimated variance component for sires within breed and year was 0 for both traits. Therefore, the random sire effect was removed from the model, and all effects were tested against the residual variance component. Litter size (total number of lambs born) and gender were added to the prolificacy model to describe individual lamb birth and weaning weights, and age of lamb at weaning was included as a covariate for weaning weight. Only weights of live lambs were used in the analyses.

Survival data were described with a Weibull survival model using the LIFEREG procedure. Birth date was considered d 1 of age in the survival data, and records were right-censored at orphaning and weaning. The model included effects of sire breed, production year, dam age, litter size (total number of lambs born), gender, and linear and quadratic effects of birth weight. The model was also evaluated without birth weight effects. Survival functions were estimated as $S_i(t) = \exp\{-[t_i e^{-\beta x_i}]^{1/\sigma}\}$, where $S_i(t)$ is the probability that the $i^{th}$ lamb survives to age $t$, $\beta x_i$ is the regression coefficient associated with x explanatory variables, and $\sigma$ is a scale parameter (Allison, 1995). When $\sigma > 1$, the hazard is decreasing over time; when $\sigma < 1$, the hazard is increasing over time. Risk ratios, relative to a mean birth weight of 5.2 kg, were calculated for birth weight as $\exp[-\beta_1(\text{birth weight} - 5.2) - \beta_2(\text{birth weight}^2 - 5.2^2)]$. The experimental design required all ewes to be maintained in a single summer grazing band, however some ewes (approximately 2.3%) were inadvertently mixed with other grazing bands. These lamb records, and those of lambs fostered onto ewes in other grazing bands, were right-censored at d 3 of age. Thirty death records (aborted or partially resorbed conceptuses) had missing gender and birth weight data, and were excluded from the analyses.

Table 2. Maximum likelihood estimates (fertility), least squares means (prolificacy), SE, and significance of sire breed effects on ewe fertility and prolificacy

| Sire breed | Fertility, % | Total lambs born | Lambs born alive |
|---|---|---|---|
| Columbia | 94.3 ± 1.60 | 1.92 ± 0.057 | 1.80 ± 0.062 |
| USMARC[1] | 94.4 ± 1.57 | 1.98 ± 0.057 | 1.87 ± 0.061 |
| Suffolk | 97.2 ± 1.34 | 2.00 ± 0.057 | 1.86 ± 0.061 |
| Texel | 94.3 ± 2.00 | 1.91 ± 0.061 | 1.73 ± 0.065 |
| Significance | P = 0.60 | P = 0.63 | P = 0.33 |

[1] USMARC = USMARC-Composite.

## Results

Kaplan-Meier survival functions for sire breeds show the unadjusted survival data (Figure 1, Panel a). Sire breed effects were not detected (P > 0.05) for lamb survival, with or without birth weight in the model (Figure 1, Panel b), or for fertility and prolificacy (Table 2). Sire breed effects were important (P < 0.05) for individual lamb weights at birth and weaning. Suffolk- and Columbia-sired

lambs were heaviest at birth, and Suffolk-sired lambs were heaviest at weaning (Table 3).

The scale parameter estimate for lamb survival was 3.07, indicating a decreasing hazard over time (Figure 1, Panels a-e). Linear and quadratic effects of birth weight were important for survival; lighter lambs had increased risk of death (Figure 1, Panel f). Litter size affected survival without birth weight in the model (Figure 1, Panel c; $P < 0.05$), but was not important with birth weight in the model. Production year had a significant ($P < 0.05$) effect on lamb survival regardless of the modeling of birth weight effects; poorer lamb survival was observed in 2006 (Figure 1, Panel e). Effects of lamb gender (Figure 1, Panel d) and ewe age (data not shown) were not significant ($P > 0.05$) for lamb survival, with or without birth weight in the model.

## Discussion

This study was initiated, in part, because of producer concerns and literature reports of poor survival of Suffolk-sired crossbred lambs (Leymaster and Jenkins, 1993; Smith, 1977). Relative to other breeds tested, our data give no indication of reduced survivability of Suffolk-sired crossbred lambs. Numerically, Suffolk-sired lambs had the smallest proportion of deaths (10.6%), while Texel-sired lambs had the greatest (16.6%). Inferences from these data about sire breed effects on crossbred lamb survival are valid for adult, nonprolific ewe types, but may not be valid for young ewes (≤ 2 yr old) or semiprolific ewe types (Leymaster and Jenkins, 1993). The lack of sire breed effects on ewe fertility and prolificacy in the current study is consistent with other studies that evaluated nonprolific sire breeds (Freking et al., 2000; Nugent and Jenkins, 1991). Superior growth of Suffolk-sired lambs has been clearly documented.

Nongenetic effects on lamb survival are important and have been described in the literature. Borg (2007) reported similar linear and quadratic effects of birth weight. Because nonprolific ewes were used in the current study, we anticipated an increased risk of death for heavy birth weight lambs; however, our data did not suggest an appreciable increase in risk in adult ewes. With birth weight in the model, female lambs tended to have better survival ($P = 0.10$; data not shown), which is consistent with other reports (Freking and Leymaster, 2004; Southey et al., 2001). In contrast with Southey et al. (2001) and Borg (2007), survival rates were nearly identical for lambs born as singles and twins.

## Implications

Adverse terminal sire breed effects on ewe productivity and crossbred lamb survival can negate superior performance for growth and carcass composition in terminal crossbreeding systems. This study found no differences among Columbia, USMARC-Composite, Suffolk, and Texel sire breeds for ewe productivity and crossbred lamb survival. Thus, performance of crossbred lamb growth and carcass composition can be emphasized as criteria for selecting the appropriate terminal sire breed.

Table 3. Least squares means, SE, and significance of sire breed effects on $F_1$ lamb birth and weaning weights

| Sire breed | Birth wt, kg | Weaning wt, kg |
|---|---|---|
| Columbia | $5.13 \pm 0.083^{ab}$ | $37.8 \pm 0.37^{b}$ |
| USMARC[1] | $5.02 \pm 0.083^{a}$ | $37.4 \pm 0.37^{ab}$ |
| Suffolk | $5.26 \pm 0.081^{b}$ | $39.4 \pm 0.36^{c}$ |
| Texel | $5.02 \pm 0.090^{a}$ | $36.7 \pm 0.39^{a}$ |
| Significance | P = 0.037 | P < 0.001 |

[1] USMARC = USMARC-Composite.
[a,b,c] Within a column, means without a common superscript letter differ ($P < 0.05$).

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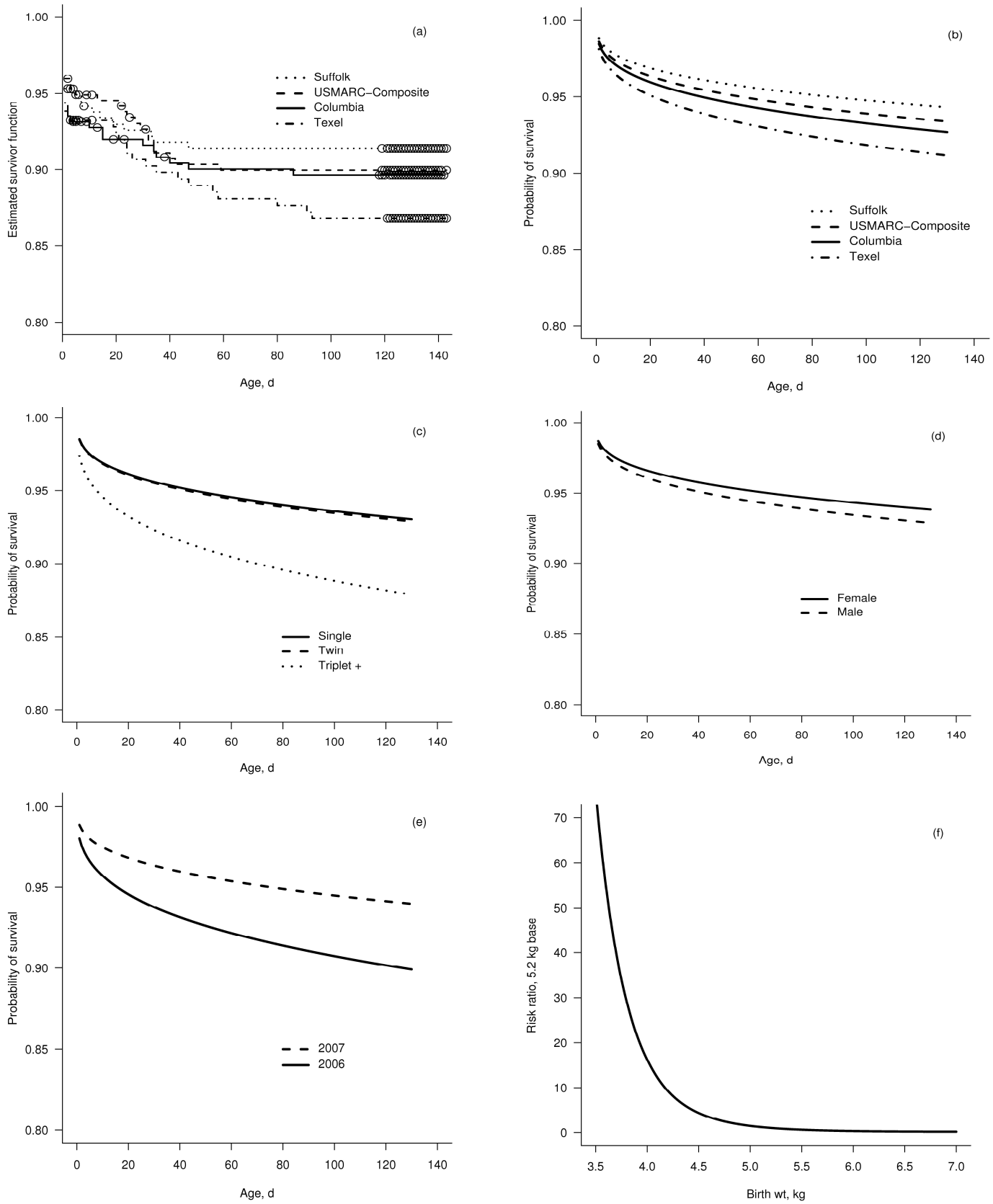


Figure 1. Panel (a): Kaplan-Meier survival functions for sire breeds, circles indicate censoring; Panel (b): survival functions for sire breeds, adjusted to a twin male from a 4-yr-old ewe in 2007 (P-value for sire breed effect = 0.38); Panel (c): survival functions for litter size, adjusted to a male from a 4-yr-old ewe in 2007 (P-value for litter size effect = 0.02); Panel (d): survival functions for gender, adjusted to a twin from a 4-yr-old ewe in 2007 (P-value for gender effect = 0.43); Panel (e): survival functions for year, adjusted to a 5.2 kg twin male from a 4-yr-old ewe (P-value for year effect = 0.01); Panel (f): risk ratios for birth weight ($P < 0.05$) after accounting for sire breed, litter size, gender, ewe age, and year effects.

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# ASSOCIATION OF MICROSATELLITE ETH10 GENOTYPES WITH GROWTH AND CARCASS TRAIT LEVELS IN BRANGUS CATTLE

**K. L. DeAtley[1], G. Rincon[2], C. R. Farber[2], J. F. Medrano[2], R. M. Enns[3], G. A. Silver[1], M. G. Thomas[1]**
[1]Department of Animal and Range Science; New Mexico State University, Las Cruces 88003;
[2]Department of Animal Science; University of California, Davis 95616;
[3]Department of Animal Science, Colorado State University, Fort Collins 80523

**ABSTRACT:** ETH10 is a GT microsatellite within the promoter of signal transducer and activator of transcription-6 (STAT6) gene on bovine chromosome 5. This protein is involved in signaling of GH. ETH10 is included in the panel of genetic markers recommended by the International Society of Animal Genetics for DNA-based parentage testing in cattle. International Brangus Breeders Association (IBBA) requires these tests for AI sires and embryo donors. Objective herein was to investigate the association of ETH10 genotypes with growth and ultrasound carcass phenotypes of cattle registered with IBBA (n = 2,222 from 1983 to 2007). Study of 13 allele and 38 genotype frequencies and estimates of deviance from Hardy-Weinberg Equilibrium revealed that individual alleles could be grouped into two different-sized classes: small which were 209, 211, 213, and 215 bp in size, or large which were 217, 219, and 221 bp in size. This procedure yielded genotypes in the categories of small/small, small/large, or large/large. Frequencies of the small/large and large/large genotypes were 44.7 and 45.3%, respectively. Genotype frequency of small/small was less than 10% so it was omitted from further analyses. Associations of genotype to phenotype were evaluated with a mixed effects model which included year of birth, sex of the animal, date of birth, genotype, breeding method, and contemporary group as fixed sources of variation and sire as a random source of variation. These effects were significant ($P < 0.05$) sources of variation for all outcomes. Cattle with small/large genotype had heavier ($P < 0.05$) birth weight than cattle of the large/large genotype (36.84 > 35.86 ± .03 kg). Concomitantly, cattle with the large/large genotype had greater percent fat within LM (3.51 < 3.67 ± 0.08%; $P < 0.05$) and more LM per body weight (0.17 > 0.16 ± 0.001 $cm^2/kg$; $P < 0.05$) than cattle of the small/large genotype. ETH10 genotypes appear to be associated with growth and ultrasound carcass trait levels in Brangus cattle. These results provide rationale for additional investigations involving STAT6 as a candidate gene in studies of the growth endocrine axis.

Key Words: Brangus, genotype, growth

## INTRODUCTION

Initial genome mapping efforts used microsatellites, even though today most efforts involve SNP. Microsatellites have been identified in both coding and non-coding regions of the genome and have been utilized to detect QTL (Sellner et al., 2007). Farber and Medrano (2003) reported that ETH10, a microsatellite included in the International Society of Animal Genetics (ISAG) parentage panel, was a GT repeat located in the promoter region of the signal transducer and activator of transcription-6 (STAT6) gene on bovine chromosome 5. Biologically, STAT6 is involved in the signaling of GH, which regulates postnatal bone and muscle growth and fat metabolism in mammals (Zhu et al., 2001).

ETH10 was strongly associated with marbling in Wagyu cattle (Barendse, 2002) and used to detect QTL for growth traits in Angus x Brahman crossed cattle (Kim et al., 2003). The International Brangus Breeders Association (IBBA) requires parentage testing for AI sires and embryo donors using the ISAG genetic marker panel which includes ETH10; therefore, the objective of this study was to conduct association analyses utilizing ETH10 genotypes and growth and ultrasound carcass phenotypes of cattle registered with IBBA.

## MATERIALS AND METHODS

Genotype and phenotype data were queried from the IBBA database (n = 2,222, 3/8 Brahman x 5/8 Angus individuals). Data were analyzed with SAS (SAS Inst. Inc., Cary, NC), which included genetic analysis tools. Frequencies of alleles and genotypes were determined using Proc Allele, which also outputted tests of Hardy-Weinberg equilibrium. If frequency of genotype category was greater than 10%, then data was considered appropriate for use in mixed effects model analyses. These mixed model associations were conducted with Proc Mixed. The genotype to phenotype association model was:

$y_{ijklmno} = \mu + A_i + B_j + C_k + D_l + E_m + F_n + G_o + e_{ijklmno}$ where

$y_{ijklmno}$ = phenotypic value of trait,

$\mu$ = population mean,

$A_i$ = fixed effect of genotype (i.e., small/large or large/large),

$B_j$ = fixed effect of contemporary group,

$C_k$ = fixed effect of breeding method (i.e., AI, ET = embryo transfer, NS = natural service),

$D_l$ = fixed effect of sex (i.e., male or female),

$E_m$ = covariate of Julian birthday (i.e., age of individual),

$F_n$ = fixed effect of year of birth (1983 to 2007),

$G_o$ = random effect of sire (i.e., mean = zero, variance = $\sigma_s^2$; Z statistic used to test if Ho: $\sigma_s^2 = 0$, and

$e_{ijklmno}$ = random residual error (mean = zero, variance = $\sigma_e^2$).

Traits analyzed with this model were 365-d weight and fat thickness over the 12th and 13th rib, % intramuscular fat of LM, rump fat, and LM area, and LM area per BW. These carcass measures were from ultrasound. Age ($E_m$) was not included in traits adjusted for age, which were birth weight, 205- and 365-d BW, carcass traits, and ADG, which was computed using the 205- and 365-d BW. Birth method ($C_k$) and individuals born by embryo transfer procedures were not included in analyses of the maternal traits of birth weight and 205-d weight. The interaction of genotype and year of birth was also evaluated in preliminary testing of models, but was omitted due to lack of significance. If genotype terms were found to be significant ($P < 0.05$), preplanned pair wise comparisons of least squares means generated with PDIFF (i.e. within option LSMEANS of the mixed procedure of SAS) were conducted.

The genotype term ($A_i$; i.e., small/large or large/large) was derived from 4 analyses. The first analysis revealed that the 217, 219, and 221 alleles were most prevalent in the population. Combinations of these three alleles as genotypes and their homozygotes were tested in the model above. Neither genotype configuration was significant. Then the alleles of 209, 211, 213, and 215 were paired as a genotype with the 217, 219, or 221 allele and tested in the model. Significance was not detected. Thus, animals were assigned genotypes composed of alleles 209, 211, 213, and 215 bp in size (i.e., small allele) or 217, 219, and 221 bp in size (i.e., large allele). This procedure yielded genotypes of small/small, small/large, and large/large.

## RESULTS

Thirteen alleles and 38 genotypes were present in the data. Allelic frequencies are shown in Table 1. Frequencies of genotypes are presented in Table 2, which also includes least squares means for trait levels by assigned genotypes. Frequency of small/small genotype was less than 10%, so it was omitted from these analyses. Genotypes appeared to be in Hardy-Weinberg equilibrium and sources of variation in the mixed effects model were significant ($P < 0.05$). Also, cattle with small/large genotype had heavier ($P < 0.05$) birth weight than cattle of the large/large genotype. Concomitantly, cattle with the large/large genotype had greater percent fat within LM ($P < 0.05$) and more LM per body weight ($P < 0.05$) than cattle of the small/large genotype.

## DISCUSSION

Kim et al. (2003) reported that ETH10 was useful in detecting QTL associated with birth weight and 365-d weight in Brahman x Angus cattle. Association results from the current study were similar for the trait of birth weight. Barendse (2002) reported an association between marbling score and ETH10 alleles in Wagyu cattle. In brief, the 223 bp allele was associated with higher marbling scores relative the 217 bp allele. However, in the Brangus cattle studied herein, the 217, 219, and 221 alleles were associated with higher marbling scores relative the small alleles of 209, 211, 213, and 215 bp. Waygu and Brangus are very divergent breeds of cattle, especially since Brangus are 3/8 *Bos indicus*. Thus, it is not surprising the alleles which were significant predictors of measures of marbling were different among these breeds. Nonetheless, results provide evidence to suggest that STAT6 could be a candidate gene for predicting differences in growth and carcass traits. ETH10 is located within the STAT6 gene, which is a protein involved in GH signaling (Zhu et al., 2001; Farber and Medrano, 2003). There currently are no reports to suggest that ETH10 influences gene function; however, the results cumulatively suggest that STAT6 could contain quantitative trait nucleotides (QTN; i.e., functional mutations) that are linked with ETH10 on bovine chromosome 5.

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**Table 1.** ETH10 allelic frequencies in Brangus (3/8 Brahman, 5/8 Angus) cattle from the IBBA database.

| Allele | Frequency, % | N |
|---|---|---|
| 199 | 0.020 | 1 |
| 203 | 0.020 | 1 |
| 206 | 0.020 | 1 |
| 207 | 0.090 | 4 |
| 209 | 10.00 | 442 |
| 211 | 6.22 | 275 |
| 213 | 12.78 | 565 |
| 215 | 3.30 | 146 |
| 217 | 34.87 | 1542 |
| 219 | 16.58 | 733 |
| 221 | 15.99 | 707 |
| 223 | 0.09 | 4 |
| 225 | 0.02 | 1 |

**Table 2.** Frequency of small/large and large/large genotypic categories and least square means for growth traits and carcass phenotypes in Brangus cattle (3/8 Brahman x 5/8 Angus; n = 2222).

| Item | Small/Large | Large/Large | SEM |
|---|---|---|---|
| Frequency, % | 44.73 | 45.32 | |
| Birth weight, kg | 35.27[a] | 34.35[b] | 0.31 |
| Adjusted birth weight, kg | 36.84[a] | 35.86[b] | 0.32 |
| 205-d weight, kg | 278.08 | 276.29 | 2.85 |
| Adjusted 205-d weight, kg | 287.07 | 288.95 | 2.18 |
| 365-d weight, kg | 456.79 | 454.31 | 3.66 |
| Adjusted 365-d weight, kg | 461.31 | 458.08 | 3.60 |
| LM area, $cm^2$ | 72.05 | 71.98 | 0.73 |
| Adjusted LM area, $cm^2$ | 71.86 | 71.83 | 0.72 |
| $12^{th}$ rib fat thickness, cm | 0.62 | 0.64 | 0.02 |
| Adjusted $12^{th}$ rib fat thickness, cm | 0.61 | 0.63 | 0.02 |
| Intramuscular fat, % | 3.56[a] | 3.71[b] | 0.08 |
| Adjusted intramuscular fat, % | 3.51[a] | 3.67[b] | 0.08 |
| Rump fat, cm | 1.01 | 1.04 | 0.05 |
| LM area/BW, $cm^2$/kg | 0.16[a] | 0.17[b] | 0.00 |
| ADG, kg/d | 1.09 | 1.08 | 0.02 |

[ab]Within a row, means without a common superscript differ ($P < 0.05$).

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# GENOTYPIC VARIATION AT THE PRION PROTEIN GENE AMONG SUFFOLK, RAMBOUILLET, AND TARGHEE SHEEP

**M.K. Thompson and C.L. Gasser**
Southern Utah University, Cedar City, UT

**ABSTRACT:** Scrapie in sheep is a member of the family of transmissible spongiform encephalopathies. Infected animals are thought to accumulate abnormal prion proteins in nervous tissues, causing extensive degeneration and eventually death. Polymorphisms of the prion protein gene influence susceptibility to scrapie. Arginine residues (R) at codon 171, and alanine residues (A) at codon 136 increase resistance to scrapie. The Suffolk breed has been found to have the greatest incidence of scrapie in the United States. The objective of this experiment was to compare genotypes at codons 136 and 171 of the prion protein gene among a sample of Suffolk, Rambouillet, and Targhee sheep from a single flock. Genotypes were compared between 14 Suffolk, 14 Rambouillet, and 15 Targhee ewes from the SUU flock. Gene and genotypic frequencies for codon 171 of the prion protein gene varied among the three breeds ($P < 0.01$). Frequency of R was 50.0%, 21.4%, and 3.3% in the Suffolk, Rambouillet, and Targhee ewes, respectively. The incidence of the RR genotype was 21.4% in the Suffolk ewes, and 0% for the Rambouillet and Targhee ewes. Frequency of the QR genotype was 57.1%, 42.9%, and 6.7% for the Suffolk, Rambouillet, and Targhee ewes respectively. No differences were detected between breeds for gene or genotypic frequencies at codon 136. Overall frequency of A across breeds was 88.4%. Genotypic differences were not associated with lambing rate or mean lambing date. The expected resistance to scrapie based on genotypes was greatest in the Suffolk ewes and least in the Targhee ewes. Based on these results, greater incidence of scrapie in Suffolk sheep would be expected to result from greater exposure rather than genetic susceptibility.

**Key Words:** codon 136, codon 171, prion protein gene, scrapie, sheep

## Introduction

Scrapie is found throughout the world. The disease was first reported in 1732 in England. However, the first diagnosis in the U.S. was not until 1947, in a flock of Suffolk sheep that were of U.K. origin and had been imported to Michigan from Canada. Since then scrapie has also been found in Cheviots, Corriedales, Dorsets, Finns, Hampshires, Merinos, Montadales, Southdowns, and several crossbreeds.

Scrapie is caused when the misfolded form of the normal prion protein (PrP) aggregates in nervous tissues. Resistance to protein misfolding is influenced by the animo acid sequence of the PrP gene. Selection for alanine (A) at codon 136 and arginine (R) at codon 171 is advisable to increase resistance to the disease.

Almost 90% of scrapie cases reported in the U.S. have been in Suffolk sheep. High prevalence of scrapie in the Suffolk breed has led many to believe that this breed may be more genetically susceptible to the disease than other breeds. Alexander et al. (2005) genotyped ewes from the Columbia, Hampshire, Rambouillet, Suffolk, and Targhee breeds. Results of the evaluation showed that the Suffolk ewes had a high percentage of individuals possessing Non-R alleles. A high incidence of the Non-R/Non-R genotype has also been found in Suffolk sheep in other countries (Ohara et al., 2007; Passos et al., 2008).

Genotypic sequence is important in resistance to different strains of the scrapie agent. Presence of R at codon 171 has been found to be influential in resistance to the strain of scrapie most commonly found in the U.S. and the European Union. Though specific scrapie strains in the U.S. are not known, many hypothesize that strain CH1641, the prototype for strain C, is most prevalent because it causes disease in sheep homozygous Non-R at codon 171 regardless of genotype at codon 136 (Bulgin, 2007).

Although management efforts in the US have been focused on increasing the frequency of R at codon 171, it may be advisable to also select for A at codon 136. Infected individuals with the genotype Non-R/R at codon 171 generally have valine (V) present at codon 136. Epidemiologists from the USDA APHIS suggest the possibility of a second scrapie strain prominent in the U.S., in which susceptibility is primarily influenced by V at codon 136 (Bulgin, 2007).

It would be reasonable to speculate that strain SSBP/1, the prototype for scrapie strain A, would be the other strain present in the U.S. because its activity is dependent on V residues at codon 136. Sheep possessing the homozygous AA genotype at codon 136 were found to be resistant to SSBP/1, or had an incubation period longer than the lifetime of the individual. Those with the AV genotype were found to have a longer incubation period, 4-6 years, for the strain. Individuals exhibiting the VV genotype had a short incubation period, 2-3 years, when exposed to the agent (Bulgin, 2007).

The objective of this study was to evaluate genotypic differences at codons 136 and 171 of the PrP gene among Suffolk, Rambouillet, and Targhee sheep in the SUU flock.

## Materials and Methods

### *Animals*

Procedures used in this study were approved by the Southern Utah University Animal Care and Use Committee. Sheep used in the project were selected from purebred Suffolk, Rambouillet, and Targhee groups maintained at the SUU Valley Farm. Genotypes for the PrP gene were previously unknown within this flock, and no selection for scrapie resistance had occurred.

### *Blood Collection/Genotyping*

Blood samples were collected from 14 Suffolk, 14 Rambouillet, and 15 Targhee ewes via jugular venipuncture in EDTA-coated Monoject tubes (Tyco Healthcare Group LP, Mansfield, MA.). Genotyping was performed by an APHIS-approved laboratory (Gene Check, Inc., Greeley, CO), where genotypes were determined using a DNA mismatch binding assay (Wagner and Dean, 2000). This procedure identified the presence or absence of A at codon 136, where absence of A would indicate the presence of V. The presence or absence of R was identified at codon 171. Absence of R was designated as Q, which could represent glutamine, histidine, or lysine.

### *Statistics*

Variation among breeds for gene and genotypic frequencies at codons 171 and 136 of the PrP gene was determined using $\chi^2$ analysis in SPSS (SPSS 13.0 for Windows, 2004, Chicago, IL). Genotypes were ranked in order from most resistant to least resistant: (1) RRAA, (2) QRAA, (3) QRAV, (4) QQAA, (5) QQAV, and (6) QQVV. Then, genotype rank was analyzed for variation among breeds using ANOVA in SPSS. Lambing rate and lambing date were analyzed for variation among breeds as well as among genotypes at codons 171 and 136 of the PrP gene using ANOVA in SPSS. When the ANOVA F-test was significant, individual mean comparisons were evaluated.

## Results

Frequency of R at codon 171 of the PrP gene differed among breeds ($P < 0.01$; Figure 1). The Suffolk ewes exhibited the greatest frequency of R, followed by Rambouillet, then Targhee. Frequency of the RR, QR, and QQ genotypes differed among breeds ($P < 0.01$; Figure 2).

Frequency of A at codon 136 of the PrP gene did not differ among breeds ($P > 0.05$). The mean frequency of A across breeds was 88.4%. Frequency of the AA, AV, and VV genotypes at codon 136 of the PrP gene did not differ among breeds ($P > 0.05$). Mean genotypic frequencies across breeds were 81.4%, 14.0%, and 4.7% for AA, AV, and VV, respectively.

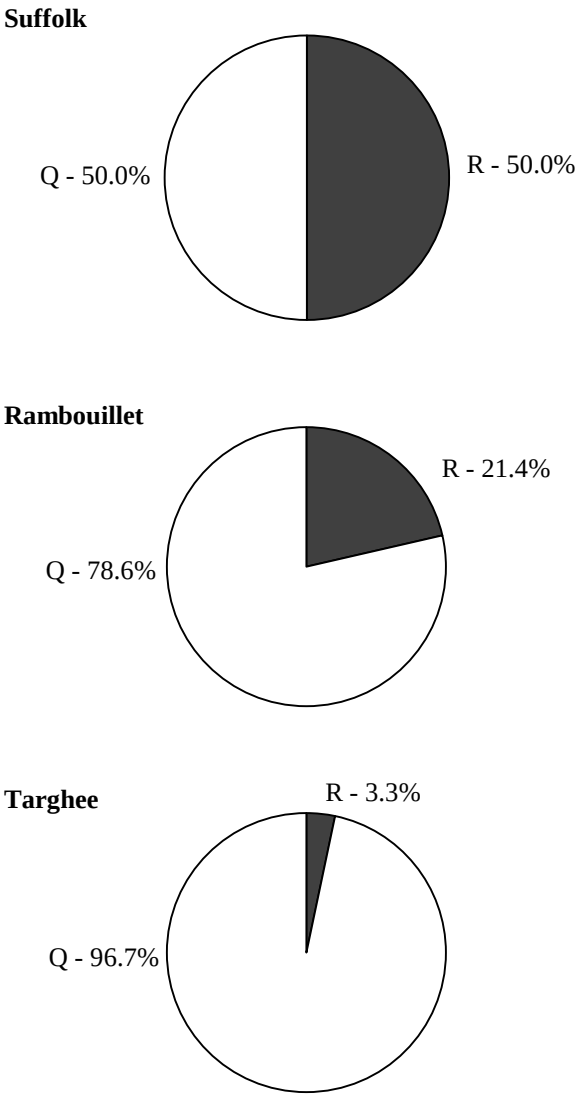


Figure 1. Frequency of arginine (R) and Non-R (glutamine, histidine, or lysine; Q) residues at codon 171 of the prion protein gene among Suffolk, Rambouillet, and Targhee ewes ($P < 0.01$).

Genotype rank for scrapie resistance differed among breeds ($P < 0.01$; Figure 3). Mean ranking for the Targhee ewes ($4.1 \pm 0.2$) was lower than both the Suffolk ewes ($2.6 \pm 0.4$; $P < 0.01$) and the Rambouillet ewes ($3.2 \pm 0.3$; $P < 0.05$).

Lambing rate did not differ across breeds or across genotypes ($P > 0.05$; mean across breeds = $1.8 \pm 0.1$). Mean lambing date also did not differ across breeds or genotypes ($P > 0.05$).

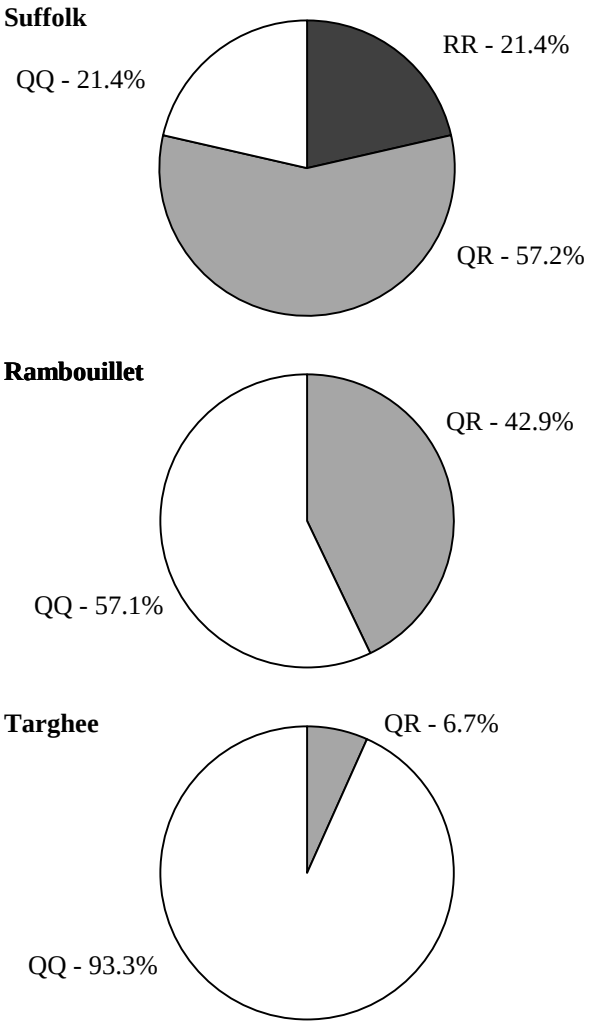


Figure 2. Frequency of arginine/arginine (RR), Non-R/R (QR; Q = glutamine, histidine, or lysine), and QQ genotypes at codon 171 of the prion protein gene among Suffolk, Rambouillet, and Targhee ewes ($P < 0.01$).

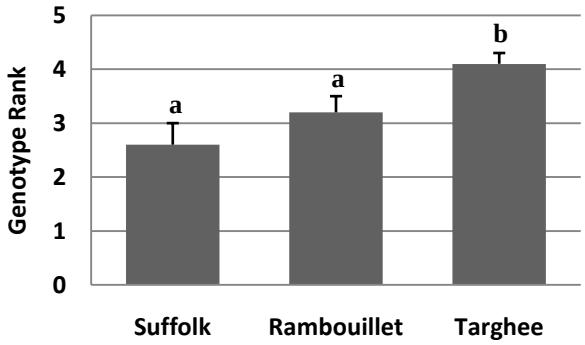


Figure 3. Mean (± SEM) genotype rank based on expected resistance to scrapie among Suffolk, Rambouillet, and Targhee ewes (1 = RRAA, 2 = QRAA, 3 = QRAV, 4 = QQAA, 5 = QQAV, 6 = QQVV). Means without a common superscript letter differ ($P < 0.05$).

## Discussion

In the present study the frequency of R at codon 171 was greatest in the Suffolk ewes at 50%. In addition RR genotypes were only found among Suffolk ewes. This was somewhat unexpected considering the prevalence of scrapie in Suffolk sheep in the U.S. Some studies of Suffolk sheep in other countries have shown a greater susceptibility to scrapie with approximately half of the sheep exhibiting the QQ genotype at codon 171 (Ohara et al., 2007; Passo et al., 2008). Results from a study by Alexander et al. (2005) working with multiple U.S. flocks showed similar results to the current experiment. This may be an indication of progress in breeding scrapie resistance into U.S. Suffolk sheep. Genotypic variation among individual flocks would certainly be influenced by specific management and breeding goals.

The predominant allele present at codon 136 in this study was A. Occurrence was similar across all breeds with an average frequency of almost 90%. This appears to be an indication of good resistance against the V-dependent strain of scrapie in these breeds. It may also indicate an extended incubation period among those that contract scrapie (Bulgin, 2007). This is consistent with other studies that have looked at the codon 136 genotype (Ohara et al., 2007; Passo et al., 2008). Predominance of A at codon 136 allows flexibility in selection for other desirable traits while maintaining resistance to scrapie.

There is some concern that selecting for scrapie resistance could compromise other production traits. Neither lambing rate nor lambing date was associated with breed or genotype in the current study. These results provide evidence that selection for PrP genotype can be performed effectively without detrimental effects on production traits. Alexander et al. (2005) found that production traits of multiple breeds were not influenced by PrP genotype at codon 171. However, the QQ genotype at codon 171 was associated with greater lamb production in Suffolk ewes.

Among these three breeds the Suffolk ewes showed the greatest resistance to scrapie. This evidence along with the evidence found in other studies suggests that prevalence of scrapie in U.S. Suffolk sheep may be due to factors other than genetic susceptibility. High incidence of the disease may have resulted from the original infection and subsequent exposure among Suffolk sheep.

It appears from these and other results that the Suffolk breed contains sufficient numbers of animals with resistant genotypes from which breeders can select for resistance to scrapie. However, the limited number of Rambouillet and especially Targhee sheep with resistant genotypes at codon 171 poses a concern. Selection for scrapie resistance within these breeds may result in decreased selection pressure for other desirable traits.

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# ESTIMATES OF GENETIC AND PHENOTYPIC PARAMETERS OF PERFORMANCE TRAITS DURING POSTWEANING IN LIMOUSIN CATTLE

**A. P. Márquez[1], J.F. Montano[1], J. Saucedo[1], H. G Gonzalez[2] and Guerrero J. N. [3]**
[1]Universidad Autónoma de Baja California, Baja California, México, Instituto de Ciencias Agrícolas.
[2] Universidad Autónoma de Ciudad Juárez, Chihuahua, México.
[3] University of California, Natural and Agricultural Resources, Holtville Ca.

**ABSTRACT:** Data came from a herd of Limousin (**L)** located at the north of México. Records of (n=27 and n=34) calves from heifers and mature cows, respectively of a total of 61 dams involving inheritance of **L** mated to **L** sires were included. The objective was to estimate genetic and phenotypic parameters of bioeconomic growth traits during postweaning involving inheritance of **L**, as a basis for developing selection criteria. For weight at 365-d (**YWT**), feed intake (**FI),** average daily gain (**ADG**), feed conversion (**FC**), and feed efficiency (**FE**) during 112 d test. A derivative-free, multiple-trait REML program (Boldman et al., 1993) was used to estimate (co) variance components for each trait. The animal model included: year of birth, age of dam, sex of the calf, with date of birth as a covariate to adjust a common age as fixed effects**;** sire and residual as random. Mean values and their standard errors were**:** 232.10±3.77, 364.76±4.61, 841.56±10.64, 159.13±2.01, 1.35±0.05, and 7.51±0.13 corresponded to weights**:** to initiate the 112 d performance test **IW**, **YWT**, **FI**, **TWG**, **ADG**, and **FE** (kg), respectively. The estimates of heritabilities ($h^2$=0.33±.04, $h^2$= 0.39±.05, $h^2$=0.36±.05, $h^2$= 0.37±.05, and $h^2$= 0.31±.05) corresponded to **YWT**, **FI**, **ADG**, **FC**, and **FE**, respectively.
**Key words: Genetic parameters, Growth traits, Postweaning, Limousin**

## Introduction

Differences in performance for most bioeconomic traits are the result of different selection goals in different breeds. These differences represents an important genetic resource for improving efficiency of beef production (Gregory et al., 1994). Best linear unbiased predictor (BLUP) and Restricted maximum likelihood (REML) of genetic values using mixed model methodology with animal models is the method of choice of animal breeders (Henderson, 1988). Restricted maximum likelihood (REML) estimation of variances and covariances and simultaneous prediction of breeding values is probably most optimum for unbalanced data. Modifications introduced by Boldman et al. (1991) to use a sparse matrix solver (Sparspak, George et al., 1980) increased the order of mixed model equations that can be used with REML The objective of this study was to estimate genetic and phenotypic parameters for growth traits during postweaning in beef cattle.

## Materials and Methods

### Description of data

This study used data that were part of a program to characterize the performance of the progeny of Limousin **L** cattle for growth traits during postweaning in a commercial herd located in Samalayuca, México at the north of the country. Cows and sires used to initiate this experiment were originally purchased in a beef herd in Kansas state. Through the time were acquired from France sires, to be used under AI matings. The traits and number of records were: (n=61) for yearling weight **YWT**, **FI**, **ADG, FC**, and feed efficiency (FE).

### Mating plans

Heifers and cows **L** were mated to **L** sires to an average weight of (300 and 466 kg), respectively to produce calves at 3 through 8 years of age. All heifers were exposed by natural service to yearling bulls. Dams two or more yr old were mated by artificial insemination (AI) for 30 days followed by natural-service exposure for 30 days for a mating season of 60 days.

### Analyses of data

**REML Analysis.** A derivative-free, multiple-trait REML program (Boldman et al., 1993) was used to estimate (co) variance components for each trait. The analytical model included: year of birth, age of dam, sex of the calf, with date of birth as a covariate to adjust a common age as fixed effects**;** sire and residual as random.

## Results and Discussion

**Overal means.** The variables of this data set are presented in **Table1**. As shown overall means and their standard errors were: 232.10±3.77, 364.76±4.61, 841.56±10.64, 159.13±2.01, 1.35±0.05, and 7.51±0.13 corresponded to **IW**, **YWT, FI**, **TWG**, **ADG**, **and FE** (kg), respectively. In a study to compare the performance of continental breeds for growth traits to 15 month age reported values (593, 564, 531**;** 1.521, 1.469, 1.399**;** 7.7, 7.5, and 7.2 kg) for final weight,

average daily gain, and feed per unit gain, for Maine Anjou, Charolais and Limousin, respectively. **Table 2** shows the estimates of heritability values **($h^2$=0.33±.04, $h^2$=0.39±.05, $h^2$=0.36±.05, $h^2$= 0.37±.05, and $h^2$= 0.31±.05)** corresponded to **YWT**, **FI**, **ADG**, **FC** (feed per gain), and **FI** (gain per feed), respectively.

**Yearling weight**

The average yearling weight and its standard error at this study was 364.76±4.61 **(Table 1**). Gregory et al. (1993) evaluated the performance of the progeny (n=254) for **YWT** of **L** dams mated naturally to sires **L**. The overall mean weight at 365-d reported for the author was 375 kg. Gregory et al. (1993) evaluated the performance of the progeny (n=254) for **YWT** of **L** dams mated naturally to sires **L**. The overall mean weight at 365-d reported for the author was 375 kg. The estimates of heritability for **YWT** in this study was **($h^2$=0.33±.04)**. This value is quite similar to the reported value for **YWT** by Greogory et al. (1993).These authors estimated heritability direct **($h^2$=0.32±.04)** for **YWT**. Van Vleck et al. (1999) estimated genetic parameters for **YWT** using 6 animal models with and without inbreeding coefficients (F) for animals with and without relationships matrix (A and A=I) for sire models. The estimates of heritability by these authors was **($h^2$=0.32±.04).** Gregory (1992) conducted an experiment involving inheritance of pure breeds beef cattle, the estimates of heritability and phenotypic deviations values were **($h^2$=0.26 and $h^2$=0.43);** ($\sigma_p$=30.05 and **$\sigma_p$ = 28.96 kg);** ($h^2$=0.27 and $h^2$=0.43)**; ($\sigma_p$=37.50, $\sigma_p$ = 34.59 kg)** for 368-d weight in females and males, respectively. Estimates of genetic and phenotypic parameters for **YWT** of beef cattle using (n=154 and n=147**;** n=6 and n=23) published studies have been summarized by Koots et al. (1994). These authors obtained unweighted and weighted mean heritabilities estimates for **YWT ($h^2$ =0.35 and $h^2$= 0.33; $h^2$=0.11 and $h^2$=0.11)** direct and maternal, respectively. Direct and maternal influences are biologically different, and the maternal influences were lower than corresponding direct trait. Henderson (1975) has shown that analytically that ignoring some

random effects in genetic evaluation can still result in unbiased estimates and prediction. Meyer (1992); however, found that ignoring maternal effects tends to inflate the genetic heritability estimates when using an animal model with real data.

**Feed intake**

The mean value of total feed intake **FI** at this study was 841.56 kg during 112-d test (Table 1). The estimates of expected **FI** are very important to calculate residual feed intake RFI. This can be predicted by using feeding standards (e.g. National Research Council, 1996) by using individual **FI** prediction models (e.g. Cornell value discovery System, 2004 or by phenotypic or genetic regression using actual feed test data (Kennedy et al 1993). Several recent studies have used the phenotypic regression approach (Archer et al. 1997; Arthur et al. 2001a,b; Crews et al.2003) to calculate RFI, generally summarized as: $y=ß_0 + ß_1(ADG) + ß_2$ (WT) + RFI; where **y** is daily feed intake, $ß_0$ is the regression intercept, $ß_1$ is the partial regression of daily gain (ADG), and $ß_2$ is the partiall regression of daily intake on body weight (BW). This equation was designed for young and growing cattle; although it is possible to equations for other beef cattle categories (Montanholi, et al. 2006). The estimates of heritability of **FI** at this study was **($h^2$=0.37±.05)**. Koots, et al. (1994) based in (n=23 and n=21) studies of ($h^2$) for feed intake **FI** (gain/feed) using unweighted and weighted mean of ($h^2$) found values ($h^2$=0.41±0.18 and $h^2$=0.34±.025) for **FI.** The estimates of $h^2$ by these authors are quite similar to the estimates **($h^2$=0.37±.05)** at this study; nevertheless the estimates of this genetic parameter was based in a limited number of observations.

**Average daily gain**

The best measure of optimum appears to be sustainable low-cost production as measured by low breakeven at weaning, feedlot and carcass endpoints. The average daily gain ADG in feedlot varies from (1.1 to 1.6 kg/d); the target industry for ADG is (1.4 kg/d) target gives central focus applicable to many commercial beef operations. Deviation from this target and optimum range is dependent on market, economic, and environmental conditions in specific commercial beef operations (Taylor et al., 1999). For average daily gain during 112 d test, the estimates of heritability in this study was **($h^2$=0.36±.05)**. The estimates of heritability for ADG at this study are in agreement to different reports for this trait that have been reviewed several times in beef cattle. Previous estimates of heritability using least squares for ADG in this study was **($h^2$=0.44±.05).** Koots et al. (1994) performed weighted least squares analyses of published genetic parameter estimates for beef production traits including factors fitted for each trait (breed, country, data origin field or experimental, feeding management, estimation method, sex, and decade of data collection). Breed and country were found to be significant in many cases. Contrary to widespread belief, heritabilities derived from field data and experimental data did not differ for growth traits. Heritabilities estimated using animal models did not differ from from those by other methods of estimates (Koots et al., 1994).

**Feed conversion**

The estimates of heritability for (FC) in this study was **($h^2$=0.37±.05).** Koots, et al. (1994) using unweigthed and weighted mean heritabilities have estimated genetic and phenotypic parameters for performance traits. These authors summarized their estimates from (n=28 and n=25) studies of heritability **($h^2$=0.36±0.22, $h^2$=0.32±0.024)** for **(**FC). Mohiuddin (1993) has summarized papers on genetics of growth traits that indicate that maternal ability for calf performance may be correlated to with the expression of genes for growth, half of which are received from the dam. Thus genetic variability for growth traits in mammals may contain the additional complexity of maternal effects. Willham (1980) indicated that the kind and relative amount of genetic variation attributable to maternal effects, especially the sign and magnitude of the genetic correlation between direct and maternal effects of traits with high importance economic, is critical in the design of optimal breeding plans for most domestic animals. Thus not only direct but also maternal effects should be considered to achieve optimal genetic gain in a selection program. Such consideration is especially important if a negative covariance exists between these two effects.

**Feed efficiency**

Rate and efficiency of gain are positively associated and under strong genetic control. It suggests that improvement can be made through selection. Taylor et al. (1999) reports that production and marketing specifications for beef cattle for feed lot efficiency as industry target (applicable to many commercial beef operations) as overall mean is (6 kg feed per kg gain) and ranges (5-7 kg feed per kg gain) when high energy rations are used. Feed efficiency (FE) measurements constitute one of the key approaches to study animal bioenergetics and metabolism (Montanholi, et al. 2007). Major studies highlight the existence of genetic variation in (FE) and the fact that most FE traits are moderately heritable, hence the potential for genetic improvement (Arthur et al., 2005). FE has been incorporated in selection objectives and breeding plans in beef cattle as an opportunity to identify biological predictors of (FE) traits (Barwick et al. , 1994). The residual feed intake RFI first described by Koch et al. (1963) which has emerged as one of key parameters to assessing feed efficiency.

The estimates of heritability unweighted based in limited numbers for feed efficiency (FE) (gain/feed) at this study was ($h^2$=0.31 ±0.05) is quite different if compared to findings of Koots, et al. (1994). These authors have summarized estimates of genetic parameters for (FE) based in (n=9 and n=9) reviews of unweighted and weighted mean heritabilities **($h^2$=0.42 ±0.16,** and **$h^2$= 0.37±0.049.** Woldehawariat et al. (1977) pooled estimates for FE, FC, and FI into a single **$h^2$** value. Koots et at. (1994) found no significant differences in the estimates values of **$h^2$** for FE, FC, and FI. Differences between the estimates of Woldehawariat et al. (1977) and Koots et at. (1994) may due to several factors. Approximately 80% of the estimates included at Koots et at. (1994) were not included by Woldehawariat et al. (977), due to the stricter criteria when selecting literature estimates, and to the increase in the number of published **$h^2$**estimates for growth traits during over the past 15 years. The inverse of the sampling variances was employed as the weighting factor calculated by Koots et at. (1994) while Woldehawariat et al. (1977) weighted each estimate by the number of sires, but these approaches should yield similar results. Additionally, contained a higher proportion of estimates from field populations. However this did not significantly affect heritability estimates for FC, FE or YWT.

**Implications**

Results of this experiment represents an important source of information to be used for producers in their breeding programs. Improvement in production traits measured by weight may result from selection within breeds. It is well documented that growth rate and feed efficiency are positively associated and under strong genetic control. The magnitude of the parameters estimated at this experiment based in limited number of observations, indicating genetic progress is possible for economically growth traits during postweaning.

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**Table 1. Overall means and their standard errors for initial weight, yearling weight, total feed consumption, total weight gain, average daily gain, and feed per unit of gain of progeny from Limousin dams mated to Limousin sires, during a 112-d performance test in feedlot.**

| Trait | Mean | SE(±) |
|---|---|---|
| Initial weight (kg) | 232.10 | 3.77 |
| Yearling weight (kg) | 364.76 | 4.61 |
| Total feed consumption (kg) | 841.56 | 10.64 |
| Total weight gain per animal (kg) | 159.13 | 2.01 |
| Daily gain per animal (kg) | 1.35 | 0.05 |
| Feed per unit of gain (kg) | 7.51 | 0.13 |

**Table 2. Estimates of heritability and their standard errors of weight at 365-d, feed intake, weight gain, average daily gain, feed conversion of progeny, and feed efficiency from Limousin dams mated to Limousin sires, during a 112-d performance test in feedlot.**

| Trait | Heritability |
|---|---|
| Yearling weight | 0.33±.04 |
| Feed intake | 0.39±.06 |
| Daily gain | 0.36±.05 |
| Feed conversion feed/gain | 0.37±.05 |
| Feed efficiency gain/feed | 0.31±.05 |

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# WEANING DATE IMPACTS ON BACKGROUNDING AND FINISHING PERFORMANCE OF MAY BORN[1] ANGUS CALVES

**M. M. Stamm[*], C. S. Schauer*, V. L. Anderson[†], and B. R. Ilse[†]**
[*] Hettinger Research Extension Center; North Dakota State University, Hettinger, ND 58639.
[†]Carrington Research Extension Center; North Dakota State University, Carrington, ND 58421.

**ABSTRACT**: This study evaluated the effects of weaning date on May born Angus calves during the grow-finish period. Forty-eight calves from the NDSU-Hettinger Research Extension Center cowherd were randomly assigned to one of two treatments (**TRT**): early weaning (**EW**; 139 d of age) or normal weaning (**NW**; 197 d of age). After weaning, calves were weighed, stratified by BW and sex, and randomly allotted to one of 12 pens (4 calves/pen; n=6) for backgrounding (EW = 91 d; NW = 42 d). Calves were fed a 43:57 forage:concentrate diet (13% CP; 1.19 Mcal $NE_g$/kg). Interim calf weights were measured on d 36, 52, and 64 and diet samples collected on d 14, 32, 54, 67, and 78. Following backgrounding, calves were finished at the NDSU-Carrington Research Extension Center. Calves were commingled into one finishing pen and fed a 14:86 forage:concentrate diet (14.7% CP; 1.39 Mcal $NE_g$/kg) for 112 d. Calves were harvested and individual carcass data collected when calf back fat was estimated at 1.27 cm. Results were analyzed as a randomized complete design using generalized least squares (PROC MIXED, SAS); LSD was used to separate TRT means ($P < 0.05$). Weaning weight was predictably less for EW calves (190 ± 1.31kg) vs. NW calves (254 ± 1.31kg; $P < 0.0001$); EW calves were 6.0% heavier than the NW calves (EW = 355 kg, NW = 335 kg; $P < 0.004$) at the end of the background period. Despite EW calves having higher feed costs during backgrounding than NW calves ($148.27 vs. $65.11; $P < 0.0001$), feed cost of gain and total cost of gain did not differ among TRT ($P = 0.11$). Weaning date did not affect DMI, ADG, G:F, and mortality rates ($P = 0.10$). No treatment differences were observed for carcass data ($P = 0.23$) or age at slaughter ($P = 0.86$). Early weaning is a viable option for producers who feed their calves for 91 d post weaning; EW calves grew as well as or better than NW calves in this study.

**Key Words**: calves, carcass, growth, and weaning.

## Introduction

The western region of North and South Dakota is characterized by a semi-arid climate, with an agricultural base consisting of dryland farming and beef cattle production. In 2005, the Dakotas had a beef cattle inventory of over 5.23 million head worth over $5.4 billion to the two states' economies (USDA NASS, 2005). The majority of cows in the region calve in the early spring (Jan - Mar.) and calves are sold at weaning (Oct.-Nov.).

During the past six years, this area was impacted by drought, drastically reducing winter-feed supplies for gestating/lactating beef cows. One management practice regional cattle producers use to spare forage for grazing gestating/lactating beef cows is to early wean calves. Peterson et al. (1987) reported that cows with early-weaned calves consumed 45.3% less hay than cows with nursing calves. The definition of early weaning varies, but calves weaned at less than 150 days of age are usually considered early-weaned (Loy et. al, 1999). Other reasons producers choose to early wean their calves include poor quality feed available, feed in short supply, cows are poor milkers or first calf heifers, and when cows calve late (Myers et al., 1999c).

Most research on early weaning has focused on early spring (Feb. - Mar.) calving cowherds (Schoonmaker et al., 2001; Story et al., 2000). Little research has evaluated the impacts of early weaning on late spring (May-June) born calves. The objective of this study was to evaluate the effects of weaning date on May born Angus calves during the growing and finishing period.

## Materials and Methods

***Study Area.*** The experiment was conducted at the NDSU Hettinger Research Extension Center's Southwest Feeders feedlot in Hettinger, ND and the NDSU Carrington Research Extension Center's feedlot in Carrington, ND.

***Animals, management, and measurements.*** The North Dakota State University Animal Care and Use Committee approved the protocols used in this study. A randomized complete design was used to evaluate the effects of weaning date on May born Angus calves during the grow-finish period. Forty-eight steer and heifer calves (average birth date = May 1 ± 3.43 d) from the NDSU Hettinger Research Extension Center's May calving cowherd were randomly assigned to one of two treatments (**TRT**): early weaning (**EW**; 139 d of age; Sept. 19, 2006) or normal weaning (**NW**; 197 d of age; Nov. 15, 2006). Brood cow age ranged from 2 to 6 years, no first calf

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*Disclaimer: Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U. S. Department of Agriculture.*

heifers were in the calving herd in 2006. The NW calves remained on pasture with their dams 57 d longer than the EW treatment group. Neither set of calves (EW and NW) were creep fed while on pasture. Early weaned calves were hauled (8 km) to the feedlot after morning gathering and weighing in the pasture. Calves assigned to the EW treatment weighed 189.5 kg at weaning. The NW calves were weaned 57 d later after morning gathering and weighing in the pasture and hauled (8 km) to the feedlot. Normal weaned calves weighed 254 kg at weaning. All calves were fed a dry hay based receiving ration for the first 14 d (NW) and 22 d (EW) post arrival at the feedlot. The receiving diet for EW calves consisted of 43.4% shell corn, 22.15% alfalfa-grass hay, 13.05% oat hay, 12.80% barley hay, 7.15% protein supplement, and 1.45% Decoquinate medicated crumbles (DM basis; 14.3% CP; 1.19 Mcal $NE_g$/kg). The receiving diet for NW calves consisted of 39.90% shell corn, 35.90% mixed hay, 12.30% oat hay, 5.20% protein supplement, 4.50% soybean meal (44% CP), 1.60% Decoquinate medicated crumbles, and 0.60% calcium carbonate (DM basis; 13.20% CP; 1.12 Mcal $NE_g$/kg). The hay sources utilized in the receiving diets were chopped (6.35 cm length) prior to feeding.

At the end of the receiving period (EW = Oct. 12, 2006; NW = Nov. 30, 2006), calves were weighed, stratified by BW and sex, and allotted randomly to one of 12 pens (4 calves/pen; six pens per TRT) for the backgrounding phase (EW = 91 d; NW = 42 d). Calves were fed a 43:57 forage:concentrate diet (13% CP; 1.19 Mcal $NE_g$/kg; growing diet; $91.25/ton DM; Table 1). Target gain for the background period was 1.14 kg/d. Diet formulations were isonitrogenous and isocaloric at the start of the study. Fence line feed bunks were read daily at 0700 hrs and slick bunk management was used to determine individual pen daily feed allotment. Calves were fed diets once daily commencing at 0900 hrs and had continual access to water. Initial and final weights were determined using average unshrunk weights from two consecutive weigh days by weighing each individual animal prior to daily feeding. Interim calf weights were measured on d 36, 52, and 64 of the background phase prior to daily feeding. Diet samples were collected on d 14, 32, 54, 67, and 78.

All calves were vaccinated with a modified live vaccine for respiratory diseases (Bovi-Shield® Gold 5; Pfizer Animal Health, NY, NY; EW = Sept. 29, 2006; NW = Nov. 16, 2006), clostridial diseases, and Mannheimia hemolytica bacterin-toxid (One Shot Ultra™ 7, Pfizer Animal Health, NY, NY). Calves were also poured with an anthelmintic (Dectomax Pour On, Pfizer Animal Health, NY, NY) at time of vaccination for internal and external parasite control. Calves were implanted with a Ralgro® implant (36 mg zeranol; Schering-Plough Animal Health Corporation, Kenilworth, NJ) at the start of the background period. During the course of the study, calves had intermittent nasal discharges due to seasonally variable weather. Calves were revaccinated on d 53 (EW calves) and d 69 (NW calves) for respiratory diseases and Hemophilus somnus using a modified live vaccine (Express™ 5-HS, Boehringer-Ingelheim Vetmedica, Inc, St. Joseph, MO).

On January 16, 2007, the backgrounded calves were sent to the NDSU Carrington Research Extension Center (NDSU-CREC) in Carrington, ND for finishing. Calves were commingled into one finishing pen and fed a 14:86 forage:concentrate diet (14.7% CP; 1.39 Mcal $NE_g$/kg; finishing diet; Table 1) for 112 d. Calves were revaccinated for respiratory (Bovi-Shield® Gold 5; Pfizer Animal Health, NY, NY) and clostridial (Ultrabac® 7; Pfizer Animal Health, NY, NY) diseases at arrival at the finishing yard. Additionally, calves were poured with an anthelmintic (Dectomax Pour On; Pfizer Animal Health, NY, NY) for internal and external parasite control at the time of vaccination. Calves (EW and NW) were not implanted during the finishing period. Calves were harvested when back fat thickness was visually estimated at 1.27 cm. On March 26, 2007 and March 27, 2007, five calves were sent each day to Barton Meats, Carrington, ND for slaughter. These calves were fed for 70 and 71 d before harvest. The remaining calves were slaughtered at Tyson Foods, Dakota City, IA on May 7, 2007. Remaining calves were on feed for 112 d. Finishing end weights were taken on all calves prior to shipping for harvest: weights were unshrunk weights (feed was present in the bunk at time of weighing). Individual carcass data of hot carcass weight (**HCW**), backfat thickness (**BF**), longissimus muscle area (**LMA**), percentage kidney, pelvic, and heart fat (**KPH**), marbling score, and yield grades (**YG**) were collected at the two commercial slaughter facilities.

***Statistical Analysis*.** All data for the background period and carcass collection were analyzed as a randomized complete design using generalized least squares (PROC MIXED, SAS Institute, Cary, NC), with pen as the experimental unit. Means were separated using the LSD procedure of SAS ($\alpha = 0.05$) when the F-test was $P < 0.05$. Performance data from the finishing period was not analyzed statistically due to the calves being commingled into one pen during the feeding period.

## Results and Discussion

Early wean calves were lighter at the start of the background period than NW calves (217 ± 0.64 kg vs. 269± 0.64 kg, respectively; $P < 0.001$). When NW calves were weaned, EW calves (242 ± 2.95 kg) were 10% lighter BW than NW calves (269 ± 2.95 kg; $P = 0.0002$). However, at the end of the background period, EW calves were 6.0% heavier than the NW calves. Early weaned calves gained 136 kg while NW calves gained only 65 kg ($P < 0.004$; Table 2). Calf weight gain was directly influenced by the number of days on feed. Early weaned calves spent 49 d more on higher energy rations (based on weaning date) as compared to the NW calves (EW= 91 d; NW = 42 d, respectively). Average daily gain was not different between TRT (1.47 vs. 1.56 kg/d, for EW and NW calves respectively; $P = 0.24$). Additionally, weaning date did not affect DMI ($P = 0.71$; Table 2) or G:F ($P = 0.16$); however, DMI as a percent of BW tended to be higher for EW calves than NW calves ($P < 0.10$; Table 2). Early wean calves had greater feed costs per head ($148.27 compared to $65.11 for NW calves; $P < 0.0001$; Table 2) due to a longer time spent in the feedlot during the background period; however,

feed cost of gain and total cost of gain did not differ between TRT ($P = 0.11$; Table 2).

During the finishing period, calves were fed an ionophore (monensin; Table 1) daily. Finishing data is reported as observations for the commingled pen. During the finishing phase, calves averaged 1.33 kg ADG, consume 9.59 kg/d DMI, and had a G:F of 0.14.

Calf age at slaughter was similar among TRT ($P = 0.86$; Table 2) and averaged 360 ± 7 d. The ten calves that were slaughtered early (d 70 and d 71) at Barton Meats in Carrington, ND, were smaller framed animals as compared to the other calves in the group. As a result, these calves were ready for market at much lighter weights as compared to the rest of the calves in this study. Calf weaning date did not negatively influence carcass characteristics for EW and NW calves: calves had similar live weights at the end of the finish period ($P = 0.24$) and HCW were not different between TRT (P = 0.23; Table 2). Additionally, dressing percentage, BF, LMA, and KPH were similar among TRT ($P \geq 0.27$; Table 2). Marbling scores for the EW calves averaged 483 and 465 for the NW calves ($P = 0.43$; Table 2). These carcass results are similar to those reported by Myers et al. (1999b) and Schoonmaker et al. (2001). The EW calves' quality grades ranged from low to high choice, with two EW calves achieving prime and one select; NW calves' quality grades ranged from select to high choice, with seven NW calves scoring select and one prime. Myers et al. (1999a) reported that 40% more early-weaned steers graded average choice or higher at harvest than did their normal weaned contemporaries. USDA YG (adjusted for HCW, BF, LMA, and KPH) were not different among TRT (P = 0.65).

## Implications

Early weaned calves were predictably younger and lighter at weaning; however, EW calves were heavier at the conclusion of the background period as compared to NW calves. Weaning date did not affect calf ADG, DMI, or G:F during backgrounding. Additionally, weaning date showed no effect on calf carcass characteristics in this study. Early weaning is a viable option for producers who feed their calves for 91 d post weaning. Additional research is warranted with larger numbers of calves over multiple years.

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**Table 1**. Calf diets fed during the grow-finish period

| | Dry Matter | |
|---|---|---|
| Item | Growing | Finishing |
| Ingredient | | |
| Shelled corn, % | 43.43 | 52.33 |
| Barley silage, % | 29.67 | --- |
| Oat hay, chopped, % | 12.87 | --- |
| Protein supplement[1], % | 6.64 | --- |
| 44% Soybean meal, % | 2.50 | --- |
| Decoquinate crumbles, % | 1.82 | --- |
| Aureomycin crumbles[2], % | 2.65 | --- |
| Calcium carbonate, % | 0.42 | 0.56 |
| Field peas, dry rolled, % | | 14.17 |
| Wet distillers grains, % | | 9.65 |
| Corn silage, % | | 7.95 |
| Naked oats, % | | 7.61 |
| Straw, chopped, % | | 6.32 |
| Monensin supplement[3], % | | 1.41 |
| Nutrient Density | | |
| Crude protein, % | 13.00 | 14.68 |
| Net energy for gain, Mcal/kg[4] | 1.19 | 1.39 |

[1] 27% Commercial supplement (as fed): 27% CP, Ca min 2.0% , P min 0.7%, K min 0.7%, Vitamin A min 59.4 KIU/kg, Vitamin $D_3$ min 3.74 KIU/kg, Vitamin E min 0.22 KIU/kg, and Monensin 495 mg/kg.

[2] Calves fed Aureomycin crumbles at 22 mg/kg BW for the first 8 d in feedlot and when the calves had nasal discharges on d 44-46, d 64-66, and d 90-91.

[3] Monensin supplement (as fed): 13.43% CP, Ca min 5.0%, P min 0.2%, K min 2.0%, and Monensin 1980 mg/kg.

[4] Calculated analysis.

**Table 2.** The influence of weaning date on backgrounding calf performance and carcass characteristics

| Item | EW [1] | NW [1] | SEM [2] | *P*-value [3] |
|---|---|---|---|---|
| Backgrounding calf performance | | | | |
| Initial Wt, kg | 217 | 269 | 0.64 | < 0.0001 |
| Final Wt, kg | 355 | 335 | 3.66 | 0.004 |
| Days on Feed, d | 91 | 42 | | |
| Weight gain, kg | 136 | 65 | 2.44 | < 0.0001 |
| ADG, kg | 1.47 | 1.56 | 0.05 | 0.24 |
| DMI, kg | 8.05 | 7.95 | 0.19 | 0.71 |
| DMI, % BW | 2.82 | 2.65 | 0.07 | 0.099 |
| G:F | 0.08 | 0.09 | 0.01 | 0.16 |
| Feed costs, $/hd | 148.27 | 65.11 | 3.03 | < 0.0001 |
| Veterinary medicine costs, $/hd | 15.37 | 14.50 | 3.50 | 0.86 |
| Feed cost of gain, $/kg | 1.14 | 1.03 | 0.04 | 0.11 |
| Total cost of gain, $/kg | 1.25 | 1.28 | 0.09 | 0.76 |
| Morbidity, % | 12.50 | 33.33 | 0.08 | 0.096 |
| Mortality, % | 4.17 | 0 | 0.03 | 0.35 |
| Carcass characteristics | | | | |
| Age at slaughter, d | 361 | 359 | 6.97 | 0.86 |
| Days on Feed, d | 225 | 168 | | |
| Live weight, kg | 489 | 474 | 8.45 | 0.24 |
| Hot carcass weight, kg | 295 | 284 | 6.0 | 0.23 |
| Dressing percent[4], % | 63.5 | 63.2 | 0.31 | 0.44 |
| Backfat thickness, cm | 0.22 | 0.21 | 0.02 | 0.68 |
| Longissimus muscle area, $cm^2$ | 76.13 | 71.49 | 2.77 | 0.27 |
| Kidney, pelvic, and heart fat, % | 2.41 | 2.50 | 0.53 | 0.29 |
| Marbling score[5] | 483 | 465 | 14.8 | 0.43 |
| USDA Yield Grade[6] | 3.04 | 3.19 | 0.22 | 0.65 |

[1] Early wean (EW) and Normal Wean (NW).
[2] Standard Error or Mean; n=6.
[3] *P*-value for separation of treatment means.
[4] Dressing percentage calculation: hot carcass weight divided by shrunk weight (adjusted for 5% shrink).
[5] Marbling score conversion to USDA Quality Grade: 300-399 = Select; 400-449 = Low Choice; 450-499 = Average Choice; 500-549 = High Choice; $600^+$ = Prime.
[6] Yield grade calculations: Preliminary Yield Grade minus carcass weight adjustment minus longissimus muscle area adjustment minus kidney, pelvic, and heart fat adjustment.

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# USING A YEAST PREPARATION AND FIBROLYTIC ENZYME AS REPLACEMENTS FOR GROWTH HORMONE AND ANTIBIOTIC IN NATURAL BEEF PRODUCTION

D.G. Landblom[1], C.J. Wachenheim[2], and T.A. Petry[3]

[1]North Dakota State University - Dickinson Research Extension Center, Dickinson, North Dakota 58601
[2]North Dakota State University - Agribusiness and Applied Economics Department, Fargo, North Dakota 58105
[3]North Dakota State University – Cooperative Extension Service, Fargo, North Dakota 58105

**Abstract**: Spring-born x-bred steers (n=80; 279.6 kg) were assigned to an 84d field pea-co-product growing study to evaluate replacing growth hormone and ionophore with phosphorylated mannan oligosaccharide (MOS) and fibrolytic enzymes (FIB). In a complete randomized design, a control (C) rearing method (Revelor-IS implant + Rumensin) was compared to three natural (N) replacement rearing methods: 1) MOS, 2) FIB, and 3) MOS + FIB. The objectives were to identify backgrounding performance, efficiency, and economics and to document subsequent carryover effect in a commercial feedyard on finishing performance, carcass closeout, and economics. Steers were double vaccinated and the C group was implanted with trenbelone acetate. The C steer diet included monensin sodium at the rate of 30g/T and MOS and FIB were fed at the rate of 10mg/h/d. Steers were backgrounded in North Dakota and finished in Kansas (Decatur County Feedyard); harvest end point was determined using MicroBeef Technologies' ECM system. Treatment differences were determined using SAS PROC MIXED. Backgrounded C steers gained an average 0.313kg/h/d faster ($P < 0.01$) than steers receiving yeast and enzyme; an 18.9% improvement. Treatment differences for ADFI were similar ($P > 0.10$). The C group tended to be more efficient ($P = 0.18$). Backgrounding ending weight was greater for the C steers, i.e. 423, 391, 393, and 389 kg for the C, MOS, FIB and MOS+FIB, respectively ($P < 0.01$). Feed cost/kg gain was also significantly lower for the C group ($P <0.01$). During finishing, the backgrounding weight advantage of the C steers carried over into finishing. Control steers were heavier at final harvest ($P < 0.01$) and were harvested 4.9 days earlier than the N backgrounded steers. Hot carcass weight of the C steers was also heavier ($P < 0.01$); however, no other carcass measurements differed ($P > 0.10$). Closeout margins favored the C steers. Net returns were $54.22, -$33.62, -$20.65, and -$48.69 for the C, MOS, FIB, and MOS+FIB, respectively. Pea-co-product yield was acceptable. Natural programs require substantial premiums to offset reduced performance.

**Keywords:** Natural Beef, Yeast Extract, Enzyme

## Introduction

The cattle feeding industry has experienced significant growth in "natural beef" as cattle producers respond to increasing consumer concerns over the use of growth promoting hormones and antibiotics in cattle feeding. Alternatives to antibiotics and growth hormones have the potential to be replaced with phosphorylated mannan oligosaccharides and fibrolytic enzymes that in separate research investigations have been shown to reduce stress, enhance immune response, inhibit intestinal binding, enhance ruminal degradation of fiber, and increase feed intake, average daily gain, and feed efficiency (Anderson and Schoonmaker, 2004; Spring et al., 2000; Newman, 1994; Grieshop, 2002. Cellulase enzymes have been shown to effect digestive function, energy intake, and growth performance in cattle (Howes et al., 1998; Lewis et al., 1999; Zinn and Salinas, 1999; Zinn and Ware, 2002; Ware et al., 2002; Johnson and Shivas, 1999).

The research objective is to determine the effectiveness for using mannan oligosaccharide and fibrolytic enzymes (cellulase and xylanase) to replace hormone implant and ionophore during backgrounding and to document the subsequent effect on finishing performance.

## Materials and Methods

Eighty spring-born crossbred steers averaging 280 kg were weaned the first week of November and fed in an 84d receiving-backgrounding study using a complete randomized design with four treatments and four pen replicates per treatment. The investigation was conducted using sixteen 32' X 112' pens at the Dickinson Research Extension Center's feedlot located near Manning, North Dakota. Each feedlot pen was equipped with continuous steel fence, anti-siphoning frost-free water fountains, slotted sheet metal windbreak, and a tree windbreak oriented northwest of the feedlot.

*Treatments*-
1. Control - Revelor-IS® + Rumensin®
2. Natural - Fibrolytic Enzyme (Fibrozyme®10 gm/head/day; no implant or ionophore)
3. Natural - Mannan Oligosaccharide (Bio-MOS®10 gm/head/day; no implant or ionophore)
4. Natural – Bio-MOS® + Fibrozyme® (10 gm/head/day; no implant or ionophore)

Mannan oligosaccharide and fibrolytic enzyme preparations were blended with cracked corn, shredded beet pulp, corn oil, and molasses (Table 1) as a carrier and top-dressed over chopped hay at the rate of 454 gm per head per day to provide 10 grams per head per day of each additive. The field pea-co-product receiving-backgrounding feed was prepared as a pelleted complete feed (Table 2) that was top-dressed over medium quality alfalfa-bromegrass hay (CP - 9.1%; ADF – 35.0%; NDF – 59.4%; TDN – 57.4; NEg Kcal/lb – 0.31). After backgrounding, the steers were transferred to Decatur County Feedyard, Oberlin, Kansas for finishing and final harvest. End point was determined using MicroBeef Technologies' ECM system.

Receiving, backgrounding, and finishing data were analyzed using pen as the experimental unit for both growth and carcass closeout data. The MIXED procedure of SAS was used to separate means using a non-repeated measures procedure.

## Results and Discussion

Eighty-four day backgrounding performance, feed efficiency, and partial feeding economics are shown in Table 3. Control steers that were implanted with Revelor-IS® and fed diets containing Rumensin® medication gained an average 0.31 kg faster ($P < 0.01$) than steers fed a microbial additive and enzyme, an18.9% improvement in average daily gain. Average daily feed intake did not differ between treatments ($P = 0.85$). Feed per pound of gain tended to be improved in the control group; however, the advantage measured did not differ ($P = 0.198$). Feed cost per kg of gain amounting to $0.836, $1.035, $1.00, and $1.071 for the control, Bio-MOS®, Fibrozyme®, and Bio-MOS+Fibrozyme®, respectively, was significantly lower for the control group.

Had the natural reared steers in this study been marketed at the end of the backgrounding phase, natural steers would have returned -$13.55, -$9.56, and -$21.46 less for the Bio-MOS®, Fibrozyme®, and Bio-MOS+Fibrozyme®, respectively.

Within the parameters of the project, field peas and co-product ingredients fed in both the conventional and natural programs yielded excellent steer performance.

The weight advantage observed among conventionally raised steers, during the backgrounding phase, carried over to the final harvest weight. Control steers gained faster ($P < 0.05$), were heavier ($P< 0.01$), consumed more feed ($P<0.01$), and hot carcass weight was heavier ($P <0.01$) than steers backgrounded with natural additives. Except for hot carcass weight, all other carcass measurements did not differ, i.e. fat depth ($P = 0.535$), REA ($P = 0.532$), yield grade ($P = 0.787$), quality grade ($P = 2.14$), and percent of carcasses grading Choice or higher ($P = 0.807$).

The total carcass value was greater for steers that were reared conventionally ($P <0.01$). Marketing analysis comparing conventional and natural production resulted in a profit of $54.22 per head for control steers whereas net losses were realized for all carcasses from naturally reared steers. Compared to conventional Revelor-IS® implanted steers fed Rumensin® medication, losses per head among naturally reared steers were -$33.62, -$20.65, and -$48.69 per carcass for Bio-MOS®, Fibrozyme®, and Bio-MOS+Fibrozyme®, respectively.

## Implication

Producers growing cattle for natural markets need to be prepared to feed cattle longer to attain similar market weight, and will need premiums ranging from $87.00 to $102.00 per head from natural markets to offset lost revenue available using conventional rearing methods.

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Table 1. Conventional and natural topdressed supplement ingredient composition (As Fed).

| | **Conventional** | **Bio-MOS** | **Fibrozyme** | **Fibrozyme +Bio-MOS** |
|---|---|---|---|---|
| Cracked Corn, % | 46.0 | 44.9 | 44.9 | 43.8 |
| Shredded Beef Pulp, % | 46.0 | 44.9 | 44.9 | 43.8 |
| Corn Oil, % | 3.0 | 3.0 | 3.0 | 3.0 |
| Molasses, % | 5.0 | 5.0 | 5.0 | 5.0 |
| Bio-MOS, % | --- | 2.2 | --- | 2.2 |
| Fibrozyme, % | --- | --- | 2.2 | 2.2 |

Table 2. Pelleted conventional and natural supplement ingredient composition (As Fed).

| | **Conventional** | **Natural** |
|---|---|---|
| Soybean Hull, % | 30.753 | 30.80 |
| Field Peas, % | 20.00 | 20.00 |
| Corn, % | 15.00 | 15.00 |
| Barley Malt Sprouts, % | 10.00 | 10.00 |
| Wheat Midds, % | 10.00 | 10.00 |
| Distillers Dried Grain w/ Solubles, % | 8.00 | 8.00 |
| Decoquinate-6%, % | 0.54 | --- |
| Monensin (80 gm/lb), % | 0.40 | --- |
| **Analysis:** CP, % | 15.10 | 15.1 |
| TDN, % | 70.20 | 70.25 |
| Fat, % | 2.65 | 2.65 |
| Fiber, % | 15.57 | 15.58 |
| Acid Detergent Fiber, % | 18.03 | 18.05 |
| NEm, Mcal/lb. | 0.785 | 0.785 |

[a]Beet Molasses, 5.0%; Calcium Carbonate, 0.50%; Salt, 0.50%; Dicalcium Phosphate 21%, 0.10%; Feedlot Trace Mineral Premix, 0.075%; Feedlot Vitamin Premix, 0.025%.

Table 3. Eighty-four day natural versus conventional backgrounding.

| | *Control – Medicated* | *Bio-MOS®* | *Fibrozyme®* | *Fibrozyme + Bio-MOS®* | *SEM* | **P-Value** |
|---|---|---|---|---|---|---|
| ***Growth*:** | | | | | | |
| No. Steers | 20 | 20 | 20 | 20 | | |
| Days on Fed | 84 | 84 | 84 | 84 | | |
| Start Wt, kg | 284.2 | 278.8 | 277.8 | 278.0 | 3.16 | 0.432 |
| Final 84d Wt., kg. | 423.4[w] | 390.8[x] | 393.1[x] | 389.3[x] | 4.63 | <0.01 |
| Gain, kg | 139.1[w] | 112.0[x] | 115.3.[x] | 111.3[x] | 3.44 | <0.01 |
| ADG, kg | 1.66[w] | 1.33[x] | 1.38[x] | 1.33[x] | 0.041 | <0.01 |
| Feed:Gain, kg | 6.06 | 7.12 | 6.86 | 7.11 | 0.371 | 0.198 |
| Feed Cost/Day, $ | 1.388 | 1.377 | 1.380 | 1.425 | 0.0275 | 0.603 |
| Feed Cost/kg. Gain, $ | 0.8361[w] | 1.035[x] | 1.00[x] | 1.071[x] | 0.0088 | <0.01 |
| Net/Hd, $ | 34.58 | 18.03 | 25.02 | 13.12 | | |
| **Difference Versus Control, $** | --- | -13.55 | -9.56 | -21.46 | | |

Table 4 Natural versus conventional finishing growth, feed intake, and efficiency.

| | *Conventional* | | | *Natural* | | |
|---|---|---|---|---|---|---|
| | Control – Medicated | Bio-MOS® | Fibrozyme® | Fibrozyme + Bio-MOS® | SEM | P-Value |
| Days on Feed | 116.3 | 122.2 | 120.1 | 121.2 | | |
| Start Wt., kg | 410.3[w] | 381.3[x] | 383.6[x] | 376.3[x] | 18.50 | <0.01 |
| Harvest Wt., kg | 615.1[w] | 576.0[x] | 583.7[x] | 572.4[x] | 15.67 | <0.01 |
| Gain, kg | 204.8 | 194.7 | 200.1 | 196.1 | 5.80 | .308 |
| ADG, kg | 1.76 | 1.59 | 1.67 | 1.62 | 0.399 | .022 |
| Fd/Hd/Day, kg | 9.95[w] | 9.64[x] | 9.60[x] | 9.55[x] | 0.170 | <0.01 |
| Feed:Gain, kg | 5.65 | 6.06 | 5.75 | 5.90 | 0.122 | .231 |

Table 5 Natural versus conventional carcass closeout values.

| | *Conventional* | | | *Natural* | | |
|---|---|---|---|---|---|---|
| | Control – Medicated | Bio-MOS® | Fibrozyme® | Fibrozyme + Bio-MOS® | SEM | P-Value |
| Hot Carcass Wt., kg | 390.4[w] | 361.8[x] | 366.8[x] | 362.2[x] | 9.83 | <0.01 |
| Fat Depth, cm | 1.32 | 1.32 | 1.32 | 1.27 | 1.04 | .535 |
| Ribeye Area, $cm^2$ | 84.6 | 80.0 | 79.9 | 81.5 | 2.61 | .532 |
| Yield Grade | 2.95 | 2.80 | 3.05 | 2.80 | 0.2508 | .787 |
| Quality Grade | 4.35 | 3.4 | 4.8 | 5.05 | 0.8091 | .214 |
| Percent Choice, % | 75.0 | 70.0 | 65.0 | 58.8 | 12.26 | .807 |

Table 6 Natural versus conventional finishing economics.

| | *Conventional* | | | *Natural* | | |
|---|---|---|---|---|---|---|
| | Control – Medicated | Bio-MOS® | Fibrozyme® | Fibrozyme + Bio-MOS® | SEM | P-Value |
| Total Carcass Value, $ | 1243.55[w] | 1149.52[x] | 1153.66[x] | 1130.98[x] | 3.58 | <0.01 |
| Feeder Calf Cost, $ | 680.77 | 667.73 | 665.55 | 665.55 | | |
| Bkg. Feed. and Yardage, $ | 141.85 | 140.89 | 141.13 | 144.91 | | |
| Feedlot Cost/Head, $ | 325.71 | 333.52 | 326.63 | 328.21 | 6.73 | 0.784 |
| Transportation, $[a] | 41.00 | 41.00 | 41.00 | 41.00 | | |
| Net Return (Loss), $ | 54.22 | -33.62 | -20.65 | -48.69 | | |

[a]Transportation from Dickinson, North Dakota to Oberlin, Kansas

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# COW NUTRITION IMPACTS FEEDLOT PULL RATE

**J. T. Mulliniks[1], S. H. Cox[1], S. L. Ivey[1], C. P. Mathis[1], J. E. Sawyer[2], M. K. Petersen[1]**

[1]New Mexico State University, Las Cruces, NM
[2]Texas A&M University, College Station, TX

**ABSTRACT:** Nutritional stress in gestating cows grazing winter range has been implicated in feedlot calf morbidity and profitability. A 3-yr study was conducted at Corona Range and Livestock Research Center, NM to evaluate prenatal nutrition on calf (n = 82) performance after weaning. Cows were supplemented with 1) 36% CP cottonseed base supplement (**CON**) fed 3x/week at 2.3kg/feeding, 2) NMSU small self fed supplement comprised of 25% feather meal, 25% blood meal, and 50% mineral package (40% CP, **SMP**), or 3) cows fed CON according to perceived environment stress (**VAR;** negative control) by manager. Supplementation started November and ended 2 wks prior to calving. Management after calving was similar for all cows. In 3 yrs, average supplement consumption was 0.63, 0.23, and 0.04 kg/d for CON, SMP, and VAR, respectively. Cow nutrition did not effect calf BW at weaning ($P$ = 0.57; 252, 247, 252 ± 5 kg), end of preconditioning ($P$ = 0.46; 281, 275, 282 ± 5 kg), or feedlot ($P$ = 0.31; 508, 518, 532 ± 10 kg) for CON, SMP, and VAR, respectively. Hot carcass weights were similar among treatments ($P$ = 0.32; 320, 326, 336 ± 7 kg for CON, SMP, and VAR, respectively). Calves from dams fed CON and VAR required 50% more medical treatments (pulls) than SMP calves ($P$ = 0.10; 12, 6, 12 treatments for CON, SMP, and VAR, respectively). Feedlot medicinal costs were \$20.44, \$13.79, \$34.86 ± \$9.18/hd for CON, SMP, and VAR, respectively ($P$ = 0.21). Feedlot feed cost of gain was similar among treatments ($P$ = 0.62; \$23.92, \$22.03, \$22.77 ± \$1.10/100 kg for CON, SMP, and VAR, respectively). These results imply that the range of prenatal nutrition in this study had no affect on calf body weight at weaning, during preconditioning, or in the feedlot. However, calves from cows fed SMP had a tendency toward lower medicine costs and improved health status due to fewer pulls in the finishing phase.

Key words: beef calves, feedlot, prenatal nutrition

## INTRODUCTION

The effect of prepartum nutrition on reproduction have been widely reported in the literature (Corah, et al., 1975; Bellows et al., 1982; Wiley et al., 1991). However, the effect of prepartum nutrition on calf-well being from weaning through the feedlot is not well known. Limiting nutrients during gestation can decrease cow body weight, body condition, and affect calf growth (Bellows et al., 1982; Richards et al., 1986). Consequently, calves may experience reduced passive immunity when precalving nutrient availability is low (Blecha et al., 1981). Passive immunity transfer at calving has been speculated to have long term affects on calf profitability from weaning through the finishing phase. Wittum and Perino (1995) reported that calves classified as having lower plasma protein (< 4.8 g/dL) concentration at 24 hr postpartum had a greater risk of morbidity and respiratory tract morbidity while in the feedlot than calves with higher plasma protein at 24 hours postpartum. Feedlot morbidity may cost even more than mortality when expenses associated with medical treatments are combined with reduced income from premature sale because of chronic cattle and reduced performance during and after illness (Smith, 1998). However, nutrient restriction during pregnancy may have to be extreme to affect calf performance in the feedlot. NRC (2000) reports that calf birth weight is not affected when BCS at calving is between 3.5 and 7. Therefore, the fetus has a natural protection against prepartum protein under nutrition of the dam by mobilization of maternal nutrient reserves (Martin et al., 1997). Stalker et al (2006) found that feeding protein supplements to cows during gestation has no benefit in the feedlot phase for steer calves. The objective of this study was to evaluate the effects of differing approaches to prepartum nutrition on calf well-being from weaning through the feedlot phase. A secondary objective was to evaluate the interaction of year with cow supplementation on calf performance in the backgrounding and finishing phases.

## MATERIALS AND METHODS

This study was conducted for 3 consecutive years at New Mexico State University's Corona Range and Livestock Research Center, Corona, NM. The data was compiled from three independent studies: 1) Sawyer et al., 2005, 2) Mathis et al., 2007, and 3) extension data from New Mexico State's Ranch to Rail. The ranch's average elevation is 1,900 m. Annual precipitation averages 400 mm, with approximately 70% of annual precipitation occurring from May to October (Sawyer et al., 2005). All animal handling and experimental procedures were in accordance with guidelines set by the New Mexico State University Institutional Animal Care and Use Committee.

### *Prepartum Supplementation*

A complete description of the methodology used for cow supplementation was described in Sawyer et al. (2005). Briefly, cows (n = 150) were 2.5 to 8.5 years of age and were primarily Angus with some Hereford influence. Each year, cows were stratified by breed and weight at weaning, and then randomly assigned to one of six replications. Each replication was randomly assigned to a pasture and pasture was randomly assigned a treatment.

Cows were supplemented with 1) 36% CP cottonseed meal basal supplement (**CON**) fed 3x/week at 2.3kg/feeding, 2) NMSU small self fed supplement comprised of 25% feather meal, 25% blood meal, and 50% mineral package (40% CP, **SMP**), or 3) cows fed CON according to perceived environment stress (**VAR;** negative control) by manager. Strategically supplements were fed for 27 d (yr 1), 62 d (yr 2), and 93 d (yr 3). Prepartum supplementation ended 2 wks prior to the expected initiation of parturition. In all years, supplementation ended in the first week of February. Management of cows after parturition was similar in all years.

The hand-fed supplement fed to CON and VAR was a 36% CP range cube. Supplements were composed of 57% cottonseed meal, 21% wheat midds, 10% soybean meal, 9% molasses, 1.2 % urea and fortified with trace minerals and vitamin A. The SMP supplement (NMSU"S small supplement package) was formulated to contain 40% CP, and was composed of 25% feather meal, 25% blood meal, 27% minerals, 19% salt and 4% distillers dried grains. Feeding rate, duration of supplementation, and total consumption for each supplementation by year is shown in Table 1.

**Table 1**[1]. Feeding rate, duration of the supplementation period, and total amount of supplement fed to cows receiving different supplemental feeds during three years.

| Item | CON | SMP | VAR |
|---|---|---|---|
| *Year 1* | | | |
| Rate, g/d | 953 | 281 | 454 |
| Duration, d | 27 | 27 | 9.5 |
| Total fed, kg | 25.7 | 7.6 | 4.3 |
| *Year 2* | | | |
| Rate, g/d | 757 | 172 | 454 |
| Duration, d | 62 | 62 | 8 |
| Total fed, kg | 46.9 | 10.7 | 3.6 |
| *Year 3* | | | |
| Rate, g/d | 454 | 249 | 0 |
| Duration, d | 93 | 93 | 0 |
| Total fed, kg | 42.2 | 23.2 | 0 |

[1]Data from Sawyer et al. (2005)

Cows were weighed and body condition scores (BCS) were assigned on a 1-9 scale (1 = emaciated, 9 = obese) at weaning (October) of each year, at the initiation of supplementation period (January, December, and November for yrs 1, 2, and 3, respectively), and at end of supplementation (February). Body weight responses and BCS of gestating cows averaged across 3 yrs to the three supplementation strategies are shown in Table 2.

*Backgrounding Phase*

Detailed methodology for yr1 and yr 2 was previously described by Mathis et al. (2007). All calves were preconditioned, conforming to VAC-45 the management guidelines (Anonymous. 2005). Vaccinations occurred at branding and 16 to 21 d prior to weaning. However, calves in yr 3 received a Pasturella vaccination post weaning, whereas, calves in yr 1 and yr 2 did not. At weaning steers were weighed and randomly assigned to one of two treatments: 1) high-input feeding (**HIF**) or 2) low-input supplementation (**LIS**). The HIF calves received alfalfa hay in a drylot in yr 1 and yr 2 or grazed native range in yr 3 and were fed 15.8% CP corn/wheat midds-based backgrounding pellet (1.83 Mcal/kg ($NE_m$) and 1.10 Mcal/kg ($NE_g$)) offered at 3.00 – 3.30% of BW. The LIS calves were fed a 32% CP range cube (composition similar to 36% CP cubes fed to cows) 3 × weekly at 0.20% of BW and grazed native range pastures. All calves were weighed near the mid point (d 19 or 21) and at the end of the backgrounding phase (d 42 to 45).

Each year, weaning and final backgrounding prices were individually applied to each calf based on prices in the New Mexico Weekly Weighted Average Feeder Cattle Report (USDA CB LS 795) for the week of weaning and at the end of preconditioning with no premiums for backgrounding. In each year purchased feed, hay cost, grazing fee, and labor costs were applied to each calf.

*Finishing Phase*

A complete description of methods can be found in Mathis et al. (2007) for yr 1 and yr 2. Nevertheless, steers were managed similar in yr 3. Steers were entered into the New Mexico Ranch to Rail Program and were fed at a commercial feedlot (Double A Feeders, Clayton, NM). Initial BW and price of steers for the finishing phase were calculated from the final BW and price of steers from the backgrounding phase.

Steers were received at the feedlot in mid-November each year, and were managed according to the procedures in place at the feedlot. Feedlot staff diagnosed morbidity by subjective visual appraisal. At receiving, steers were administered a growth-promoting implant and preventive pharmaceuticals based on judgment of feedlot management.

Steers were processed for a secondary application of growth hormone at 74 – 94 d. At this time, an interim weight was recorded and steers were individually assigned to a marketing group using ultrasound technology and computer software form the Cattle Performance Enhancement Co. (CPEC, Oakley, KS). Steers were harvested between March and early July in a commercial facility (National Packing Co., Liberal, KS). Hot carcass weight (HCW) was recorded at slaughter and carcass traits were evaluated by an independent data collection service (Cattle Trail LLC, Johnson, KS) following chilling. Steers were sold through the National Beef Grid and premiums and discounts were applied using HCW, USDA yield and quality grade.

*Statistical Analysis*

Data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) with calf as the experimental unit using the Kenward-Roger degrees of freedom method. The model included fixed effects of dam's supplement, year, preconditioning treatment, and their interactions. Feedlot days on feed, weaning weight, and end of preconditioning weight were used as covariant when appropriate. Categorical data, which included pull rate and death loss, utilized Chi-square in the FREQ procedure of SAS. Significance was determined at $P \leq 0.10$.

**Table 2**[1]. Body weight and body condition responses of gestating cows to different supplementation strategies.

| Item | CON | SMP | VAR | SE[a] | P |
|---|---|---|---|---|---|
| *Body Weight Responses* | | | | | |
| Wean BW, kg[b] | 488 | 492 | 490 | 17 | 0.98 |
| Initial BW, kg[c] | 522 | 521 | 534 | 14 | 0.78 |
| Final BW, kg | 522 | 523 | 521 | 14 | 0.99 |
| Change[d], kg | -0.2[e] | 1.8[e] | -12.6[f] | 3.9 | 0.06 |
| Change, % | 0.1[e] | 0.5[e] | -2.2[f] | 0.8 | 0.09 |
| *Body Condition Responses* | | | | | |
| Initial BCS | 5.0 | 4.9 | 5.0 | 0.1 | 0.49 |
| Final BCS | 4.9[e] | 4.9[e] | 4.6[f] | 0.1 | 0.12 |
| BCS change | -0.1[e] | -0.1[e] | -0.4[f] | 0.1 | 0.10 |
| Total Feed Consumed, kg | 38.2 | 13.7 | 2.6 | -- | -- |

[1]Data from Sawyer et al. (2005)
[a]n = 6
[b]Cow weight at weaning in October
[c]Cow weight at initiation of supplementation
[d]Cow weight change (Final – Initial)
[e,f,g] Means differ, P < 0.10

## RESULTS AND DISCUSSION

*Backgrounding Phase*

Prepartum treatment × preconditioning treatment and prepartum treatment × preconditioning treatment × year did not influence calf performance. Weaning and final backgrounding weights were similar ($P \geq 0.46$) for all calves from CON, SMP, and VAR supplemented dams (Table 3). Average daily gains were also similar for steers ($P = 0.87$) among cow prepartum treatments (0.57, 0.60, and 0.59 ± 0.07 kg for CON, SMP, and VAR, respectively).

A prepartum supplementation by year interaction ($P = 0.04$) occurred for ADG in the backgrounding phase. Rank appeared to randomly change among all calves whose dams were fed CON, SMP and VAR during the three years, however, calves from SMP dams had the most consistent ADG over the three years.

Weaning weights decreased (Table 4) over the three years with yr 1 as the heaviest weaning weight ($P < 0.01$; 275, 249, and 228 ± 4 kg for yr 1, yr 2, and yr 3, respectively). However, final backgrounding weights increased across the three years ($P < 0.01$; 265, 280, and 285 ± 3 kg for yr 1, yr 2, and yr 3, respectively).

*Finishing Phase*

Calf initial and final BW was similar ($P \geq 0.31$) among cow prepartum treatments (Table 3). Therefore, ADG in the finishing phase was not different ($P = 0.82$; 1.38, 1.41, and 1.38 ± 0.05 kg for CON, SMP, and VAR, respectively). Stalker et al. (2006) found similar results when beef cows were either supplemented or not supplemented during gestation. Feedlot feed cost of gain was also similar among treatments ($P = 0.62$; $23.92, $22.03, $22.77 ± $1.10/100 kg for CON, SMP, and VAR, respectively). Prepartum supplementation did not affect days on feed ($P = 0.39$), HCW ($P = 0.32$), fat thickness ($P = 0.54$), rib-eye area ($P = 0.27$), or marbling score ($P = 0.70$; Table 4).

Medicinal costs and pull rate in the feedlot can have a substantial affect on feedlot net profit. Therefore, lower medicine costs and fewer treated calves may increase profitability. Medicine cost/hd ($P = 0.16$) and percent death loss ($P = 0.79$) were similar however, calves from SMP dams were treated for sickness half as many times than calves from CON and VAR dams ($P = 0.10$; 12, 6, and 12 pulls for CON, SMP, and VAR, respectively; Table 3). A difference in composition of the prepartum supplements might have contributed to the difference in medicine cost and feedlot pulls. Feedlot net profit ranged from breakeven (SMP) to $33/hd loss (CON) and was similar for all treatments ($P = 0.87$; $-33.93, $-0.38, and $-18.14 ± $50.52/hd for CON, SMP, and VAR, respectively).

Initial BW ($P < 0.01$), final BW ($P = 0.09$), and HCW ($P = 0.09$) were highest in yr 3 compared to yr 1 and yr 2 (Table 4). However, steers from yr 2 were on feed for fewer days ($P = 0.04$) while having the highest fat thickness ($P = 0.03$). Medicine cost/hd and number of steers treated declined over the three years ($P \leq 0.02$). Consequently, with less treated steers and lower medicine costs in yr 3, net profit was the highest in yr 3 and the lowest in yr 1 ($P = 0.04$). However, feed cost of gain did not follow this trend and was the highest in yr 3 and lowest in yr 1 ($P < 0.01$).

Applying the unit feed costs for CON, SMP, and VAR for total cow supplement consumption pooled across years resulted in per cow costs of $10.08, $4.70, or $0.60/cow, respectively (Sawyer et al., 2005). Considering the cost/cow for prepartum supplementation and the calf net profit in the feedlot, feeding SMP during gestation appears to be a promising cost effective method of strategic supplementation while maintaining cow BW, lowering winter feed costs, and decreasing pull rate for calves in the feedlot.

## IMPLICATIONS

Range cow prepartum supplementation may play an important role in calf well-being from birth to the feedlot. However, these results allude to a range of prenatal nutrition that will have no affect on calf development and lifetime weight gain. Environmental stress during gestation and type of weaning preconditioning program might play a bigger role in calf performance than prenatal nutrition. However, this study revealed that calves born from SMP supplemented dams were pulled fewer times and had the tendency to be more profitable implying that there maybe nutrient or ingredient formulations for prepartum supplements that when fed to range cows results in more favorable calf feeding outcomes.

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**Table 3**. Effects of prepartum nutrition on calf performance from weaning through the finishing phase.

| Item | CON | SMP | VAR | SE | P |
|---|---|---|---|---|---|
| *Backgrounding Performance* | | | | | |
| Weaning Wt, kg | 252 | 247 | 252 | 5 | 0.57 |
| Final BW, kg | 281 | 275 | 282 | 2 | 0.46 |
| Total ADG, kg | 0.57 | 0.6 | 0.59 | 0.07 | 0.87 |
| *Feedlot Performance* | | | | | |
| Initial Wt, kg | 266 | 266 | 264 | 2 | 0.58 |
| Final BW, kg | 508 | 518 | 532 | 12 | 0.31 |
| Days on Feed | 172 | 172 | 182 | 6 | 0.39 |
| Total ADG, kg | 1.38 | 1.41 | 1.38 | 0.05 | 0.82 |
| Hot Carcass Wt, kg | 321 | 327 | 336 | 7 | 0.32 |
| Fat Thickness, cm | 1.27 | 1.35 | 1.41 | 0.09 | 0.54 |
| Rib-eye area, $cm^2$ | 30.81 | 32.11 | 31.52 | 0.6 | 0.27 |
| Marbling Score | 492 | 482 | 497 | 14 | 0.70 |
| Pulls, 1 or more | 12 | 6 | 12 | -- | 0.10 |
| % Death Loss | 3.45 (1/29) | 7.14 (2/28) | 4 (1/25) | -- | 0.79 |
| Medicine Cost, $/hd | 20.44 | 13.79 | 34.86 | 9.18 | 0.16 |
| Net Profit, $/hd | -33.93 | -0.38 | -18.14 | 49.04 | 0.87 |
| Feed Cost of Gain, $/100 kg | 23.92 | 22.03 | 22.77 | 1.10 | 0.43 |

**Table 4.** Effect of year on calf performance from weaning through the finishing phase.

| Item | Yr 1 | Yr2 | Yr 3 | SE | P |
|---|---|---|---|---|---|
| *Backgrounding Performance* | | | | | |
| Weaning Wt, kg | 275 | 249 | 228 | 4 | < 0.01 |
| Final BW, kg | 265 | 280 | 285 | 3 | < 0.01 |
| Total ADG, kg | 0.42 | 0.75 | 0.61 | 0.05 | < 0.01 |
| *Feedlot Performance* | | | | | |
| Initial Wt, kg | 253 | 269 | 273 | 2 | < 0.01 |
| Final BW, kg | 529 | 499 | 530 | 13 | 0.09 |
| Days on Feed | 176 | 164 | 185 | 7 | 0.04 |
| Total ADG, kg | 1.39 | 1.33 | 1.46 | 0.05 | 0.17 |
| Hot Carcass Wt, kg | 332 | 314 | 337 | 8 | 0.09 |
| Fat Thickness, cm | 1.47 | 1.61 | 1.40 | 0.09 | 0.03 |
| Rib-eye area, $cm^2$ | 31.21 | 32.09 | 31.16 | 0.6 | 0.38 |
| Marbling Score | 505 | 468 | 497 | 6 | 0.11 |
| Pulls, 1 or more | 13 | 11 | 6 | -- | 0.02 |
| % Death Loss | 4.88 (4/24) | 0 (0/26) | 0 (0/32) | -- | 0.01 |
| Medicine Cost, $/hd | 50.72 | 14.53 | 3.85 | 8.46 | < 0.01 |
| Net Profit, $/hd | -89.17 | -45.22 | 81.93 | 49.04 | 0.04 |
| Feed Cost of Gain, $/100 kg | 20.71 | 22.25 | 25.62 | 1.05 | < 0.01 |

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# FEEDLOT PERFORMANCE OF LAMBS FROM DIFFERENT BACKGROUNDING SYSTEMS

**R. R. Redden[1], R. W. Kott[1], L.M.M. Surber[1], J. A. Boles[1], A. W. Layton[2], and P. G. Hatfield[1]**
[1]Department of Animal and Range Sciences, Montana State University, Bozeman, MT 59717
[2]Montana Department of Livestock, Bozeman, MT 59717

**ABSTRACT:** Backgrounding lambs after weaning may provide producers with alternatives to traditional marketing of lambs directly to feedlots. However, limited data is available on lamb feedlot performance after backgrounding. Our objective was to evaluate lamb feedlot performance after backgrounding treatments. Late April and early May born Hampshire/Suffolk sired lambs (n = 72) weaned on August 23, 2007 from Western white-face range ewes were randomly assigned to 1 of 4 backgrounding treatments. Lamb backgrounding treatments were: ad libitum access to 80% alfalfa: 20% barley pellets (**PELLET**); cool season grass paddock grazing (**GRASS**); remain with ewe flock on fall dormant range (**LATE WEAN**); wean for 96 h and returned to ewe flock (**RANGE**). After 29 d of backgrounding, lambs were assigned to feedlot pens (6 lambs/ pen and 3 pens/backgrounding treatment) and finished on a corn based diet. Lambs weights were taken at approximately 120 d of age, after backgrounding (d 0, beginning of finishing period), at the beginning of the 70% concentration ration (d 19), and at the end of the feedlot period (d 71). Pen intake was measured. Rib eye area (**REA**) and fat depth (**FD**) were measured using ultrasound at each date that lambs were weighed. Lambs backgrounded on PELLET were heavier ($P < 0.10$) than all other treatments after the backgrounding period (d 0) and at the end of the feedlot period (d 71). Lambs backgrounded with PELLET had the greatest intakes and ADG ($P < 0.10$) during the feedlot period. At d 0 and 71, PELLET and GRASS lambs had larger ($P < 0.05$) REA than RANGE and LATE WEAN lambs. Lambs fed PELLET in the backgrounding period had greater ($P < 0.05$) FD than all other lambs at d 0; however no difference ($P > 0.10$) in FD was seen at d 71. In conclusion, lambs backgrounded on PELLETS had higher feedlot ADG than RANGE and LATE WEAN lambs. In addition, GRASS and PELLET lambs deposited larger REA in the feedlot than RANGE and LATE WEAN.

**Keywords:** Backgrounding, Lambs, Feedlot, Ultrasound

## Introduction

The states including CO, MT, SD, UT, and WY raise 34% of the US lambs and approximately 75% of those operations wean in the fall (NASS, 2005). Typically weaned lambs will directly enter a feedlot and be harvested in 60 to 120 d. Backgrounding can serve to extend the period in which feeder lambs are marketed, delay feedlot placement, and produce low cost gains before the finishing period (SID, 2002). Mathis et al. (2007) reported that calves backgrounded in the fall on native New Mexico range had better ADG than calves backgrounded on processed feed in a drylot.

Little data to our knowledge exists on lamb feedlot performance following fall backgrounding treatments. Therefore our objective was to compare feedlot performance, feedlot tissue deposition via ultrasound measurements, and carcass quality of lambs subjected to 1 of 4 backgrounding systems.

## Materials and Methods

*Research Sites, Pastures, and Animals:* Seventy-two lambs were selected at weaning (August 23, 2007) from the 2007 lamb crop. Ewe and wether lambs selected were terminal sire crosses (Suffolk/Hampshire X Western Whiteface) raised at the Red Bluff Research Ranch near Norris, MT. Lambs were assigned to 1 of 4 backgrounding treatments (n = 18) in such a manner that BW and sex were similar among treatments. Treatments were: lambs not removed from the ewe herd until the beginning of the feedlot period (**LATE WEAN**); lambs removed from the ewes for 4 d then returned to graze native range with the ewe herd (**RANGE**); lambs weaned and moved to grass paddocks (**GRASS**); lambs weaned and allowed ad libitum access to 80% alfalfa: 20% barley pellets in a drylot (**PELLET**).

Lambs were born in late April to early May, and then grazed native range at the Red Bluff Research Station until traditional weaning in early September. Red Bluff Research Ranch elevations range from 1,402 to 1,889 m, and annual precipitation ranges from 35.5 to 43.1 cm. Vegetation is a typical foothill bunchgrass type. Bluebunch wheatgrass (*Agropyron spicatum*) and Idaho fescue (*Festuca idahoensis*) are the major grasses. Rubber rabbit brush (*Chrysothamnus nauseosus*), fringed sagewort (*Artemisia frigida*), lupine (*Lupinus spp.*), milkvetch (*Astragalus spp.*) and western yarrow (*Achillea millefolium*) are commonly occurring shrubs and forbs (Harris et al., 1989).

Fort Ellis Research Center near Bozeman, MT has an approximate elevation of 1,500 m, and in 2007 precipitation was approximately 47 cm. Sheep pastures consists of predominantly smooth brome (*Bromus inermis*), crested wheat (*agropyrom cristatum*), and Kentucky blue (*poa pratensis*) grasses. Prior to trial, paddocks A and B (1.3 and 3.5 acres, respectively) were grazed in the spring and early summer. Fall grass growth produced the forage available for the GRASS backgrounded lambs.

*Backgrounding Period.* On September 6, 2007 all

lambs except LATE WEAN were moved from Red Bluff to Fort Ellis. At Fort Ellis PELLET, RANGE, and GRASS lambs were maintained on paddock B for 4 d. Then on September 10, RANGE lambs were returned to the ewe herd at the Red Bluff Research Ranch, PELLET lambs were moved to a drylot pen with self-feeders containing 80% alfalfa: 20% barley pellets, and GRASS lambs were moved to paddock A. The lambs remained on their backgrounding treatments for 29 d.

*Feedlot Period.* On October 9, 2007 all lambs were removed from their respective backgrounding treatment, orally drenched with an anthelmintic (Valbazen; Pfizer Animal Health, Exton, PA), and allowed to graze paddock B to drop parasite load. On October 11 lambs were held off feed and water for 12 h and shrunk lamb body weights were obtained. Lambs were vaccinated against Clostridial perfringens C and D (Bar-Vac CDT**;** Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO). Lambs within backgrounding treatments were randomly assigned to a pen (6 lambs per pen) so that each treatment pen had similar lamb weights. Self-fed troughs allowed ad libitum access to lamb feedlot starting ration. Step-up rations started at 30% concentrate and moved up 10% in concentrate for every 26.67 kg of pen intake (~4.45 kg·lamb$^{-1}$). Finishing lamb rations were held constant at 70% concentrate. Total step-up and finishing pen intakes were recorded.

On October 30, 2007 at the end of the step-up period, lamb unshrunk weights were recorded and lambs were vaccinated against Clostridial perfringens C and D. On November 29 lamb unshrunk weights were recorded. On December 18 lambs were removed from the feedlot pens and weighed. Lambs were then held off feed and water over night and shrunk weights were measured. Percent shrink was averaged on each lamb and a pencil shrink was applied to unshrunk weights taken during the feedlot period.

Lamb health was monitored during the feedlot period. Lambs showing signs of acidosis were drenched with sodium bicarbonate saturated in water. One RANGE lamb died during the step-up period and its data was removed from the study.

*Feed Ration.* Feedlot rations were hand mixtures of whole corn, 80% alfalfa: 20% barley pellets, and a supplement pellet. Supplement pellets were designed to be fed at 0.227 kg·lamb$^{-1}$·day$^{-1}$ and it provided 20% CP, 1.09% Ca, 0.53% P, 1.44% Na, 2.73% Cl, 1.15% K, 0.25% Mg, 190 ppm Mn, 154 ppm Fe, 14 ppm Cu, 210 ppm Zn, 2.4 ppm Se, 0.45 ppm Co, 1.66 ppm I, 88 IU/kg vitamin E, 114 KIU/kg vitamin A, 11 KIU/kg vitamin D, and 264 ppm of lasalocid (Bovatec, Alpharma Animal Health, Bridgewater, NJ). Step-up rations were mixed at a rate of 1.3 kg·lamb$^{-1}$·day$^{-1}$. Lamb finishing ration intakes were measured and feed was mixed according to pen intake.

*Carcass Evaluation.* Twenty lambs (5 lambs per treatment) of similar weight (53 kg) were selected for determination of carcass characteristics. On December 20, 2007 lambs were taken to a local abattoir and harvested the next morning. After an approximate 24 h chill, carcass weight, kidney fat, 12$^{th}$/13$^{th}$ rib fat depth (FD), and 12$^{th}$/13$^{th}$ rib-eye area (REA) were recorded.

*Ultrasound Evaluation.* Ultrasound measurements of REA and back FD were taken at 12$^{th}$/13$^{th}$ rib transverse using an Aloka SSD-500V real-time ultrasound device with a 3.5 MHz, 12.5-cm linear array transducer and standoff guide. On September 6, October 10, October 30, November 29, and December 18, 2007 lamb REA was measured via ultrasound. On October 30 and November 29, and December 18, 2007 lamb FD was measured via ultrasound. All ultrasound measurements were collected and interpreted by the same technician. Ultrasound measurements were compared to the carcass data from each treatment. Technician bias was -0.018 and 0.12 cm for REA and FD, respectively. Standard error of prediction was 0.63 and 0.17 cm for REA and BF, respectively. Standard error of repeatability was 0.55 and 0.07 cm for REA and BF, respectively.

*Statistical Analysis.* Data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Lamb was the experimental unit for ultrasound and carcass measurements and pen was the experimental unit for all other measurements. Feedlot measurements and lamb ultrasound measurements were analyzed within day and all models included backgrounding treatment as the variable. Data are presented as least squares means with differences considered significant at $P < 0.10$. All animal procedures were approved by the Montana State University Institutional Animal Care and Use Committee *(P*rotocol #AA-030).

## Results and Discussion

*Feedlot performance.* No differences ($P > 0.35$; Table 1) among backgrounding treatments were detected for intake, ADG, or gain:feed during the step-up (d 0 to 19) or finishing period (d 19 to 71). However, when combined (d 0-71) PELLET lambs had the greatest intakes ($P < 0.10$). Average daily gain was greater ($P < 0.10$) for PELLET lambs than RANGE and LATE WEAN. Gain:feed was greater ($P < 0.10$) for GRASS than RANGE. In addition, GRASS lambs tended ($P = 0.14$) to have greater gain:feed than LATE WEAN lambs.

*Growth.* Lambs backgrounded on PELLET were heavier ($P < 0.10$; Table 2) than all other treatments after backgrounding (d 0). After lambs were stepped up onto the 70% concentrate diet (d 19) PELLET and GRASS lambs were heavier ($P < 0.05$) than RANGE and LATE WEAN lambs. At d 49 and 71, PELLET lambs were heavier ($P < 0.05$) than all other backgrounding treatments.

*Ultrasound data.* After lamb backgrounding (d 0), PELLET and GRASS lambs had larger ($P < 0.05$; Table 3) REAs than RANGE and LATE WEAN lambs. After the step-up period (d 19), PELLET backgrounded lambs had a larger ($P < 0.10$) REA than RANGE backgrounded lambs. No differences ($P > 0.50$) for lamb growth were seen among treatments at d 49 of feedlot period. At the conclusion of the feedlot period (d 71), PELLET and GRASS backgrounded lambs had larger ($P < 0.05$) REAs than RANGE and LATE WEAN backgrounded lambs. Fat depth was the greater ($P < 0.05$) for the PELLET than all other backgrounded lambs at the end of the step-up phase (d 19). At d 49, LATE WEAN lambs tended ($P = 0.17$) to have greater FD than RANGE lambs. At the conclusion of

the feedlot, there were no differences (P > 0.50) in FD among treatments.

*Carcass data.* Lambs of similar weight from each treatment were selected to be harvested (n = 20). No differences ($P > 0.10$; Table 4) were seen among treatments for chilled carcass weight or REA. Fat depth was thicker ($P < 0.10$) for GRASS, RANGE, and LATE WEAN than for PELLET lambs.

*Discussion:* Lamb weights during the backgrounding period were greatest for the PELLET lambs and they maintained their heavier weights throughout the feedlot period. Feedlot ADG was again greater for the PELLET lambs than the RANGE and LATE WEAN lambs. However, GRASS lamb ADG was similar to PELLET lambs. GRASS lambs had improved gain:feed over RANGE lambs. Ultrasound measurements of REA showed that GRASS and PELLET lambs deposited more lean tissue than RANGE and LATE WEAN lambs. Ultrasound measurements of FD showed that PELLET lambs had the greatest fat deposition during the backgrounding period, due to their treatment effect ad libitum access to 80% alfalfa: 20% barley pellets. However, the three other treatments caught up to the PELLET lambs FD by the end of the feedlot period. Of the other three backgrounding treatments, GRASS lambs performed the best during the step-up period and maintained numerically higher feedlot weights. In addition, body composition data from ultrasound measurements showed that GRASS lambs utilized their feed resources to better deposit lean muscle.

*Conclusions:* Our study showed that backgrounding lambs on PELLET allowed for more rapid feedlot lamb gains as compared to RANGE and LATE WEAN backgrounding. The study also showed that of the low input backgrounding strategies (RANGE, LATE WEAN, and GRASS); lambs backgrounded on grass transitioned onto a feedlot diet better, had larger REA and had better feed conversion in a feedlot than range backgrounded lambs.

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Table 1. Effects of backgrounding treatments on lamb pen (n = 12) intake (kg/d), ADG (kg/d), and gain:feed (g:kg) during feedlot periods[1]

| | Treatment[2] | | | | |
|---|---|---|---|---|---|
| | Grass | Late Wean | Pellet | Range | SE |
| Step-up | | | | | |
| Intake | 1.31 | 1.24 | 1.34 | 1.15 | 0.08 |
| ADG | 0.16 | 0.10 | 0.08 | 0.06 | 0.05 |
| Gain:feed | 120 | 79 | 49 | 48 | 23.9 |
| Finishing | | | | | |
| Intake | 1.66 | 1.68 | 1.79 | 1.65 | 0.06 |
| ADG | 0.24 | 0.25 | 0.28 | 0.25 | 0.02 |
| Gain:feed | 144 | 148 | 156 | 152 | 9.15 |
| Total | | | | | |
| Intake | 1.56[a] | 1.56[a] | 1.67[b] | 1.51[a] | 0.04 |
| ADG | 0.22[ab] | 0.20[a] | 0.23[b] | 0.20[a] | 0.01 |
| Gain:feed | 139[a] | 132[ab] | 135[ab] | 131[b] | 2.72 |

[ab] Row means with different superscripts differ ($P < 0.10$).

[1] *Step-up* 19 d period during which lambs were adjusted to 70% concentrate ration.
*Finishing* 52 d period that lambs remained on the 70% concentrate ration.
*Total* was the entire feeding period.

[2] Treatments were applied to lambs for 29 d after weaning.
*Grass* lambs were maintained on grass paddocks at the Fort Ellis Research Center.
*Late Wean* lambs were not weaned from dams.
*Pellet* lambs were self-fed alfalfa:barley pellets.
*Range* lambs were weaned from dams for 4 d and returned to range.

Table 2. Effects of backgrounding treatments on feedlot lamb pen (n = 12) weights (kg)

| | Treatment[2] | | | | |
|---|---|---|---|---|---|
| Period[1] | Grass | Late Wean | Pellet | Range | SE |
| Weaning | 32 | 31 | 31 | 31 | |
| Feedlot | | | | | |
| Day 0 | 33[a] | 33[a] | 35[b] | 33[a] | 0.59 |
| Day 19 | 36[b] | 35[a] | 36[b] | 34[a] | 0.60 |
| Day 49 | 44[a] | 43[a] | 46[b] | 42[a] | 0.94 |
| Day 71 | 48[a] | 47[a] | 51[b] | 46[a] | 0.87 |

[ab] Row means with different superscripts differ ($P < 0.10$).

[1] *Weaning* represents removal of lambs from ewes.
*Day 0* lambs were removed from backgrounding treatments and began step-up rations.
*Day 19* lambs finished the transition period and began the finishing ration.
*Day 49* was a median measurement of the finishing period.
*Day 71* was the conclusion feedlot period.

[2] Treatments were applied to lambs for 29 d after weaning (n = 12).
*Grass* lambs were maintained on grass paddocks at the Fort Ellis Research Center.
*Late Wean* lambs were not weaned from dams.
*Pellet* lambs were self-fed 80% alfalfa: 20% barley pellets.
*Range* lambs were weaned from dams for 4 d and returned to range.

Table 3. Ultrasound measurements of rib-eye area and fat thickness measured at the last rib of backgrounded lambs[1]

| | Treatment[2] | | | | |
|---|---|---|---|---|---|
| | Grass | Late Wean | Pellet | Range | SE |
| Rib-eye area, $cm^2$ | | | | | |
| Weaning | 8.18 | 7.61 | 7.93 | 7.83 | 0.27 |
| Day 0 | 9.49[a] | 8.47[b] | 9.96[a] | 8.43[b] | 0.30 |
| Day 19 | 11.04[ab] | 10.43[ab] | 11.13[a] | 10.39[b] | 0.30 |
| Day 49 | 15.35 | 15.49 | 15.94 | 15.15 | 0.39 |
| Day 71 | 16.53[a] | 15.46[b] | 16.46[a] | 15.27[b] | 0.37 |
| Fat depth, cm | | | | | |
| Day 19 | 0.28[a] | 0.27[a] | 0.36[b] | 0.26[a] | 0.01 |
| Day 49 | 0.37[ab] | 0.40[a] | 0.38[ab] | 0.34[b] | 0.02 |
| Day 71 | 0.53 | 0.51 | 0.51 | 0.50 | 0.02 |

[ab] Row means with different superscripts differ ($P < 0.10$).

[1] *Weaning* represents removal of lambs from ewes.
*Day 0* lambs were removed from backgrounding treatments and began step-up rations.
*Day 19* lambs finished the transition period and began the finishing ration.
*Day 49* was a median measurement of the finishing period.
*Day 71* was the conclusion feedlot period.

[2] Treatments were applied to lambs (n = 72) for 29 d after weaning.
*Grass* lambs were maintained on grass paddocks at the Fort Ellis Research Center.
*Late Wean* lambs were not weaned from dams.
*Pellet* lambs were self-fed alfalfa:barley pellets.
*Range* lambs were weaned from dams for 4 d and returned to range.

Table 4. Effects of backgrounding treatments on lamb carcass (n = 20) data taken after 71 d feedlot period

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| | Grass | Late Wean | Pellet | Range | SE |
| Chilled carcass, kg | 26.5 | 26.3 | 26.6 | 25.8 | 0.46 |
| Rib eye area, $cm^2$ | 17.2 | 16.1 | 16.0 | 15.9 | 0.77 |
| Fat depth, cm | 0.46[a] | 0.48[a] | 0.33[b] | 0.46[a] | 0.05 |
| Kidney pelvic fat, kg | 1.16 | 0.97 | 1.16 | 1.00 | 0.05 |

[ab] Row means with different superscripts differ ($P < 0.10$).

[1] Treatments were applied to lambs for 29 d after weaning.
*Grass* lambs were maintained on grass paddocks at the Fort Ellis Research Center.
*Late Wean* lambs were not weaned from dams.
*Pellet* lambs were self-fed alfalfa:barley pellets.
*Range* lambs were weaned from dams for 4 d and returned to range.

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# EVALUATION OF IMPLANTABLE RFID MICROCHIPS FOR READABILITY AND MEASUREMENT OF BODY TEMPERATURE IN MATURE HORSES

**L.E. Wallace*, J. A. Paterson, L. Gagnon, R. Clark, K. Davis, M. Miller**
Montana State University, Bozeman, MT, USA

**ABSTRACT:**Experiments were conducted to first determine the readability of two implantable RFID microchips (134.2 kHz; Allflex, 12 mm in length and DestronLifechip with Bio-Thermo™ technology, 15 mm in length) placed in 38 mature (>3 yr of age) horses (19/treatment). The second experiment was a 2 x 2 factorial to measure body temperature with the Destron microchip vs. a digital rectal thermometer reading when horses were rested or immediately after 30 min of moderate exercise. Both brands of microchips were implanted via a supplied syringe needle into the nucal ligament approximately 46 cm from the poll and 2.5 cm down from the base of the mane. For Exp. 1, the microchips were read with a handheld Bluetooth scanner (Destron) every two wk for a 10 wk period and then again 344 d after trial initiation. For Exp. 2, 10 mature horses from Exp. 1 which had the implanted DestronBio-Thermo™ microchip were used. Measurements were collected (Destron scanner) every Friday afternoon for 10 wk. Results from Exp. 1 showed that readability was higher ($P<0.05$) for the Allflex microchip (100%) compared to the Destron microchip (96.3%) after 344 d. For Exp. 2, no body location by exercise interactions ($P>0.10$) were measured. No differences ($P=0.48$) were detected between rectal temperatures (avg 37.7°C) using a digital thermometer compared to temperatures measured from the nucal ligament (avg 37.8°C) with the Destron microchip in either a resting or exercised state of activity. Body temperatures were lower ($P<0.01$) for resting horses (37.3°C) compared to exercised horses (38.2°C). Results from these two studies showed that an implantable RFID microchip could be read for at least 344 d. The DestronBio-Thermo™ microchip implanted in the nucal ligament provided similar body temperature measurements as did measuring rectal temperature with a digital thermometer. It was concluded from this study that the DestronBio-Thermo™ implantable microchip could be used for both RFID, as well as an accurate means of determining body temperature in horses.

Keywords: Implantable RFID, Equine ID, Body Temperature

## Introduction

The development of livestock identification systems are based on industry needs which include disease control and eradication, disease surveillance and monitoring, emergency response to foreign animal diseases, consumer concerns over food safety, and emergency management programs (USDA,2007). Under the National Animal Identification System (NAIS) every animal classified as livestock, including equine will be individually identified (USDA, 2008). Being able to quickly trace horses that may have been in contact with infected animals is imperative to containing an outbreak and preventing further spread of harmful contagious diseases (ESWG, 2007).

Horses have been routinely identified with radio frequency identification (RFID) technology since the early 1980's (ESWG, 2007).Besides disease traceback, this technology has many benefits for the horse industry (Evans, 2008). RFID microchips are the most efficient way to identify horsesfor proof of ownership, identification and management purposes (ESWG, 2007).Implantable microchips provide a reliable method of permanent, unalterable animal identification (AVMA, 2007).

Microchips with bio-thermal capabilities have the benefit of recording temperatures as well as providing individual identification, making this technology more attractive to horse owners (ESWG, 2007; Evans, 2008). According to standards established by the USDA (2008), injectable RFID transponders must remain functional for the expected life of the animal (20 yr for horses), with a failure rate of no more than one percent per year being allowed. However, there is limited long-term research evaluating this new technology.

The objectives of this study were to: 1) determine the readability rates of two commercially available implantable RFIDmicrochips; and 2)compare body temperature measurements of the horses using the DestronLifeChipBio-Thermo™ microchip vs. a rectal digital thermometer reading when horses were rested or immediately after 30 min of exercise.

## Materials and Methods

Two experiments were conducted to compare two brands of equine RFID (134.2 kHz) microchips;Allflex, 12 mm in length; and DestronLifechip with Bio-Thermo™ technology, 15 mm in length. The microchips wereplaced in 38 mature (>3 yr of age) horses (19/treatment). Each horse was identified by color, markings, sex and name; then correlated to a unique 15-digit microchip number. The procedures for implantation of both types of microchips were identical and followed the internationally-recognized site for equine microchips (Ingwersen, 2000; ESWG, 2007; Evans, 2008).

A licensed veterinarian implanted the microchipsvia a supplied pre-packaged, one time use, syringe needle into the fibrous tissue of the horse's nucal ligament on the left side of the neck, approximately 46 cm from the poll and 2.5 cm downward from the base of the mane. Similar to the procedures of Evans (2008), the

needle and syringe were oriented perpendicular to the skin at the implantation site and the needle was inserted to the depth of its hub. The microchip was injected at that depth in the nuchal ligament and the needle was withdrawn. The microchips were scanned using a handheld Bluetoothwand-reader (Destron)immediately after implantation to verify that no damage to the transponder occurred during the insertion process. A hand-held palm pilot (HP Pocket PC) with Bluetooth capability was used to capture and record the RFID and temperature data in Excel using TWedgeCE_ARM (www.tec-it.com).

For Exp. 1, 38 microchips (n=19) were read every two wk for a 10 wk period and then again 344 d after trial initiation (ave 6.9 scanning opportunities/horse).

For Exp. 2, 10 mature horses from Exp. 1 which had the implanted DestronLifechip with Bio-Thermo™were used. Measurements were collected every Friday afternoon for 10 wk. Temperatures were recorded from both the microchip and a digital rectal thermometer while the horses were at rest. The horses were then walked for a five to 10 min warm-up session, then trotted and loped for an additional 15 to 20 min. Immediately after the exercise session, the microchip and rectal temperatures were recorded again.

***Statistical Analysis***

Data collected were analyzed using the GLM and Mixed Linear procedures of SAS (2003). Differences in readability of the two microchip brandsin Exp. 1 were analyzed using LS means (PROC GLM). The second experiment was designed as a 2 x 2 factorial and was analyzed using PROC MIXED (Littell et al., 1998). Main effects were: method of determining body temperature (microchip vs. digital thermometer), state of activity (resting vs. exercised), and the interaction.

## Results and Discussion

Results from Exp. 1 showed that readability was higher ($P<0.05$) for the Allflex microchip (100%) compared to the Destron microchip (96.3%) after 344 d. Two of the Destron microchips failed to read. The first failed microchip read attempt was successfully read at d 0 but could not be read over the duration of the first 10 weeks (d 6, 15, 29 and 43). However, on d 344 the same microchip was successfullyread. The second microchip with the failed read attempt was not able to be read on d 6 and d 344 but was successfully read on the other 11 scanning opportunities. Evans (2008) recordedresults that were intermediate to results documented in this study with overall read rates of 99.6% on 1,915 CA racehorses.

For Exp. 2, no body location by exercise interactions ($P>0.10$) were measured. No differences ($P=0.48$) were detected between rectal temperatures (avg 37.7°C) using a digital thermometer compared to temperatures measured from the nucal ligament (avg 37.8°C) with the Destron microchip in either a resting or exercised state of activity (Table 1). Data are also presented over the 344 d (Fig. 1). With regard to body temperature recording, Evans (2008) recorded inconsistent microchip readings using the DestronBio-Thermo™ microchip when horses were at rest. However, when the horses were exercised the accuracy of the temperature recording improved.Body temperatures on the average were lower ($P<0.01$) for resting horses (37.3°C) compared to exercised horses (38.2°C; Table 1).

No adverse reactions at the microchip implantation sites were recorded over the duration of the experiments. Similar results were found by Conill et al. (2000) in 343 fattening calves implanted in the ear, armpit and upper lip. Evans (2008) recorded adverse reactions in only 0.05% of the 1,915 horses in his study. However, incidences of a foreign body reactions and tumor formation have been reported in other animal species (Johnson, 1996; Tillmann et al., 1997; Vascellari, 2006).

## Implications

Results from these two studies showed that Allflex and Destron implantable RFID microchips could be read for at least 344 d. The DestronLifechip with Bio-Thermo™ implanted in the nucal ligament provided similar body temperature measurements as did measuring rectal temperature with a digital thermometer. It was concluded from this study that the Destron Bio-Thermo™ implantable microchip could be used for both RFID, as well as an accurate means of determining body temperature in horses.

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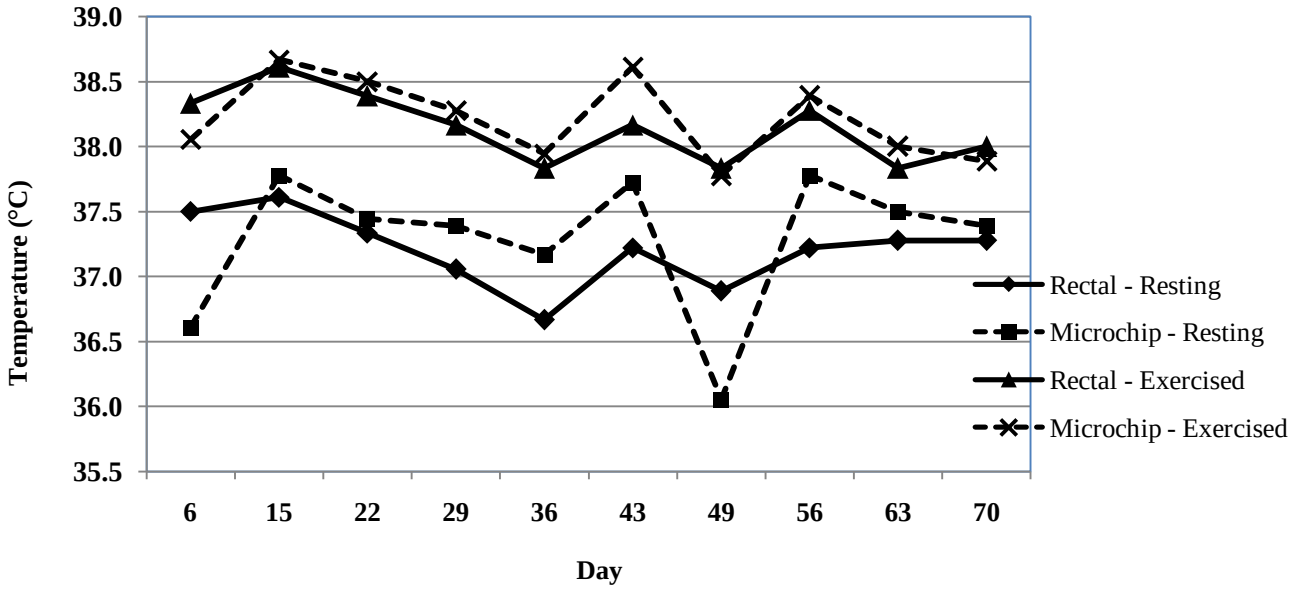
**Table 1.** Comparison of average horse temperature(°C) by measurement location (microchip vs. digital thermometer)and average horse temperature by activity level (resting vs. exercised).

| Measurement Location | | Activity Level | |
|---|---|---|---|
| Microchip* | Digital** | Resting | Exercised |
| 37.8 | 37.7 | 37.3[a] | 38.2[b] |

[ab]Main effect for activity level (P<0.05).
*Microhip implanted in the nuchal ligament.
**Digital thermometer measurement taken from the rectum.

**Figure 1.** Average microchip temperature (°C) compared to the average rectal temperatureof horses at rest and after30 min of exercise. (Main effect due to Activity , P<0.05)



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# EFFECTS OF WINTER GRAZING SYSTEM AND SUPPLEMENTATION DURING LATE GESTATION ON PERFORMANCE OF BEEF COWS AND PROGENY

**R. N. Funston, J. L. Martin, D. C. Adams, and D.M. Larson**
University of Nebraska West Central Research & Extension Center, North Platte, NE 69101

**ABSTRACT:** The objective of this study was to evaluate cow winter system and supplementation on cow and progeny performance. Composite cows (n = 108 yr 1; n = 114 yr 2; n = 120 yr 3) grazed range (**WR**) or corn residue (**CR**) during winter and received 0.45 kg/d (DM) 28% CP cubes (**PS**) or no supplement (**NS**). Steer calves (n = 51 yr 1; n = 58 yr 2) entered the feedlot 10 d post-weaning and were harvested 221 d later. Heifer calves (n = 55 yr 1, n = 54 yr 2) were wintered in drylot and grazed summer range. Pre-calving BW and BCS were greater ($P < 0.001$) for PS than NS in both winter systems. Calf birth weight was greater ($P = 0.01$) for CR than WR. Pre-breeding BW and BCS were greater ($P < 0.001$) for CR than WR cows and PS than NS ($P < 0.001$, $P = 0.06$) cows. At weaning, CR cows were heavier ($P < 0.001$) than WR cows but similar BCS ($P = 0.83$). Weight and BCS were not affected ($P > 0.80$) by PS. Calf weaning BW was greater ($P < 0.01$) for PS cows that grazed WR. Pregnancy rate was not affected by treatments ($P > 0.20$). Final BW and12$^{th}$ rib fat tended ($P < 0.09$) to be greater for steers from cows on CR than WR. Yield grades, HCW, ADG, and LM area were similar ($P > 0.10$). Steers from PS cows graded a higher proportion ($P = 0.02$) USDA Choice or better. The DMI was greater ($P = 0.04$) for heifers from WR than CR cows and PS of cows reduced G:F of heifer progeny ($P = 0.02$). Heifers born to cows that grazed CR and not supplemented were heavier at breeding ($P = 0.07$) and pregnancy diagnosis ($P = 0.03$) compared to heifers from WR cows that were not supplemented and heifers from cows on CR that were supplemented. Heifers born to CR cows were younger ($P = 0.10$) at puberty than progeny of WR cows. There were more ($P = 0.10$) heifers pubertal before breeding from dams receiving PS on WR. Pregnancy rate was not affected ($P = 0.16$) by dam treatment. Grazing CR resulted in greater cow BW and BCS throughout the year, increased steer final BW, and reduced heifer age at puberty versus grazing WR. Calf weaning BW and percent of heifers pubertal before breeding increased with PS of WR cows, while PS improved steer quality grade in both systems.

**Keywords:** Fetal programming, Heifer development, Maternal nutrition

## Introduction

Protein supplementation of spring calving beef cows grazing dormant Sandhills range during late gestation does not improve cow reproductive performance (Stalker et al., 2006) despite the fact nutrient requirements are greater than nutrient content of the grazed forage (NRC, 2000). Supplementation does increase progeny weaning weight and fertility of heifer progeny (Stalker et al., 2006; Martin et al., 2007). Corn crop residue provides a winter grazing alternative more economical than feeding harvested forage (Adams et al., 1996). Decreasing harvested forage needs can reduce breakeven costs of weaned calves or finished steers (Anderson et al., 2005).

The fetal programming hypothesis states postnatal growth and physiology can be influenced by stimulus experienced *in utero* (Barker et al., 1993). Previous research (Stalker et al., 2006; Martin et al., 2007), provide evidence for fetal programming of reproductive tissue and endocrine metabolism of progeny from cows grazing dormant winter range without supplementation. The objectives of the current study were to determine the effects of grazing dormant Sandhills range or corn crop residue on performance of cows and their progeny, and to determine if supplementing cows grazing either dormant Sandhills range or corn crop residue affects performance of cows or their progeny.

## Materials and Methods

### *Cow Management*

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved all procedures and facilities used in this experiment. A 3-yr study utilized composite Red Angus x Simmental cows and their progeny at Gudmundsen Sandhills Laboratory (**GSL**), Whitman, NE and West Central Research and Extension Center, North Platte, NE. Cows were used in a 2 x 2 factorial treatment arrangement to determine effects of grazing dormant Sandhills winter range (**WR**) or corn crop residue (**CR**) and receiving supplement (**PS**) or no supplement (**NS**) on cow and progeny performance. Pregnant, spring-calving cows (n = 108) between 3 and 5 yr of age were stratified by age and weaning weight of their previous calf and assigned randomly to treatment in yr 1. Cows remained on the same treatment for the length of the study, unless removed due to reproductive failure or injury. Pregnant 3-yr-old cows were stratified by age and weaning weight of their previous calf and assigned randomly to treatment to replace cows removed from the study and to increase numbers as forage availability allowed. Data are reported for 2005 (n = 108), 2006 (n = 114), and 2007 (n = 120). Current results include 3 yr of data through weaning, 2 yr of feedlot and carcass data for steers, and 2 yr of data through pregnancy diagnosis for heifers.

Cows that grazed winter range were divided into four, 32-ha upland pastures, two pastures received protein supplement, two did not. Cows that grazed cornstalks were maintained in two fields, one field received protein supplement.

On a pasture or field basis, cows received the equivalent of 0.45 kg/d of 28% CP supplement three times/wk or no protein supplement from December 1 until February 28. The supplement contained 62.0% dried distillers grains plus solubles, 10.6% wheat middlings, 9.0% cottonseed meal, 5.0% dried corn gluten feed, 5.0% molasses, 3.0% calcium carbonate, 2.0% urea on a DM basis. Additionally, the supplement was formulated to meet vitamin and trace mineral requirements of the heifers and supply 80 mg· animal$^{-1}$·d$^{-1}$ monensin (Rumensin, Elanco Animal Health, Indianapolis, IN).

After winter grazing, cows were managed in a common group and fed hay harvested from subirrigated meadows and protein supplement. Cows returned to upland range in late May and remained in a common group throughout the breeding season until the subsequent winter grazing period. Cows were exposed to fertile bulls at a ratio of approximately one bull to 25 cows for 60 d each year.

Pre-calving, pre-breeding, and weaning BW and BCS were recorded each year. Cows were not limit fed prior to weighing. A subset of cows (n = 12-15 per treatment) were assigned randomly to one of four weigh-suckle-weigh groups. Milk production data was collected each year in late May, prior to the grazing season, and at weaning. Pregnancy was diagnosed via rectal palpation and/or transrectal ultrasonography 60 or more d following the end of the breeding season.

***Calf Management***

Treatments included only dam winter grazing system and late gestation protein supplementation, no further treatments were applied to calves. Approximately 14 d following weaning, calves were transported to West Central Research and Extension Center (**WCREC**), North Platte, NE. After arrival, steers were limit fed a starter diet containing 35% ground alfalfa hay, 40% wet corn gluten feed, 7.5% supplement, and 17.5% dry rolled corn (DM basis) at 2.0% of BW (DM basis) for 5 d, prior to being weighed on 2 consecutive days. At this time, an initial implant containing 20 mg estradiol benzoate and 200 mg progesterone (Synovex S, Ft. Dodge Animal Health) and moxidectin (Cydectin, Ft. Dodge Animal Health) were administered. Approximately 100 d prior to estimated harvest date, steers were implanted with 24 mg estradiol and 120 mg trenbolone acetate (Revelor S, Intervet). Steer calves were penned by dam treatment and replication, and were adapted over 21 days to a finishing diet including 48% dry rolled corn, 40% wet corn gluten feed, 7% ground alfalfa hay, and 5% supplement (DM basis).

Steers were harvested when estimated visually to have 1.3 cm fat thickness over the 12$^{th}$ rib, and were fed for an average of 221 d. Steers were harvested at a commercial abattoir, and carcass data were collected.

Heifers remained in a single group for approximately 30 d following transport to WCREC. They were acclimated to a diet consisting of corn gluten feed and low quality forage. In yr 1, heifers were fed 25% WCGF and 75% prairie hay (DM basis) *ad libitum*. In yr 2, heifers were allowed *ad libitum* intake of 20% wet corn gluten feed and 80% of a forage mix including wheat straw and alfalfa hay ground together. Interim BW and blood samples were collected every 14 d. Subsequently, heifers from WR cows in yr 1 and a subset of heifers from each treatment in yr 2 were assigned randomly to 1 of 4 pens containing Calan gates (American Calan, Northwood, NH) to evaluate individual feed efficiency.

Following completion of the individual feeding period (minimum 84 d) in early May each year, heifers returned to GSL. Heifers were exposed to bulls (1:25 bull:heifer) for a 45-d breeding season. Pregnancy diagnosis was performed via transrectal ultrasonography approximately 45 d following completion of the breeding season.

***Statistical Analysis***

Continuous data were evaluated using PROC MIXED of SAS (SAS Inst., Inc., Cary, NC). The statistical model included winter grazing system, protein supplementation, and the interaction. Cow age was included as a covariate for cow performance traits. Year was included as a random variable in all analyses, and pen within year for individually-fed heifer data. Binomial data, including reproductive performance and quality grade, were analyzed using Chi-square procedures in PROC GENMOD of SAS. Individual animal was considered the experimental unit for analysis of continuous data, treatments within year was the experimental unit for binomial data.

## Results and Discussion

Cow BW and BCS after the winter grazing period and prior to calving were affected (Table 1; $P < 0.01$) by the winter grazing system and protein supplementation. Heavier BW and higher BCS were recorded for PS and cows grazing CR. These results are similar to those of Stalker et al. (2006), who reported cows grazing winter range lost 29 kg and 0.6 BCS if not supplemented, but maintained both is they received 0.45kg/d of 42% CP supplement during this period. Calving date was also later ($P = 0.03$) for NS cows grazing WR but not CR.

Calf birth BW was greater ($P = 0.01$) if their dams grazed corn residue than winter range, and tended to increase ($P = 0.10$) with protein supplementation. This is somewhat surprising because previous research using the same cow herd did not find differences in calf birth BW due to supplementation of dams grazing winter range (Stalker et al., 2006; Martin et al., 2007). Despite relatively small magnitude of differences, winter grazing system and protein supplementation did affect birth BW of calves in the current study.

Pre-breeding cow BW and BCS were increased (Table 1; $P \leq 0.06$) by winter grazing of corn residue and protein supplementation. The interaction of grazing system and supplementation was no longer significant ($P = 0.67$), but groups ranked nearly the same as they had before calving. Milk production did not differ by treatment ($P = 0.11$) in

May but was greater ($P < 0.01$) in November for cows that previously grazed CR, calf BW was increased ($P < 0.01$) in May by protein supplementation when cows grazed WR but not CR.

At weaning, actual and adjusted calf BW were greater ($P \leq 0.07$) for calves from PS cows grazing winter range. Similar effects of dam supplementation during winter grazing on calf weaning BW were reported in previous studies (Stalker et al., 2006, Martin et al., 2007). Cow BW and BCS at weaning were not affected by supplementation ($P > 0.80$), but cows that grazed corn residue the previous winter were heavier ($P < 0.001$) than WR at weaning despite similar ($P = 0.83$) BCS. Pregnancy rate was not affected ($P > 0.20$) by PS or winter system. Stalker et al. (2006) also reported no benefit of PS on winter range on subsequent pregnancy rates.

Effects of dam treatment on steer progeny feedlot performance are shown in Table 2. Feedlot initial BW differed ($P < 0.001$) due to the interaction of dam grazing system and supplementation. However, feedlot ADG was similar ($P > 0.31$) between treatments. There was a tendency ($P = 0.08$) for steers from cows that grazed corn residue to have heavier final live BW compared to steers born to cows that grazed winter range, but hot carcass weight was not different due to dam treatment ($P > 0.12$). External fat thickness measured over the 12$^{th}$ rib tended (P = 0.09) to be greater for steers whose dams grazed corn residue compared to cows that grazed winter range, and tended to be greater ($P > 0.08$) for steers from supplemented versus unsupplemented cows. Concomitant differences in calculated yield grade and REA were not ($P > 0.22$) found. A greater proportion ($P = 0.01$) of steers born to supplemented cows achieved USDA quality grades of Choice or higher. However, dam grazing system did not affect ($P = 0.96$) quality grade. These data suggest a potential fetal programming effect of late gestation cow supplementation on subsequent steer progeny intramuscular fat deposition. Using only cows that grazed winter range, Stalker et al. (2006) were unable to identify any significant differences in steer progeny feedlot or carcass data. However, they did note a tendency ($P = 0.16$) for increased proportions of steers grading Choice or higher if their dams were supplemented with protein during late gestation, with a comparable magnitude of difference as the current study.

Heifer progeny from cows in the current study achieved similar (Table 3; $P = 0.14$) ADG from weaning until breeding regardless of dam treatment. Heifers born to cows that grazed CR and not supplemented were heavier at breeding ($P = 0.07$) and pregnancy diagnosis ($P = 0.03$) compared to heifers from WR cows not supplemented and heifers from cows on CR that were supplemented. It is not clear why heifers born to cows on CR that were not supplemented would be heavier than heifers from supplemented cows. Heifers born to CR cows tended to be younger ($P = 0.10$) at puberty than progeny of WR cows, weight at puberty was not affected ($P = 0.17$) by dam treatment. It is important to note heifers from WR cows were individually fed in yr 1 while heifers from CR cows were not. In yr 2 heifers from both systems were individually fed. The difference in environment in yr 1 may have contributed to apparent differences in age at puberty. There were more ($P = 0.09$) heifers cyclic before breeding from dams receiving PS on WR but not in heifers from dams on CR. Final pregnancy rate was not affected ($P = 0.16$) by dam treatment. Previous research indicated a fetal programming effect of late gestation maternal nutrition on heifer progeny fertility independent of age at puberty and percent cycling before the breeding season (Martin et al., 2007).

There were no differences ($P > 0.19$) in DMI, ADG, or residual feed intake (**RFI**) due to dam protein supplementation. However, heifers from unsupplemented cows gained more efficiently (G:F; $P = 0.02$) than heifers from supplemented cows. Dry matter intake was greater ($P = 0.04$) for heifers born to cows that grazed WR than cows that grazed CR, but ADG, RFI, and G:F were similar ($P > 0.12$) between grazing systems. Previously, RFI and DMI appeared to be affected by late gestation supplementation dependent upon postpartum dam treatment (Martin et al., 2007).

Grazing corn residue resulted in greater cow BW and BCS throughout the production year, increased steer final BW, and reduced heifer age at puberty versus grazing WR. Calf weaning BW and percent of heifers pubertal before breeding increased with PS of WR cows, while PS improved steer quality grade in both systems.

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**Table 1.** Effects of grazing WR or CR and PS during the last trimester of gestation on cow performance and reproduction[1]

| | Treatment[1] | | | | | Treatment *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| Trait | PS/ WR | NS/ WR | PS/ CR | NS/ CR | SEM | Sys. | Supp. | S*S |
| n | 86 | 84 | 86 | 86 | - | - | - | - |
| Pre-calving BW, kg | 501[a] | 468[b] | 530[c] | 519[d] | 20 | <0.001 | <0.001 | 0.02 |
| Pre-calving BCS | 5.11[a] | 4.75[b] | 5.34[c] | 5.20[a] | 0.05 | <0.001 | <0.001 | 0.03 |
| Calf birth date, d | 83[a] | 89[b] | 82[a] | 84[a] | 2 | 0.24 | 0.02 | 0.03 |
| Calf birth BW, kg | 35.8 | 34.9 | 37.2 | 36.3 | 0.45 | 0.01 | 0.10 | 0.46 |
| Pre-breeding BW, kg | 452 | 442 | 478 | 472 | 12 | <0.001 | 0.06 | 0.67 |
| Pre-breeding BCS | 5.22 | 4.99 | 5.36 | 5.22 | 0.05 | <0.001 | <0.001 | 0.32 |
| Pre-breeding calf BW, kg | 90[a] | 85[b] | 92[a] | 92[a] | 2 | <0.001 | 0.01 | 0.01 |
| May 24 hr milk, kg | 5.4 | 5.3 | 6.0 | 5.7 | 1.0 | 0.11 | 0.41 | 0.69 |
| Nov. 24 hr milk, kg | 2.5 | 2.8 | 3.8 | 3.8 | 0.4 | <0.01 | 0.69 | 0.55 |
| Calf weaning BW, kg | 235[a] | 220[b] | 235[a] | 235[a] | 7 | 0.01 | 0.03 | <0.01 |
| Calf adj. 205 d BW, kg | 220[a] | 211[b] | 222[a] | 221[a] | 6 | 0.01 | 0.03 | 0.07 |
| Cow weaning BW, kg | 479 | 473 | 496 | 499 | 8 | <0.001 | 0.80 | 0.30 |
| Cow weaning BCS | 5.13 | 5.07 | 5.08 | 5.14 | 0.07 | 0.83 | 0.06 | 0.20 |
| Pregnancy rate, % | 96.4 | 92.6 | 97.7 | 95.3 | -- | 0.46 | 0.20 | 0.96 |

[1]PS = dams supplemented with 0.45 kg/d 28% CP during gestation; NS = dams not supplemented; CR = dams grazed winter corn residue; WR =dams grazed winter range.
[2]Sys = winter system; Supp = supplementation treatment; S*S = winter system by supplementation treatment interaction.
[abc]Within a row, means without a common superscript differ at $P < 0.05$.

**Table 2.** Effects of dam grazing system and PS during the last trimester of gestation on gain and carcass merit of steers

| | Treatment[1] | | | | | Treatment *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| Trait | PS /WR | NS /WR | PS /CR | NS / CR | SEM | Sys. | Supp. | S*S |
| n | 29 | 27 | 22 | 32 | - | - | - | - |
| Beginning feedlot BW, kg | 238[a] | 220[b] | 234[a] | 242[a] | 11 | <0.01 | 0.12 | <0.001 |
| ADG, kg/d | 1.52 | 1.51 | 1.56 | 1.52 | 0.14 | 0.42 | 0.31 | 0.54 |
| Final live BW, kg | 578 | 556 | 582 | 582 | 10 | 0.08 | 0.21 | 0.21 |
| HCW, kg | 367 | 350 | 367 | 367 | 6 | 0.15 | 0.13 | 0.14 |
| 12$^{th}$ rib fat, cm | 1.2 | 1.1 | 1.3 | 1.2 | 0.08 | 0.09 | 0.08 | 0.82 |
| REA, cm$^2$ | 89.9 | 90.0 | 92.2 | 90.7 | 1.94 | 0.36 | 0.67 | 0.64 |
| Yield grade | 2.69 | 2.47 | 2.69 | 2.67 | 0.12 | 0.33 | 0.24 | 0.34 |
| Quality grade, % Choice | 77.8 | 66.7 | 86.4 | 53.1 | - | 0.98 | 0.02 | 0.22 |

[1]PS = dams supplemented with 0.45 kg/d 28% CP during gestation; NS = dams not supplemented; CR = dams grazed winter corn residue; WR =dams grazed winter range.
[2]Sys = winter system; Supp = supplementation treatment; S*S = winter system by supplementation treatment interaction.
[abc]Within a row, means without a common superscript differ at $P < 0.05$.

**Table 3.** Effects of dam grazing system and PS during the last trimester of gestation on growth and reproduction of heifers

| | Treatment[1] | | | | | Treatment *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| Trait | PS/ WR | NS/ WR | PS/ CR | NS/ CR | SEM | Sys. | Supp. | S*S |
| n | 28 | 25 | 32 | 24 | - | - | - | - |
| Act. weaning BW, kg | 228 | 217 | 232 | 226 | 6 | 0.05 | 0.01 | 0.33 |
| Adj. 205 d BW, kg | 214 | 204 | 215 | 215 | 4 | 0.04 | 0.07 | 0.11 |
| Gain while in Calan gates, kg/d | 0.79 | 0.79 | 0.77 | 0.85 | 0.23 | 0.50 | 0.14 | 0.14 |
| Pre-breeding BW, kg | 335 | 322 | 333 | 346 | 14 | 0.11 | 0.98 | 0.07 |
| Pubertal prior to breeding, % | 89.3 | 70.4 | 79.4 | 87.5 | - | 0.74 | 0.56 | 0.09 |
| Age at puberty, d | 364 | 358 | 343 | 353 | 9 | 0.10 | 0.79 | 0.33 |
| Pregnancy diagnosis BW, kg | 368[ab] | 356[a] | 365[a] | 381[b] | 17 | 0.09 | 0.71 | 0.03 |
| Pregnancy diagnosis BCS | 5.82 | 5.82 | 5.81 | 5.91 | 0.08 | 0.49 | 0.36 | 0.39 |
| Pregnancy rate, % | 92.6 | 76.0 | 87.5 | 91.3 | - | 0.62 | 0.45 | 0.16 |

[1]PS = dams supplemented with 0.45 kg/d 28% CP during gestation; NS = dams not supplemented; CR = dams grazed winter corn residue; WR =dams grazed winter range.
[2]Sys = winter system; Supp = supplementation treatment; S*S = winter system by supplementation treatment interaction.
[abc]Within a row, means without a common superscript differ at $P < 0.05$.

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# EFFECT OF CALVING SEASON AND WINTERING SYSTEM ON COW PERFORMANCE

**W.A. Griffin[*†], T.J. Klopfenstein[†], D.C. Adams[‡], G.E. Erickson[†], L.A. Stalker[‡], and R.N. Funston[‡]**

[†]University of Nebraska-Lincoln

[‡]University of Nebraska West Central Research and Extension Center, North Platte

**ABSTRACT:** A two year study using two hundred forty-one (5/8 Red Angus, 3/8 Continental) cows was conducted to evaluate the effect of calving season and wintering system on cow performance. Cows were assigned to one of five treatments: 1) spring calving cows (SP) wintered on native range, 2) SP wintered on cornstalks, 3) summer calving cows (SU) wintered on native range, 4) SU wintered on cornstalks, and 5) fall calving cows (FA) wintered on cornstalks. Calves were weaned 220, 298, and 247 d of age for SP, SU, and FA, respectively. Cow BW and BCS were recorded at three times during the year: 21 d before calving (pre-calving), 59 d post calving (pre-breeding), and weaning. Data were analyzed as a completely randomized design. There was no difference in cow BW ($P = 0.27$) or BCS ($P = 0.11$) at pre-breeding, pre-calving, or weaning when comparing wintering systems. Rebreeding rate was increased for cows wintered on cornstalks (92.3 vs. 88.3%; $P = 0.04$). When evaluating calving season, BW at pre-breeding was lowest for SP, intermediate for SU and greatest for FA (485 vs. 548 vs. 574 kg; $P = 0.02$). At weaning BW was lowest for SP (482 kg; $P = 0.02$) but SU and FA were not different (511 vs. 505; $P = 0.23$). Cow BW pre-calving was greatest for FA (635 kg; $P = 0.04$) but SP and SU were not different (526 vs. 557 kg; $P = 0.13$). At pre-breeding SP had the lowest BCS (5.2; $P < 0.01$) when compared to SU and FA which were not different (6.0 vs. 6.0; $P = 0.64$). At pre-calving BCS was lowest for SP, intermediate for SU, and greatest for FA (5.2 vs. 6.1 vs. 6.8; $P = 0.03$). Calving season had no effect on BCS at weaning ($P = 0.29$). Rebreeding performance was lowest for FA (84.1%; $P = 0.01$) when compared to SP and SU (93.1 vs. 93.5%; $P = 0.66$). In the current study, wintering system had no effect on cow BW or BCS; however, wintering cows on cornstalks increased rebreeding rate. Calving season also influences cow BW, BCS, and rebreeding performance.

**Key Words:** Calving season, Cow-calf systems, wintering system

## Introduction

The amount of harvested feed required to maintain cows in the Nebraska Sandhills is directly related to calving date (Adams et al., 1996; Clark et al., 2004). Traditionally, cows are bred to calve in February and March which leads to lactation occurring in early spring. In early spring, range resources are dormant and low in protein and energy (Geisert et al., 2008). To meet nutrient requirements of the cows, producers feed hay and other purchased feeds that can lead to increased cost for spring calving cows (Stockton et al., 2007). However, changing calving date could decrease the use of harvested forages and purchased feed resources by matching the cow's requirements with the time of year that forage resources are greater in protein and energy, potentially decreasing cost for cow-calf producers. The use of corn residue can be advantageous to beef production systems by providing low cost feed that does not compete with grain demand (Guteirrez-Ornelas, 1989). As corn price increases there is potential for increased corn acres leading to increased cornstalk availability. The use of cornstalks in cow-calf production could increase the capacity of a ranch as cows are moved from the ranch in the winter. This allows producers to utilize most of their forage in the spring and summer months and not have to stockpile winter grass. Secondly, the use of cornstalks offers some flexibility for cow-calf producers when managing drought. Traditionally, drought has caused producers to decrease herd numbers or dry lot cows using harvested forage and purchased feeds. Instead of culling or dry lotting cows, cornstalks offers producers an inexpensive feed that can help maintain herd numbers and prevent use of harvested forages and purchased feeds. Therefore, the objectives of this study were to 1) determine the effect of calving season and 2) wintering program on cow performance.

## Material and Methods

*Cow Management.* Two hundred forty one cows (5/8 Red Angus, 3/8 Continental) from the Gudmundsen Sandhills Laboratory (Whitman, NE) were assigned to one of five treatments. Treatments were: 1) spring calving cows (SP) wintered on native range, 2) SP wintered on cornstalks, 3) summer calving cows (SU) wintered on native range, 4) SU wintered on cornstalks, or 5) fall calving cows wintered on cornstalks. Average calving dates were March 25$^{th}$, June 16$^{th}$, and August 7$^{th}$ for SP, SU, and FA, respectively.

Spring calving cows wintered on native range were allowed to graze native sandhills range from mid-May until the end of February. At the beginning of March, SP wintered on range were fed meadow hay until mid-May. Spring calving cows wintered on cornstalks were allowed to graze native Sandhills range from mid-May until mid-October when cows were transported to cornstalks in the Platte river valley. At the end of February, SP wintered on cornstalks were returned to the ranch and fed meadow hay until mid-May. Summer calving cows wintered on native range were allowed to graze native Sandhills range for the entire year. However, SU cows wintered on cornstalks

were transported to cornstalks in mid-October and returned to the ranch at the end of March. Summer calving cows wintered on cornstalks were allowed to graze native Sandhills range from April until the beginning of October. During late winter to early spring SU and FA were not fed hay; however, SU calving cows wintered on range were supplemented 1.14 kg/hd daily of 28% CP dried distillers grain cube to meet protein requirements (Table 1). Additionally, SU wintered on cornstalks and FA were supplemented 0.45 kg/hd daily.

Table 1. Composition of 28% CP distillers grain cube[a]

| Item, % DM-basis | |
|---|---|
| Dried distillers grains plus solubles | 62 |
| Wheat midds | 11 |
| Cottonseed meal | 9 |
| Corn gluten feed | 5 |
| Molasses | 5 |
| Urea | 2 |
| Calcium carbonate | 3 |
| Binder | 3 |

[a]Formulated to have 22000 IU/kg of Vitamin A and 36 mg/kg Rumensin (Elanco Animal Health Greenfield, IN).

At calving, calves were assigned a calving difficulty score from 1 to 5(1= no assistance, 2= minor assistance; 3=difficult assistance, 4 = caesarean section, 5 = abnormal presentation) and a calf vigor score from 1 to 5 (1=nursed unassisted, 3 = nursed with assistance, and 5 = dead at birth). Calves from SP cows were weaned on October 31$^{st}$ (220 d of age). Calves from SU and FA were weaned on April 11$^{th}$, when calves were 298 and 247 d of age, respectively. For SU and FA April 11th was when cows grazing cornstalks during the winter returned to the ranch.

For each system, cow BW and BCS were recorded at three different periods during the year: 21-d before calving (pre-calving), 59-d post calving (pre-breeding), and at weaning. Calf BW was recorded at birth, dam pre-breeding, and weaning.

*Statistical Analysis.* Data from this study were analyzed as a completely randomized design using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Experimental unit for this study was group of cows within treatment. To determine the effect of calving date on cow performance the model included calving season, yr, and calving season by yr interaction. Contrast statements were used to evaluate the difference between calving season (SP vs. SU, SP vs. FA, and SU vs. FA). Spring calving cows and SU were used to determine the difference between wintering on cornstalks and wintering on native Sandhills range, since FA were only wintered on cornstalks. The model to test for differences between wintering systems included wintering system, yr, and yr by wintering system interaction. In all analyses, yr and yr by treatment interaction were not significant ($P > 0.10$); therefore, results are presented as main effects of calving date and wintering system. Data are presented as least squares means with differences considered significant at $P < 0.05$.

**Results and Discussion**

*Calving Season.* Calving difficulty ($P = 0.17$; Table 2) and calf vigor ($P = 0.54$) were not different among calving seasons. Pre-calving BW was similar for SP and SU ($P = 0.13$); however, FA was 108 and 78 kg heavier than SP ($P = 0.02$) and SU ($P = 0.04$). Body weight at pre-breeding was greatest for FA (574 kg) when compared to SP ($P < 0.01$) and SU ($P = 0.02$). Additionally, SU was 63 kg heavier ($P < 0.01$) than SP. Cow BW at weaning was lowest for SP ($P = 0.02$) when compared to SU and FA which were not different ($P = 0.23$).

Pre-calving BCS differed ($P = 0.03$) among calving seasons with FA having the greatest followed by SU and SP (Table 2). At pre-breeding, SP had the lowest BCS ($P < 0.01$) compared to SU and FA which were not different ($P = 0.64$). There was no difference ($P = 0.29$) in BCS at weaning among calving seasons.

There was a tendency for later calving cows to have increased birth BW compared to SP calving cows ($P = 0.13$ and $P = 0.08$ for SU and FA, respectively; Table 2). Spring calves were 18 kg and 14 kg lighter at pre-breeding than summer ($P < 0.01$) and fall ($P = 0.02$) calves, respectively. Calf weaning BW was similar ($P = 0.87$) for SP and FA calves; however, because of increased days of age, summer calves were 16 kg and 15 kg heavier than fall ($P = 0.12$) and spring ($P = 0.08$) calves, respectively. Calf ADG from birth to weaning was 0.20 kg/d and 0.13 kg/d greater for spring calves ($P = 0.03$) when compared to summer and fall calves, respectively, Weaning BW were not different ($P = 0.12$) for summer and fall calves. Adjusted 205 d weaning BW for calves was greatest for spring calves ($P < 0.01$) when compared to summer and fall calves which were not significantly different from each other ($P = 0.08$) even though calves from FA were 14 kg heavier.

Percent of cows to calve tended ($P = 0.07$) to be lower in FA vs. SP and was lower ($P = 0.04$) in FA vs. SU (Table 2). Rebreeding rate was similar ($P = 0.66$) for SP and SU; however, SP and SU were 8.7 and 9.1 percentage units greater ($P < 0.01$) than FA, respectively. Weaning rate as a percent of live calves was similar ($P = 0.11$) among calving seasons.

Differences in BW and BCS for the cows throughout the different periods of the year were expected because of how cow requirements (NRC, 1996) and nutrients from forage resources match up throughout the year. When SP enter into peak lactation, metabolizable protein (MP) is adequate and meets the cows requirements; however, at weaning when cows need to be increasing BW and storing nutrients to prepare for calving and milk production, MP of native Sandhills range is at the lowest point of the year. However, when comparing the SU and FA MP requirements to MP availability of the range, SU MP requirements are not met by range from December to June, however, from July to November (peak lactation through weaning) range MP exceeds SU requirements for MP. For FA, MP availability of the range is below MP requirements from calving to weaning; however, at weaning MP requirements exceed FA MP demand. Additionally, energy status is an extremely important factor that can

affect cow performance. During peak lactation which is April and May for SP, energy requirements would be the greatest. In April and May range TDN content peaks (Geisert et al., 2008). When comparing SU and FA, energy requirements are greatest during July and August for SU and September and October for FA. In the months of September and October range nutrient value has declined to dormant season nutrient levels. Additionally, rebreeding performance is directly related to the energy status of the cow (Randel; 1990). Therefore, FA having the lowest rebreeding performance could have been due to decreased energy availability for FA during the breeding season.

*Wintering System.* Calf vigor ($P = 0.29$; Table 3) and calving difficulty ($P = 0.82$) were not different between cows wintered on Sandhills native range or cornstalks. Additionally, cow BW and BCS at pre-calving ($P > 0.16$), pre-breeding ($P > 0.11$), and weaning ($P > 0.27$) were not different between wintering systems (Table 3).

Wintering system did not influence calf BW at birth ($P = 0.74$), at start of the breeding season ($P = 0.45$), or at weaning ($P = 0.26$). Additionally, calf ADG ($P = 0.33$) from birth to weaning and adjusted 205 d weaning BW ($P = 0.34$) was not different between wintering systems. Neither percent of cows to calve nor percent of live born calves to wean were influenced ($P > 0.20$) by wintering system, however, rebreeding rate was greater ($P = 0.04$) for cows wintered on cornstalks compared to cows wintered on native Sandhills range (Table 3).

Body weight and BCS for cows grazing cornstalks in the winter was similar when compared to cows grazing native Sandhills range. Similar results were presented by Anderson et al. (2003) who found that BW and BCS were not different between cows wintered on cornstalks or stockpiled pasture. However, in this study cows grazing cornstalks had an increased rebreeding percent. When evaluating cow numbers relative to rebreeding performance there are four more cows that were determined to be open in the group wintered on native range. However, when comparing the number of live born calves weaned, which was not significantly different, cows wintered on native range weaned 3 more calves than cornstalks. The increase in rebreeding for cornstalks was a net of 1 calf when compared to the numerical increase for calves weaned from cows were wintered on range. Therefore, given the extremely small difference in number of animals across treatments this response related to rebreeding performance is perhaps a consequence of too few experimental units since cow BW and BCS were not different across wintering treatments.

## Implications

Results from this study imply that calving season can have an effect on cow BW and BCS throughout the production year. Additionally, season of calving may also have an impact on rebreeding performance. When wintering cows on cornstalks a producer can expect similar BW and BCS score changes compared to cows wintered on native range; however, wintering program may have an effect on rebreeding performance.

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Table 2. Effect of calving season on cow performance

| Item | SP[b] | SU[c] | FA[d] | SEM | *P* value[a] SP vs. SU | SP vs. FA | SU vs. FA |
|---|---|---|---|---|---|---|---|
| n | 100 | 84 | 57 | --- | --- | --- | --- |
| Calf vigor[e] | 1.03 | 1.01 | 1.01 | 0.03 | 0.59 | 0.54 | 0.83 |
| Calving difficulty[f] | 1.05 | 1.01 | 1.00 | 0.03 | 0.17 | 0.17 | 0.67 |
| Cow BW | | | | | | | |
| Pre-calving, kg | 527 | 557 | 635 | 16 | 0.13 | 0.02 | 0.04 |
| Pre-breeding, kg | 485 | 548 | 574 | 4 | < 0.01 | < 0.01 | 0.02 |
| Weaning, kg | 482 | 511 | 505 | 4 | < 0.01 | 0.02 | 0.23 |
| Cow BCS | | | | | | | |
| Pre-calving | 5.2 | 6.1 | 6.8 | 0.1 | 0.01 | < 0.01 | 0.03 |
| Pre-breeding | 5.2 | 6.0 | 6.0 | 0.1 | < 0.01 | < 0.01 | 0.64 |
| Weaning | 5.1 | 5.0 | 5.0 | 0.1 | 0.29 | 0.65 | 0.65 |
| Calf BW | | | | | | | |
| Birth, kg | 36 | 38 | 40 | 1 | 0.13 | 0.08 | 0.27 |
| Pre-breeding, kg | 89 | 107 | 103 | 2 | < 0.01 | 0.02 | 0.21 |
| Weaning, kg | 233 | 248 | 232 | 6 | 0.08 | 0.87 | 0.12 |
| Adj. weaning, kg[e] | 184 | 145 | 159 | 4 | < 0.01 | 0.03 | 0.08 |
| Calf ADG[f], kg/d | 0.90 | 0.70 | 0.77 | 0.02 | < 0.01 | 0.03 | 0.09 |
| Calved, % | 96.8 | 99.4 | 90.4 | 1.8 | 0.20 | 0.07 | 0.04 |
| Calves weaned, % | 97.5 | 94.8 | 91.2 | 2.3 | 0.26 | 0.11 | 0.27 |
| Rebreeding, % | 93.1 | 93.5 | 84.4 | 1.1 | 0.66 | 0.01 | 0.01 |

[a]*P* value = differences across treatments determined using contrast statements.
[b]SP = spring calving cows (average calving date = March 25th).
[c]SU = summer calving cows (average calving date = June 16th).
[d]FA = fall calving cows (average calving date = August 7th).
[e]Calf vigor = 1=nursed unassisted, 3 = nursed with assistance, and 5 = dead at birth.
[d]Calving difficulty = 1= no assistance, 3=hard assistance, and 5 = abnormal presentation.
[e]Adj. weaning = calf weaning weight adjusted to 205 d.
[f]Calf ADG = ADG for the calf from birth to weaning.

Table 3. Effect of wintering system on cow performance

| Item | Cornstalks | Native range | SEM | *P* value |
|---|---|---|---|---|
| n | 92 | 92 | --- | --- |
| Calf vigor[a] | 1.03 | 1.00 | 0.02 | 0.29 |
| Calving difficulty[b] | 1.02 | 1.02 | 0.02 | 0.82 |
| Cow BW | | | | |
| Pre-calving, kg | 565 | 580 | 12 | 0.33 |
| Pre-breeding, kg | 535 | 537 | 3 | 0.46 |
| Weaning, kg | 502 | 493 | 3 | 0.27 |
| Cow BCS | | | | |
| Pre-calving | 5.9 | 6.2 | 0.1 | 0.16 |
| Pre-breeding | 5.7 | 5.8 | 0.4 | 0.11 |
| Weaning | 5.0 | 5.1 | 0.1 | 0.29 |
| Calf BW | | | | |
| Birth, kg | 38 | 38 | 1 | 0.74 |
| Pre-breeding, kg | 99 | 100 | 2 | 0.45 |
| Weaning, BW | 234 | 241 | 5 | 0.26 |
| Adj. weaning[c], kg | 160 | 165 | 3 | 0.34 |
| Calf ADG[d], kg/d | 0.78 | 0.80 | 0.02 | 0.33 |
| Calved, % | 95.8 | 95.2 | 1.4 | 0.69 |
| Calves weaned, % | 92.9 | 96.1 | 1.8 | 0.20 |
| Rebreeding, % | 92.3 | 88.3 | 0.8 | 0.04 |

[a]Calf vigor = 1=nursed unassisted, 3 = nursed with assistance, and 5 = dead at birth.
[b]Calving difficulty = 1= no assistance, 3=hard assistance, and 5 = abnormal presentation.
[c]Adj. weaning = calf weaning weight adjusted to 205 d.
[d]Calf ADG = ADG for the calf from birth to weaning.

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# INFLUENCE OF GRAZING DEFERMENTS FOLLOWING SUMMER FIRE ON EWE PERFORMANCE AND FORAGE QUALITY*

**R. C. Waterman and L. T. Vermeire**

USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT 59301

**ABSTRACT:** Complete rest or grazing deferment is a general recommendation following fire in the western U.S. to encourage vegetative recovery. However, effects of grazing deferments on animal performance have not been determined. Ewe performance and forage quality were evaluated for 70-d grazing trials with deferments until spring (May 17), early summer (June 21), or late summer (August 2) in the yr following summer fire in the Northern Great Plains. Within each deferment, three 1.5 ha plots were each grazed by 12 ewes (including two rumen-cannulated ewes). Ewes were weighed on d 0, 35, and 70 to evaluate BW changes to determine if length of time in a plot influenced performance. Forage quality was assessed by complete rumen evacuations, subsequent grazing and collection of rumen extrusa on d 15, 31, 51, and 68 of each grazing period. There was an interaction between deferment × weigh day ($P < 0.01$) indicating ewe BW differed across deferments. However, BW gains for the initial 35 d in each 70-d grazing trial were similar. Body weight gains the last 35 d ($P < 0.01$ for deferment × period [1st and 2nd 35 d)] were similar to initial 35 d for spring but remarkably lower for early summer and ewes actually lost BW the last 35 d in late summer deferment. Forage quality characteristics declined with later deferments and as time progressed within each grazing period. Forage CP (% OM) was highest ($P < 0.01$) during spring grazing (9.7%) and decreased to similar concentrations of 6.7 and 6.3% for early and late summer grazing, respectively. No differences ($P > 0.05$) were observed for extrusa NDF. However, in vitro NDF disappearance decreased ($P < 0.01$) from spring to late summer. Results show that ewe BW decreases as post-fire grazing deferment extends through summer and potential tradeoffs between livestock performance and plant recovery should be evaluated.

**Key Words:** Ewe, Forage Quality, Grazing Deferment, Summer Fire, Weight Gain

## Introduction

Historically, rangeland fires in the Northern Great Plains occur every 0-35 yr (Schmidt et al., 2002) with some magnification of fire intensity occurring due to fire suppression that has interrupted historic fire regimes. Consequently, land management agencies traditionally have favored multiple year deferments (minimum 2 yr) prior to allowing domestic livestock to reenter rangelands for grazing. Recently, a more methodical approach using site monitoring has been implemented aiding the decision of whether grazing can recommence (BLM, 2007). Minimal research is available documenting the association between livestock production and forage quality of rangeland vegetation in the yr following summer fire. Objectives of this research were: 1) to evaluate weight change in ewes during one of three 70-d grazing periods and 2) characterize forage nutritional quality from rumen extrusa collected from ruminally-cannulated ewes.

## Material and Methods

### *Study Area and Management*

This study was conducted at the Fort Keogh Livestock and Range Research Laboratory (**LARRL**) located approximately 1.6 km west of Miles City, MT (46°22´N 105°5´W) from May 2007 through October 2007. The LARRL Institutional Animal Care and Use Committee approved all animal handling and experimental procedures used in the present study (no. 101106-1). The LARRL encompasses 22,500 ha of Northern Great Plains rangelands and has an average elevation of 730 m, which includes rolling hills and barren land set apart by roughly eroded ridges, peaks, and mesas with small intersecting streams that seasonally drain into large permanent rivers meandering through broad, nearly level valleys. Experimental plots were on a silty ecological site, dominated by perennial cool-season grasses. The most abundant graminoids were western wheatgrass (*Pascopyrum smithii*, 27%), needle-and-thread (*Hesperostipa comata*, 17%), threadleaf sedge (*Carex filifolia*, 15%), and blue grama (*Bouteloua gracilis*, 12%). Fringed sage (*Artemisia frigida*) and pricklypear (*Opuntia polyacantha*) cactus were frequent. Fringed sage comprised less than 3% of the biomass. All forbs combined were generally less than 8% of the biomass. Average current-year forage production for the site is 930 kg/ha with near-average spring precipitation.

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Average daily temperatures range from -10°C in January to 24°C in July with daily maximum temperatures occasionally exceeding 37°C during summer and daily minimums occasionally dropping below -40°C during winter. Average annual precipitation is 341 mm with the majority of precipitation occurring from April through September from convectional thunderstorms. Actual precipitation was measured at study site via all-weather rain gauges (Analytical Scientific, Ltd. San Antonio, Texas) measured immediately following a precipitation event.

***Data Collection***

At the end of each deferment a 70-d grazing trial was initiated in spring (May 17), early summer (June 21), or late summer (August 2) in the yr following summer fire. Ninety white-face crossbred ewes were randomly allotted by weight to one of three plots (10 ewes/plot) within each 70-d grazing period. Ewes were weighed on d 0, 35, and 70 of each grazing trial. Ewes were gathered the day prior to weighing and held off of feed and water for a 12-h shrink.

Twelve ruminally-cannulated ewes (2/plot) were also used in the study to provide rumen extrusa samples to estimate and describe nutritional chemical composition of forages grazed by experimental ewes. Diet extrusa samples were collected on May 4, May 19, June 6, and June 24 for spring, and June 6, June 24, August 14, and August 28 for early summer; and on August 14, August 28, September 20, and October 10 for late summer grazing trials. On day of extrusa sampling, ruminally-cannulated ewes grazing concomitantly with other ewes were gathered and ruminal contents were completely evacuated and stored in 19-L plastic buckets, and ruminal walls were sponge dried to remove any residual moisture as described by Lesperance et al. (1960). Ewes were then released into experimental plots and allowed to graze for 45 to 60 min. After the grazing bout, extrusa was removed from the rumen and thoroughly mixed. An aliquot was saved for analysis and original ruminal contents were replaced. Collected extrusa samples (1 from each ewe) were frozen at -20°C, lyophilized, ground to pass a 1-mm screen, and stored until analysis for DM, OM (AOAC, 1990), and NDF (Goering and Van Soest, 1970). Sub-samples of ground extrusa were placed in glass, square bottom vessels with metal rod inserts and dried in an oven at 60°C for 12 h. Upon removal from a drying oven, vessels were capped with lids and subsequently placed on a roller grinder for 24 h (Mortenson, 2003). Nitrogen was determined by combustion techniques using a C-N analyzer (CE Elantech, Inc., Lakewood, NJ). Nitrogen values were multiplied by 6.25 to obtain CP, which was then expressed on an OM basis.

At 0700 on the day of in vitro analyses, rumen extrusa boluses were collected from 5 ruminally-cannulated ewes on alfalfa hay diets and placed directly into Dewar flasks (Nalgene 4150-200- StevenJo & Steph Rochester, NY 14625) that had been incubated to 39°C for 24 h. Outside temperatures were -18 °C when in vitro incubations occurred, requiring prompt handling of rumen contents to insure the integrity of microorganisms essential for in vitro analysis. Samples were immediately transported to LARRL and rumen boluses were strained through four layers of cheesecloth into a 6-L Erlenmeyer flask that had been pre-warmed in a 39°C water bath under continuous $CO_2$ flushing. Next, 500 mL of rumen liquor was measured out into a graduated cylinder and was then combined with 500 mL of pre-made phosphate buffer [70.8% $Na_2HPO_4$ and 29.2% $KH_2PO_4$; Menke et al., (1979)] and 1000 mL of McDougal's buffer (Tilley and Terry, 1963) already in vessels of a DAISY$^{II}$ apparatus (ANKOM Technology Corp., Fairport, NY) maintained at 39°C. Vessels also contained samples [250 mg of sample/bag (F57; 5 × 5.55 $cm^2$ ANKOM Technology Corp., Fairport, NY)]. Vessels were purged with $CO_2$ for 30 s and a lid was secured onto the jar and immediately placed back into the DAISY$^{II}$ apparatus (process was repeated for each of four vessels). Samples were then subjected to in vitro incubation for 48 h at 39°C. At the end of 48-h, incubation bags containing samples were removed and rinsed under RO water until effluent was clear. Bags were then placed into ANKOM$^{220}$ Fiber Analyzer (ANKOM Technology Corp., Fairport, NY) and NDF disappearance was determined. Bags were then dried at 60°C for 48 h to determine residual DM weights followed by placement into a muffle furnace at 550°C for 8 h to determine residual OM weights. In vitro NDF disappearance (IVNDFD) was calculated as the OM which disappeared from the initial weight inserted into the bag.

***Statistical Analysis***

Animal weight data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with plot as the experimental unit. Grazing period (70-d period beginning in May, June, and August) and sampling period and their interactions were included in the model. The REPEATED statement included sampling period and compound symmetry was used as the covariance structure. Estimates were considered significant if $P \leq 0.05$.

Forage quality data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with animal within plot as the experimental unit. Grazing period (period beginning at end of deferment) and sampling period and their interactions were included in the model. The REPEATED statement included sampling period and compound symmetry was used as the covariance structure. Estimates were considered significant if $P \leq 0.05$.

## Results and Discussion

Monthly temperature and precipitation for 2006 and 2007 along with their 70-yr averages are presented in Figure 1. Temperatures were slightly cooler than the 70-yr average in both yr. Fall and spring precipitation following fire were 133 and 123% of average, providing good conditions for forage production. Summer precipitation was less than 22% of the average during the grazing trial. However, 90% of forage is generally produced by July 1 because of prevailing weather and dominance by cool-season perennial grasses in the region (Heitschmidt and Vermeire, 2005). Total current-year production was 1239 kg/ha, based on caged plots excluding sheep.

Forage quality and quantity are the most important factors that influence domestic rangeland livestock production. Forage DM, as expected, increased ($P < 0.05$) from spring through late summer and CP declined respectively over this same period (Table 1). Additionally, IVNDFD was greater ($P < 0.05$) in spring and early summer than in late summer. Forage DM increased ($P < 0.05$) within each 70-d grazing period, with corresponding declines in CP concentrations (Table 2).

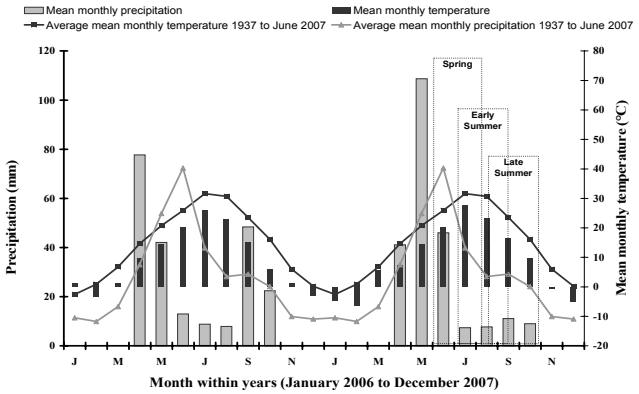


**Figure 1.** Monthly precipitation and average monthly temperature from January 2006 to December 2007 (bars) and 70-yr average (line) for Miles City, MT. Annual precipitation was 220, 231 mm, respectively for 2006 and 2007 with a 70-yr average annual precipitation of 341 mm. Initiation of 70-d grazing trials starting in May for spring, June for early summer, and August for late summer in 2007 as indicated by dashed lines in 2007. Information obtained from Western Regional Climate Center (WRCC, 2006) for average monthly and annual temperature and average annual precipitation.

Also, IVNDFD was higher ($P < 0.05$) at d 15, 31, and 51 than on d 68 of each grazing period (Table 2). An interaction for forage OM indicated that spring forage increased in OM as the 70-d grazing period progressed and early summer OM concentrations remained similar throughout the grazing period; however, late summer forage OM concentrations declined substantially in the later portion of the grazing period (Figure 2). These finding agree with others (Adams and Short, 1988; Johnson et al., 1998; Grings et al., 2005) that rangelands in the Northern Great Plains decline in forage quality from spring to late summer.

Body weight measurements indicated that ewes going onto trial were similar ($P < 0.05$) on d 0 and 35 for the spring and early summer 70-d grazing trial, but ewes in the late summer trial were 5 kg heavier ($P < 0.05$) on d 0 and 35. By d 70 no differences ($P > 0.05$) in BW for spring, early summer and late summer ewes were measured (Table 3).

In Table 4, weight gain (kg) and ADG (kg/d) for the initial and final 35-d and overall 70-d grazing period for spring, early summer, and late summer ewes is presented. No differences ($P > 0.05$) in weight gain or ADG was measured in the initial 35-d of each grazing period. However, differences ($P < 0.05$) in weight gain were measured in the final 35-d of each grazing period indicating that as deferment into summer was extended ewe performance declined.

**Table 1.** Forage characteristics of extrusa samples collected from ruminally-cannulated ewes grazing rangeland during three periods [deferred grazing until May (Spring); June (Early summer); or August (Late summer)] following summer fire the previous year

| | Forage Characteristics[1] | | | |
|---|---|---|---|---|
| Item | DM (%) SE = 0.09 | CP (% OM) SE = 0.35 | NDF (% OM) SE = 2.00 | IVNDFD[2] (% OM) SE = 1.44 |
| Spring | 92.6[a] | 9.7[a] | 70.0 | 61.2[a] |
| Early summer | 93.1[b] | 6.7[b] | 73.0 | 58.8[a] |
| Late summer | 93.6[c] | 6.3[b] | 68.2 | 51.4[b] |

[*]Means within columns with different superscript differ by $P \leq 0.05$
[2]In vitro NDF disappearance (IVNDFD)

**Table 2.** Forage characteristics of extrusa samples collected from ruminally-cannulated ewes grazing rangeland during three periods [deferred grazing until May (Spring); June (Early summer); or August (Late summer)] following summer fire the previous year at specific days into period

| | Forage Characteristics[1] | | | |
|---|---|---|---|---|
| Item | DM (%) SE = 0.09 | CP ( % OM) SE = 0.34 | NDF (% OM) SE = 1.59 | IVNDFD[2] (% OM) SE = 1.49 |
| D15 ± 1.7 | 92.7[a] | 8.8[a] | 70.6 | 60.1[a] |
| D31 ± 2.3 | 93.0[b] | 7.3[bc] | 71.5 | 58.5[a] |
| D51 ± 1.2 | 93.2[bc] | 7.7[c] | 71.5 | 57.9[a] |
| D68 ± 0.3 | 93.4[c] | 6.8[b] | 67.9 | 51.9[b] |

[1] Means within columns with different superscript differ by $P \leq 0.05$
[2]In vitro NDF disappearance (IVNDFD)

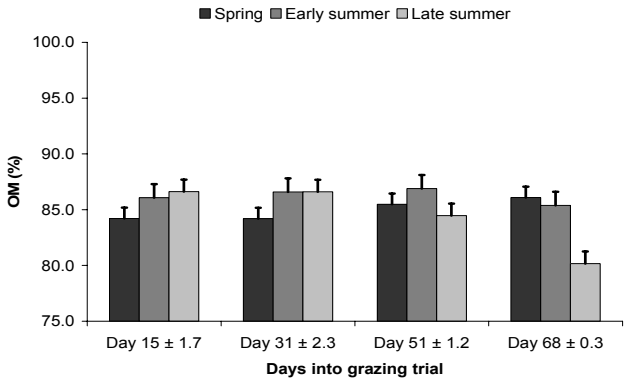


**Figure 2.** Forage OM of extrusa samples collected from ruminally-cannulated ewes grazing rangeland during three periods [deferred grazing until May (Spring); June (Early summer); or August (Late summer)] following summer fire the previous year.

## Implications

Decisions to graze rangelands in the Northern Great Plains the year following a summer fire will greatly depend on forage availability and stability of soils. However, decisions based on reseeding (rehabilitation; excluding heavy forest sites) should be less of a priority since perennial cool- and warm-season graminoids comprise the majority of biomass and 90% of that biomass is produced by July 1. Animals will obtain greater gains in spring and those gains will diminish as summer progresses. Livestock gains in Northern Great Plains rangelands commonly decrease with the advance of summer, regardless of fire. The reduced gains we observed indicate effects on animal performance should be accounted for in deferment decisions.

**Table 3.** Initial, mid-period, and final weights of sheep during three grazing periods [deferred grazing until May (Spring); June (Early summer); or August (Late summer)] following summer fire the previous year

| | Weight (kg)* SE = 0.88 | | |
|---|---|---|---|
| Item | Day 0 | Day 35 | Day 70 |
| Spring | 44.0[a] | 46.6[a] | 49.2 |
| Early summer | 45.0[a] | 47.2[a] | 48.8 |
| Late summer | 50.4[b] | 53.3[b] | 49.2 |

*Means within columns with different superscript differ by $P \leq 0.05$

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**Table 4.** Sheep weight gain and ADG during the 1st and 2nd 35 d of three grazing periods [deferred grazing until May (Spring); June (Early summer); or August (Late summer)] following summer fire the previous year

| | Weight * | | | | | |
|---|---|---|---|---|---|---|
| | Day 0 to 35 | | Day 35 to 70 | | Day 0 to 70 | |
| Item | Gain (kg) SE = 0.45 | ADG (kg/d) SE = 0.01 | Gain (kg) SE = 0.45 | ADG (kg/d) SE = 0.01 | Gain (kg) SE = 0.44 | ADG (kg/d) SE = 0.006 |
| Spring | 2.6[a] | 0.07[a] | 2.6[a] | 0.07[a] | 5.2[a] | 0.07[a] |
| Early summer | 2.6[a] | 0.07[a] | 1.1[b] | 0.03[b] | 3.8[b] | 0.05[b] |
| Late summer | 2.9[a] | 0.08[a] | -4.1[c] | -0.12[c] | -1.2[c] | -0.02[c] |

*Means within columns with different superscript differ by $P \leq 0.05$ within gain (kg) and ADG (kg/d)

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# BEEF COW PERFORMANCE FOLLOWING RUMEN-PROTECTED CHOLINE SUPPLEMENTATION DURING THE PERIPARTURIENT PERIOD[1]

**J. R. Jaeger[2], S. R. Goodall[3], K. R. Harmoney[2], and KC Olson[4]**

[2]Western Kansas Agricultural Research Center, Kansas State University, Hays, [3]Feed Industries Consultant, Erie, CO, [4]Kansas State University, Manhattan.

**ABSTRACT:** Our objective was to determine if rumen-protected choline (RPC) supplementation during the peri-parturient period could improve beef cow performance. Angus x cows (n = 181) were stratified by age, BW, and body condition score (BCS), and assigned randomly to one of two groups: control (CON) and RPC. Treatments were initiated 50 d before expected calving date and continued for 70 d. Cows were maintained in separate groups and received forage sorghum hay *ad libitum* and 0.68 kg/hd/d ground milo; RPC was added to the grain supplement to provide 4 g/hd/d choline for RPC-treated cows. Backfat (BF) over the 13th rib, marbling (MB), and longissimus muscle depth (LMD) were measured via ultrasound. Cow BW and BCS and ultrasound measurements were collected on d 0, d 50, at calving, and on d 120. Calf birth weight and ADG at 56 d of age were recorded. During the pre-calving period RPC tended (P=0.06) to have greater ADG than CON (0.45 vs. 0.35 kg, respectively). Pre-calving RPC supplementation did not affect (P>0.20) BCS, BF, MB, or LMD. Post-calving weight loss by CON was less (P<0.01) than that by RPC (-0.71 vs. -1.11 kg/d, respectively); moreover RPC lost MB and CON gained MB (P<0.01; -0.03 vs. 0.46, respectively). Post-calving BCS, BF, and LMD of CON and RPC were similar (P>0.20). Over the entire study, RPC lost more weight than CON (P<0.01). Supplementation of RPC had no effect (P=0.99) on calf birth weight but CON calves tended (P=0.08) to have greater ADG at 56 d of age than RPC calves (1.00 vs. 0.93 kg, respectively). Proportion of cows cycling at estrous synchronization was similar (P=0.65) between treatments. Conception rate to AI tended (P=0.11) to be greater for RPC than for CON (53 vs. 41%, respectively). There was a weak tendency (P=0.17) for more RPC that were anestrus at initiation of synchronization to conceive to AI compared to CON (55 vs. 35%, respectively). Final pregnancy rate did not differ (P=0.49) between treatments. Supplementation with RPC increased weight loss by cows but tended to improve conception to AI.

**Key Words:** Beef Cows, Choline, Parturition, Performance

## Introduction

Choline (Vitamin B4) can become a component of acetylcholine, phosphatidylcholine, or supply methyl groups through conversion to betaine. Methyl groups are required for a variety of metabolic reactions including methionine recycling and liver fatty acid mobilization.

Choline is rapidly degraded in the rumen (Sharma and Erdman, 1988). Therefore ruminants have limited intestinal absorption of methyl groups due to ruminal degradation of choline and betaine.

Supplementation of dairy cows with rumen-protected choline (**RPC**; 12 g/hd/d) from 28 d prepartum to 63 d postpartum resulted in increased milk yield and accelerated body weight loss after calving (Hartwell et al, 2000). Increasing dietary RPC in periparturient dairy cows also resulted in increased liver glycogen and liver esterified lipids secretion (Piepenbrink and Overton, 2003). In contrast, Janovick Gueretzky and coworkers (2006) observed no production or metabolic benefits from feeding 15 g/hd/d RPC to dairy cows pre- and postpartum.

To date, there has been no research that examined RPC supplementation to pre- or postpartum beef cows. However, several research trials have evaluated the effect of RPC on feedlot cattle performance and subsequent carcass characteristics.

Our objectives were: 1) to determine the effect of feeding RPC to beef cows during late gestation; and 2) to determine the effects of feeding of RPC after calving on reproductive performance by lactating cows and growth performance of calves.

## Materials and Methods

*Animals*

Angus crossbred cows (n = 181; age = 3 to 11 yr) were stratified by age, BW, and body condition score (BCS; 1 = emaciated, 9 = very obese; Wagner et al., 1988) and assigned randomly to one of two treatment groups: control (**CON**) and rumen-protected choline (RPC). Treatments were initiated 50 d before the expected beginning of the calving season and continued for 120 d. During the treatment period, cows were maintained in separate groups and received forage sorghum hay *ad libitum* and 0.95 kg/hd/d supplement. Supplement contained rumen-protected choline (4 g/hd/d choline) and SQM trace mineral (Quali Tech, Chaska, MN) for RPC-treated cows and SQM trace mineral only for control cows (Table 1). Body

[1]Contribution no. 08-331-A, Agric. Exp. Sta., Manhattan. The authors would like to acknowledge Quali Tech for their donation of trace mineral and rumen-protected choline, and Pfizer Animal Health for their donation of Lutalyze and EAZI-BREED CIDR.

weight was measured and BCS estimates were collected on d 0, on 50, at calving, and on d 120. Backfat (**BF**) thickness, marbling (**MB**), and longissimus muscle depth (**LMD**) were measured in the region of the 12th and 13th ribs on d 0, d 50, at calving, and on d 120 via ultrasound using an Aloka 500V (Aloka Co., Ltd, Wallingford, CT) B-mode instrument equipped with a 3.5-MHz general purpose transducer array (UST 5021-125 mm window). Images were collected with Cattle Performance Enhancement Company (**CPEC**, Oakley, KS) software. Backfat thickness, LMD, and MB were estimated with procedures that incorporated image analysis software (Brethour, 1994) that are an integral component of the CPEC product. Marbling scores were coded such that 4.0 = slight$^{00}$ (low select) and 5.0 = small$^{00}$ (low choice).

Treatment effect on calf performance was assessed by measuring calf BW on the day of birth and when all calves averaged 56 d of age.

Table 1. Supplement composition

| | Treatment group | |
|---|---|---|
| Ingredient, % DM | Control | RPC |
| Rolled milo | 69.05 | 69.05 |
| Soybean meal (44%) | 25.00 | 25.00 |
| Trace mineral supplement (5.95 %) | | |
| Zinc | 0.08 | 0.08 |
| Manganese | 0.08 | 0.08 |
| Copper | 0.03 | 0.03 |
| Choline | 0.00 | 0.54 |

*Blood Collection and RIA*

Blood was collected via coccygeal venipuncture at -10 and 0 d before estrous synchronization with the Co-Synch + CIDR system, which occurred 26 d after termination of supplementation. Blood samples were allowed to clot and were stored at 4° C for 24 h. Serum was collected after centrifugation and stored at -20° C until analysis for $P_4$ concentration. Serum concentrations of $P_4$ were determined by RIA (Skaggs et al., 1984) with intra- and interassay CV of 4.54% and 5.40% and an assay sensitivity of 0.5 ng/mL. Cows were considered to be estrous-cycling if the concentrations of $P_4$ in serum were elevated (≥ 1.0 ng/mL) at one or both sampling times.

*Breeding and Pregnancy Diagnosis*

Fixed-time AI (**FTAI**) was performed at 56 h after CIDR (Pfizer Animal Health, New York, NY) removal and $PGF_{2\alpha}$ (Lutalyse; Pfizer Animal Health, New York, NY) administration. All cows were injected with GnRH (100 µg of Cystorelin i.m.; Merial, Duluth, GA) at the time of insemination using semen from 1 of 3 sires and AI was performed by 1 of 2 experienced technicians. Cows were exposed to 4 fertile bulls 10 d after FTAI for 35 d (45-d breeding season).

Pregnancy rate to FTAI was determined by transrectal ultrasonography (Aloka 500V equipped with a 5.0-MHz linear-array transducer, Aloka, Wallingford, CT) 33 d after FTAI. Final pregnancy rates were determined 120 d after the end of the breeding season.

*Statistical Analyses*

Data were grouped to examine changes that occurred during the 50 d prepartum period or during the postpartum period. Weight change (average daily gain or loss), calf birth weight, calf ADG at 56 d of age, and change in backfat thickness, LMD and MB were evaluated by analysis of variance using the PROC ANOVA procedure of SAS (SAS Inst. Inc., Cary, NC). Estrous cyclicity before estrous synchronization, AI sire, AI technician, pregnancy rate to FTAI, and final pregnancy rate after the end of the breeding season were analyzed using PROC CATMOD of SAS.

## Results and Discussion

Average calving date occurred on d 78 of the study. During the 50 d pre-calving period, cows receiving RPC tended to have greater (P = 0.06) ADG than CON (Table 2). In contrast, supplementation of prepartum dairy cows for 25 d prepartum with 60 g/hd/d RPC (15 g dietary choline) had no effect on BW or DMI (Janovick Guretzky et al., 2006). Zahra and coworkers (2006) also reported 56 g/hd/d RPC (14 g dietary choline) supplementation of dairy cows had no effect on prepartum DMI. Interestingly, these researchers observed that DMI of thin cows (BCS < 4) was not altered by RPC supplementation but that fat cows (BCS ≥ 4) receiving RPC had significantly higher DMI than control cows. Beef steers fed 5 g/hd/d dietary choline displayed increased ADG compared to control steers, but this response was diminished with increasing RPC levels (Bryant et al., 1999). Beef heifers fed a finishing diet containing RPC at either 0, 20, 40 or 60 g/hd/d (0, 5, 10, or 15 g dietary choline) had greater DMI than control heifers for only the first 30 d of exposure; however, ADG and gain efficiency were improved for RPC-supplemented heifers for 120 d (Bindel et al., 2000). Perhaps the BW response in dairy cows in the previous study was absent due to increased dietary levels of RPC. In our study, RPC-supplementation had no effect (P > 0.20) on BCS, BF, MB, or LMD (Table 2).

During the postpartum period, RPC supplementation had no effect (P > 0.20) on BCS, BF or LMD compared to cows receiving only trace mineral (Table 3). Postpartum weight loss of CON was less than that of RPC (P < 0.01). In addition, RPC lost more weight than CON (P < 0.01; -89.2 vs. -70.0 kg, respectively) over the entire study. Supplementation of dairy cows for 25 d prepartum through 49 d postpartum with 60 g/hd/d RPC (15 g dietary choline) had no effect on BW or BCS change (Janovick Guretzky et al., 2006). Similarly, RPC-supplementation of dairy cows from wk 5 postpartum to wk 21 postpartum had no effect on average BW or DMI (Erdman and Sharma, 1991). Cows fed RPC also lost MB during the postpartum period and CON gained MB (P < 0.01; Table 3). Pinotti and coworkers (2003) observed a postpartum increase in plasma NEFA and NEFA:cholesterol was lower in RPC than CON.

Table 2. Effect of prepartum supplementation with trace

minerals or trace minerals and rumen-protected choline on cow performance.

| Item | Treatment group | | SEM |
|---|---|---|---|
| | Control | RPC | |
| ADG (kg) | 0.35 | 0.45 | 0.03 |
| BCS change[a] | -0.40 | -0.29 | 0.06 |
| BF change (mm)[b] | -0.27 | -0.44 | 0.09 |
| LMD change (mm)[c] | -1.86 | -2.13 | 0.72 |
| Marbling score change[d] | 0.52 | 0.67 | 0.06 |

[a]Body Condition Score 1 = emaciated, 9 = very obese.
[b]Back Fat measured over 12th and 13th ribs with ultrasound.
[c]Longissimus Muscle Depth measured over 12th and 13th ribs with ultrasound.
[d]Marbling scores were coded such that 4.0 = slight00 (low select) and 5.0 = small00 (low choice).

Increased plasma NEFA leads to increased uptake by the liver where NEFA are esterified to triglycerides, oxidized to ketone bodies, or oxidized to carbon dioxide. The esterification of NEFA to triglycerides and their export into VLDL involves choline. In addition, choline serves as a methyl donor for the synthesis of carnitine and carnitine is essential for fatty acid oxidation. The lesser plasma NEFA of RPC-supplemented cows reported by Pinotti and coworkers (2003) may have resulted from more efficient liver function and improved lipid metabolism. These data could explain why RPC-supplemented cows in our study lost MB during the postpartum period and CON gained marbling.

Table 3. Effect of postpartum supplementation with trace minerals or trace minerals and rumen-protected choline on cow performance.

| Item | Treatment group | | SEM |
|---|---|---|---|
| | Control | RPC | |
| ADG (kg)* | -0.71 | -1.11 | 0.07 |
| BCS change[a] | 0.03 | 0.02 | 0.04 |
| BF change (mm)[b] | -0.14 | -0.39 | 0.12 |
| LMD change (mm)[c] | -0.82 | -0.64 | 0.58 |
| Marbling score change[d]* | 0.46 | -0.03 | 0.06 |

[a]Body Condition Score 1 = emaciated, 9 = very obese.
[b]Back Fat measured over 12th and 13th ribs with ultrasound.
[c]Longissimus Muscle Depth measured over 12th and 13th ribs with ultrasound.
[d]Marbling scores were coded such that 4.0 = slight00 (low select) and 5.0 = small00 (low choice).
*Means in row differ ($P < 0.01$).

Supplementation with RPC had no effect ($P = 0.99$) on calf birth weight which averaged 40.9 ± 2.3 kg. Conversely, calves from CON-supplemented cows tended ($P = 0.08$) to have greater ADG at 56 d of age than calves from RPC-supplemented cows (1.00 ± 0.22 vs. 0.93 ± 0.27 kg/hd/d, respectively). In contrast, Zahra and coworkers (2006) reported that periparturient dairy cows with BCS ≥ 4 had greater milk production when supplemented with 14 g RPC/hd/d compared to no RPC. Likewise, Pinotti and coworkers (2003) observed increased milk production following RPC supplementation of periparturient dairy cows with 20 g/hd/d dietary choline. In contrast, Zahra and coworkers (2006) observed that thin cows (BCS < 4) supplemented with RPC had similar milk production to control cows. Although cows in the current study had an initial average BCS of 5.4 and final average BCS of 5.3, these BCS may not have been adequate to provoke an increase in milk production that was previously observed for fleshy dairy cows.

The proportion of cows considered to be estrous-cycling at initiation of estrous synchronization was similar ($P = 0.65$; 71.6% (58/81) for CON and 74.7% (65/87) for RPC). The proportion of cows that conceived to FTAI tended to be slightly greater ($P = 0.11$) for RPC cows (52.9%; 46/87) compared to CON cows (40.7%; 33/81). There was a weak trend ($P = 0.17$) among cows that had not re-established estrual behavior before estrous synchronization for more RPC-supplemented cows to conceive to FTAI (54.5%; 12/22) than CON cows (34.8%; 8/23). In contrast, dairy cows receiving either 0, 15, 30 or 45 g/d dietary choline from wk 5 postpartum to wk 21 postpartum required more services per cow and were open more days with increasing choline intake (Erdman and Sharma, 1991). These authors also reported increased milk yield due to RPC-supplementation and concluded that reproductive responses were more related to increased milk production than to the effect of RPC. It is likely that RPC-treated cows in the current study did not have increased milk yield as judged by ADG of their calves at 56 d of age compared to CON calves. Furthermore, RPC-treated cows in the current study received RPC prepartum and displayed a tendency to have greater ADG during that period. Perhaps, improvement of cow performance prepartum played a greater role in regard to subsequent reproductive performance than the decrease in performance that occurred during the postpartum supplementation period. Final pregnancy rate was similar ($P = 0.49$) between CON (91.4%; 74/81) and RPC-supplemented (94.1%; 80/85) cows.

## Implications

Supplementation of periparturient beef cows with rumen-protected choline resulted in greater weight loss and marbling loss over the length of the study. Daily gain by beef cows fed rumen-protected choline tended to be improved during prepartum supplementation and poorer during postpartum supplementation compared to control cows. In addition, choline-supplemented cows tended to conceive to fixed-time artificial insemination in greater numbers than control cows. Although, the mechanisms responsible for intramuscular fat mobilization and improved reproductive response are not understood, these data were interpreted to suggest that choline supplementation during a specific time of the periparturient period may improve subsequent reproductive performance. Further investigation appears warranted.

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# REPRODUCTION IN YOUNG POSTPARTUM RANGE COWS SUPPLEMENTED WITH GLUCOGENIC PRECURSORS

**J. T. Mulliniks, M. E. Kemp, S. I. Valverde-Saenz, C. M. Gardner, S. H. Cox, A. Cibils, D. E. Hawkins, and M. K. Petersen**

New Mexico State University, Las Cruces, NM

**ABSTRACT:** Supplementing CP with propionate salts (**PS**) may improve returns in young range beef cows. A 3 yr study conducted at Corona Range and Livestock Research Center from February to mid-July in 2005 (n = 80), 2006 (n = 81), and 2007 (n = 80) evaluated days to first estrus, kg of calf weaned, and BW change in 2- and 3-yr-old postpartum cows grazing native range and individually fed 1 of 3, 36% CP supplements after parturition with increasing glucogenic potential (**GP**) supplied by UIP and PS. Supplements were fed at 908 g•cow$^{-1}$•d$^{-1}$ 2x weekly. Supplementation was initiated 7 d after calving for an average of 95 d. Supplements provided 1) 328 g CP, 110 g UIP, 44 g GP (**0**), 2) 328 g CP, 157 g UIP + 40 g PS (NutroCAL$^{TM}$, Kemin Industries, Inc.), 93 g GP (**40**), 3) 329 g CP, 158 g UIP + 80 g PS, 124 g GP (**80**). Body wt was recorded weekly and serum collected 2x weekly for progesterone analysis to estimate days to first estrus. Supplementation was terminated when cows reached BW nadir. Cows were exposed to bulls for 60 d or less starting May 15. Supplement and yr did not interact to influence days to first estrus or days and magnitude to BW nadir ($P \geq 0.18$). Days to first estrus exhibited a quadratic ($P = 0.04$) response to GP (78, 71, 77 ± 2 d for 0, 40, 80, respectively). Cows in 2006 required more days to BW nadir and lost more BW before achieving BW nadir ($P < 0.01$; 32, -26; 111, -40; 40, -31 ± 3 d, and ± 3 kg for 2005, 2006, and 2007, respectively). Pregnancy rates were 88, 96, 91% for 0, 40, and 80 fed cows, respectively ($P = 0.14$). Total kg of calf weaned per cow exposed to bulls (a measure of reproductive success) for the supplementation and following yr was greater ($P = 0.09$) for those fed 40 or 80 (349, 373, and 376 ± 13 kg for 0, 40, and 80, respectively). This study implies that young postpartum cows fed additional glucogenic precursors may improve reproduction and wean more calf weight per cow exposed to breeding.

Key Words: beef cattle, glucogenic precursors, protein supplementation

## INTRODUCTION

Young cows grazing primarily dormant range in New Mexico experience negative energy balance postpartum during early lactation. In low quality dormant forages, protein content tends to be more limiting to grazing animal performance than energy (Wallace, 1987). Therefore, a beef cow's nutritional needs may not be met by forage alone, and thus supplementation is necessary to meet the greater nutrient requirements. Protein supplementation has been found to enhance intake and digestibility of dormant grass and thus improve cow performance (McCollum and Horn, 1990). Once meeting degradable intake protein (**DIP**) requirements, undegradable intake protein (**UIP**) can decrease days to first estrus, BW loss (Wiley et al., 1991) and may increase first-service conception rates in first-calf heifers (Triplett et al., 1995; Vasquez et al., 2005). Undegradable intake protein supplements may also repartition nutrients away from lactation (Hunter and Magner, 1988) or promote synthesis of maternal tissues for maintenance, growth, and reproduction by improved nutrient use (Miner et al., 1990; Waterman et al., 2006). Waterman et al. (2006) and Endecott et al. (2006) found that 2-yr-old range cows fed protein supplements containing glucogenic precursors provided by UIP plus 80 or more g/d propionate salt (**PS**) while grazing dormant range decreased days to first estrus by 9 and 22 d, respectively, compared to cows fed traditional cottonseed meal-based supplements with no increase of glucogenic potential. The objectives of this study were to determine influences of increasing consumption of glucogenic precursors supplied in protein supplements on onset of cyclicity, pregnancy, weight change, and calf weaning weight. A secondary objective was to evaluate any treatment interactions with age or year.

## MATERIALS AND METHODS

This study was conducted during the spring and summer for 3 consecutive years (2005 to 2007) at New Mexico State University's Corona Range and Livestock Research Center, Corona, NM. The ranch's average elevation is 1,900 mm with an average precipitation of 400 mm. Rainfall during this study was 105% (2005), 76% (2006), and 117% (2007) of an 18-yr average (161 mm) for those months. The majority of precipitation occurs from July through September from convectional thunderstorms (Waterman, et al., 2006). Primary grass species in pastures used in this experiment were blue grama (*Bouteloua gracilis*) and wolftail (*Lycurus phleoides*) (Knox, 1998; Forbes, 1999). The annual standing forage was at least 355 kg/ha in each year in the 762-ha pasture (A. Cibils, New Mexico State University, personal communication). Forage availability was never limiting in all 3 yr even with the drought in 2006. Three ruminally cannulated cows were used to collect diet extrusa samples for analysis of CP (AOAC, 2000) and NDF (Van Soest et al., 1991) in 2006 and 2007. Extrusa samples averaged 3.3% and 8.1% CP, 78.6% and 85.9% NDF for 2006 and 2007, respectively (OM basis).

All animal handling and experimental procedures were in accordance with guidelines set by the New Mexico State University Institutional Animal Care and Use Committee. Cows (n = 281) were 2 (n = 144) and 3 (n = 97) yr of age and were primarily Angus with some Hereford influence. Management before calving was similar in all 3 yr and between age groups. Within age, cows were stratified by calving date to each treatment so that similar age and days postpartum was distributed equally across treatments. Breeding season started mid-May in all 3 yr and ended less than 60 d.

Supplements were cubed and milled at Hi-Pro Feeds, Friona, TX (2005 to 2006; Table 1a) and Alderman Cave, Roswell, NM (2007; Table 1b). Cows were individually fed at a daily rate of 908 g•cow$^{-1}$ twice weekly for 74 (2005), 120 (2006), and 80 (2007) days postpartum. Total days of supplement fed were strategically determined by monitoring average cow weight change within each year. Supplementation ended 14 days into breeding in 2005 and 2007 and 7 days prior to end of breeding in 2006. Cows had ad libitum access to water and loose macro and micro mineral mix year long. Supplements provided 1) 328 g CP, 110 g UIP, 44 g GP (**0**), 2) 328 g CP, 157 g UIP + 40 g PS (NutroCal$^{TM}$, Kemin Industries, Inc.), 93 g GP (**40**), 3) 329 g CP, 158 g UIP + 80 g PS, 124 g GP (**80**). Glucogenic potential was calculated by the equation of Preston and Leng (1987), where 40% of the UIP is considered to be glucogenic (Overton et al., 1999). NutroCal$^{TM}$ contains 80% propionate, which is assumed to be 95% glucogenic (Steinhour and Bauman, 1988).

**Table 1a**. Ingredient composition of supplements (0, 40, 80 g/d propionate salt) in 2005 and 2006.

| Item | 0 | 40 | 80 |
|---|---|---|---|
| Ingredients | | % | |
| Cottonseed meal | 56.94 | 18.15 | 21.30 |
| Urea | 1.20 | 1.20 | 1.20 |
| Wheat middlings | 21.45 | 40.10 | 32.50 |
| Fish meal | -- | 13.00 | 13.00 |
| Hydrolyzed feather meal | -- | 12.00 | 12.00 |
| Soybean meal | 10.00 | -- | -- |
| NutroCal | -- | 4.40 | 8.80 |
| Molasses | 9.00 | 9.00 | 9.00 |
| Vitamin & minerals | 1.41 | 3.35 | 3.40 |

**Table 1b**. Ingredient composition of supplements (0, 40, 80 g/d propionate salt) in 2007.

| Item | 0 | 40 | 80 |
|---|---|---|---|
| Ingredients[a] | | % | |
| Cottonseed meal | 57.82 | 59.46 | 61.31 |
| Corn gluten feed | 32.60 | 5.00 | 5.00 |
| Distillers dried grain | -- | 17.79 | 10.71 |
| Fish meal | -- | 7.07 | 9.41 |
| NutroCal | -- | 4.41 | 8.81 |
| Urea | 1.39 | -- | -- |
| Molasses | 3.00 | 3.00 | 3.00 |
| Vitamins & minerals | 5.21 | 3.29 | 1.77 |

[a]Nutrient composition same as 2005 and 2006.

Cows were weighed weekly until the end of breeding and again at weaning (Figure 1). Days to BW nadir were determined from the lowest BW after calving. Body wt change was evaluated between key intervals that included: beginning of supplementation to BW nadir, beginning of supplementation to the beginning of breeding, BW nadir to beginning of breeding, end of supplementation to end of breeding, initial BW to weaning weight. Body condition scores (1 = emaciated, 9 = obese) were assigned to each cow by visual observation and palpation at initiation of the study, at branding, and at weaning. Calf birth weights were recorded within 3 d after birth in the field using a portable scale and BW were recorded at branding and weaning. Calf branding and weaning weights were adjusted for a 55-d branding and 205-d weaning weight and no adjustments were used for sex of calf or age of dam.

Serum samples were collected twice weekly on supplemental days (Monday and Friday) via coccygeal venipuncture (Corvac, Sherwood Medical, St. Louis, MO) beginning approximately 35 d postpartum (by cow) for analysis of progesterone to determine days to first estrus (2 or more consecutive progesterone concentrations $\geq$ 1.0 ng/mL). Serum was analyzed for progesterone concentration by solid phase RIA (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) as described by Schneider and Hallford (1996). Inter- and intra-assay CV were less than 10%. Cows were diagnosed pregnant by rectal palpation at weaning or a few weeks later.

Data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with cow as the experimental unit using the Kenward-Roger degrees of freedom method. The model included fixed effects of supplement, cow age, year, and their interactions. Covariates were calving date, sex of calf, days supplemented and were used when appropriate. Three contrast statements were used to test for linear, quadratic, or 0 vs. 40 + 80 effects of increasing amounts of glucogenic precursors. The GENMOD procedure of SAS was used to analyze pregnancy data. Significance was determined at $P \leq 0.10$.

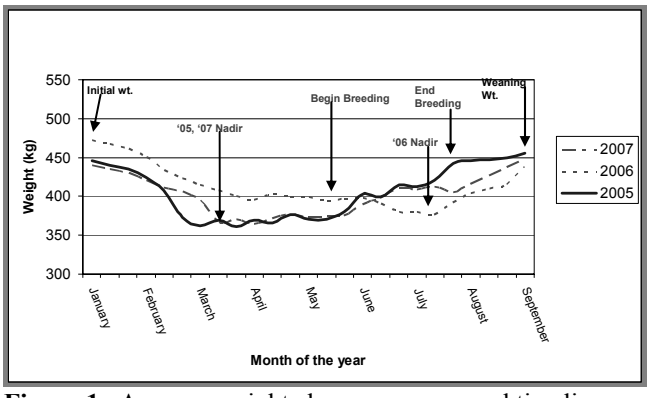


**Figure 1**. Average weight change per year and timeline.

## RESULTS AND DISCUSSION

Days to first estrus after calving exhibited a quadratic ($P = 0.04$; Table 2) response to increasing GP (78, 71, 77 ± 2 d for 0, 40, 80, respectively) demonstrating fewer days to first estrus with consumption of increasing amounts of GP. Two-year-old cows took longer to return to estrus than 3-yr-old cows ($P = 0.03$; 79 and 72 ± 2 d). In 2005 and 2006, cows returned to estrus in the same time

frame ($P = 0.82$), whereas cows in 2007 required more days to first estrus ($P < 0.01$; 72, 71, and 83 ± 3 d for 0, 40, and 80, respectively. Pregnancy rates were 88, 96, 91% for 0, 40, and 80 fed cows, respectively ($P = 0.14$). (Although, 2 cows fed 80 were considered reproductively incompetent, for non-treatment reasons, their data was still included. Pregnancy rate for the 80's without those 2 cows would have been 94%.) Calving intervals were similar ($P = 0.68$) for 2005 and 2006 cows showing that the drought of 2006 had a minimum impact on reproduction. (Calving interval data was not available for 2007 at time of writing). Supplemental GP favorably influenced days to first estrus and pregnancy.

Cow BW and BW change intervals were similar among supplement groups at all measurement times ($P \geq 0.15$; Table 3). Days to BW nadir were similar among treatment groups ($P = 0.64$; 59, 63, and 61 ± 3 d for 0, 40, and 80, respectively) and did not interact with year. However, cows in 2006 required more days to reach BW nadir than cows in 2005 and 2007 ($P < 0.01$; 32, 111, and 40 ± 14 d for 0, 40, and 80, respectively). Cows in 2006 also lost more BW from beginning of supplementation to BW nadir than cows in 2005 and 2007 ($P = 0.01$; - 26, - 40, and - 31 ± 3 kg for 2005, 2006, and 2007, respectively; Figure 1). A longer period of negative energy balance did not negatively affect days to first estrus in 2006 as had been proposed by Canfield and Butler (1990). Cows in 2006 reached first estrus 39 d before attaining positive energy balance. Cows in 2006 also lost weight throughout the duration of supplementation, and did not gain weight until the final week of the breeding season. Weight loss trend in 2005 and 2007 were similar to each other and ended 70 d earlier compared to 2006.

Cow BCS were similar among all treatment groups from beginning of supplementation to weaning ($P \geq 0.12$; Table 3). Average BCS at calving was 4.8. Two-year-old cows calved at a higher BCS than 3-yr-old cows ($P < 0.01$; 5.1 and 4.5 ± 0.05 for 2- and 3-yr-old, respectively). Cows in 2006 had a lower BCS at weaning than in 2005 and 2007($P < 0.01$; 4.4, 3.9, and 4.2 ± 0.06 for 2005, 2006, and 2007, respectively). Lower BCS did not manifest into inferior reproduction in 2006.

Calf BW at branding and weaning did not differ among supplement groups ($P \geq 0.35$; Table 3). Calves from dams fed 40 had a tendency to be heavier at branding and weaning than calves from dams fed 0 and 80. Heavier calves from cows fed 40 might have been caused by a slight increase of milk production by cows fed 40 (Mulliniks et al., 2008). Calves in 2006 had the heaviest branding weight ($P = 0.11$; 64 and 61 ± 2 kg for 2006 and 2007, respectively) and the lightest 205-d weaning weight ($P < 0.01$; 219, 159, and 218 ± 4 kg for 2005, 2006, and 2007). Observations suggest that cows in 2006 provided more milk at the expense of BW until branding than in other years. The drought in 2006 along with negative energy balance worked together to cause a decrease in milk yields and subsequently lower weaning weights. Calves from 2- and 3-yr-old cows had similar 205-d weaning weights ($P = 1.00$; 199 and 199 ± 4 kg for 2- and 3-yr-old cows, respectively).

Total kilograms of calf weaned per cow exposed to breeding bulls have been suggested to be a primary production evaluation criterion by taking into account reproductive success and calf growth potential. It is the sum of the influences of the current year's conditions, milk production, and the previous year's response to conception timing and reproductive rate. Increasing amounts of glucogenic potential, in this study, decreased days to first estrus and increased pregnancy rates providing the opportunity to wean heavier/older calves the following year. Ramsey et al. (2005) defined ranch productivity as pounds weaned per exposed female, which integrates 3 main production variables: calving percentage, calf death loss, and breeding-season length. Total kilograms of calf weaned per cow exposed to bulls for the supplementation year and the year after supplementation was greater ($P = 0.09$; Table 2) for those fed 40 or 80 vs. those fed 0 (349, 373, and 376 ± 13 kg for 0, 40, and 80, respectively). A financial comparison was calculated to show predicted margins from each supplement. Free-choice mineral ($3.98/yr) was added to the postpartum feed cost (Sawyer et al., 2005). All calves were valued at $2.20/kg at weaning. Even though supplement costs were greater for 40 and 80 ($53.94, $71.12, and $79.36/cow for 0, 40, and 80 respectively), cows fed 40 or 80 had fewer days to estrus and greater pregnancy rates, therefore total kilograms calf weaned per cow exposed the next year was greater. This resulted in a $33.58 - $35.82/cow increase in income when 80 and 40 were compared to 0. Endecott (2006) found similar results of $19.42/cow increase in income when feeding an undegradable intake protein plus 80 g of calcium propionate compared to a traditional cottonseed meal-based supplement. Because cows fed 40 were more reproductively efficient and more cost effective, feeding 40 g/d of calcium propionate is most likely a cost effective postpartum supplementation strategy.

## IMPLICATIONS

Results from this study show that supplementing undegradable intake protein with additional glucogenic precursors decreased days to first estrus and increased pregnancy rates in 2- and 3-year-old range cows. Cows fed additional glucogenic precursors appear to wean heavier calves the following year, which will increase returns beyond the expense of the higher cost supplement ingredients.

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**Table 2**. Reproduction measurements and financial comparison of cows fed increasing amounts of glucogenic precursors (0, 40, 80 g/d propionate salt).

| Supplement | Pregnancy rate (%)[a] | Pregnancy rate ratio | Days to first estrus[b] | Calving interval (d) | Total kg calf weaned/cow exposed (2yr)[c] | Postpartum Feed Cost (2yr $/cow) | Predicted income difference ($) |
|---|---|---|---|---|---|---|---|
| 0 | 88 | 67/76 | 78 | 374 | 349 | 53.94 | --- |
| 40 | 96 | 81/84 | 71 | 377 | 373 | 71.12 | 35.62 |
| 80 | 91 | 73/80 | 77 | 372 | 376 | 79.36 | 33.98 |
| SEM | -- | -- | 3 | 4 | 13 | -- | -- |

[a]($P = 0.14$)
[b]($P < 0.10$) Quadratic contrast
[c]($P < 0.10$) 0 vs. 40 + 80 contrast

**Table 3**. Effect of supplements containing increasing amounts of glucogenic precursors (0, 40, 80 g/d propionate salt) on calf weight, cow weight, and cow body condition score for 2- and 3- year-old postpartum cows grazing native range.

| | Supplement | | | | | Contrast | |
|---|---|---|---|---|---|---|---|
| Response | 0 | 40 | 80 | SEM | *P*-value | Linear | Quadratic |
| Calf BW, kg | | | | | | | |
| Branding | 63 | 64 | 60 | 4 | 0.35 | 0.27 | 0.37 |
| Weaning | 198 | 201 | 197 | 4 | 0.68 | 0.77 | 0.42 |
| Cow BW change, kg | | | | | | | |
| Begin supplementation- BW nadir | -32 | -32 | -33 | 2 | 0.88 | 0.63 | 0.90 |
| Begin supplementation - begin breeding | -3 | -8 | -3 | 3 | 0.18 | 0.98 | 0.07 |
| BW nadir - end supplementation | 37 | 35 | 38 | 2 | 0.56 | 0.71 | 0.32 |
| BW nadir - begin breeding | 15 | 9 | 13 | 3 | 0.30 | 0.71 | 0.13 |
| BW nadir - end breeding | 53 | 50 | 50 | 2 | 0.56 | 0.32 | 0.67 |
| Begin supplementation - end supplementation | 5 | 3 | 5 | 3 | 0.81 | 0.97 | 0.52 |
| End supplementation - end breeding | 16 | 14 | 11 | 2 | 0.23 | 0.09 | 0.88 |
| Initial Wt - Final Wt | -7 | -9 | -9 | 3 | 0.90 | 0.69 | 0.83 |
| Days to BW Nadir | 60 | 63 | 61 | 3 | 0.63 | 0.76 | 0.36 |
| Cow BCS | | | | | | | |
| Begin supplementation | 4.7 | 4.9 | 4.7 | 0.05 | 0.12 | 0.74 | 0.04 |
| Branding | 4.2 | 4.2 | 4.2 | 0.06 | 0.69 | 0.72 | 0.42 |
| Weaning | 4.6 | 4.7 | 4.7 | 0.06 | 0.28 | 0.17 | 0.36 |

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# EVALUATION OF CALF OUTPUT AND PROGENY CARCASS DATA OF COMMERCIAL BEEF BULLS

**A.L. Van Eenennaam†, D.J. Drake*†, and D.J.Garrick‡**
†University of California, Davis, CA and ‡Iowa State University, Ames, IA

**ABSTRACT:** Multi-sire breeding pastures preclude visual paternity identification; limiting opportunities for evaluation of sire prolificacy or progeny performance. Furthermore, few producers retrospectively receive individual carcass data resulting from the harvesting of their calves. As a result, commercial cow-calf producers have little opportunity to evaluate the performance of their herd bulls. In this study, DNA-based testing was used to assign paternity to a cohort of 205 steers from a cowherd bred to 19 registered Angus bulls that had passed a breeding soundness exam. Carcass data was collected following a feedlot phase where all steers were fed as a contemporary group. Gross income was assigned to carcasses based on carcass weight and a $2.87/kg carcass value, adjusted by the American Angus Association grid. One hundred and eighty calves were assigned to single sires (87.8 %), and all sires were excluded from the remaining 25 (12.2%) animals. The number of steer calves per bull varied from 0 to 22. The two highest output bulls accounted for nearly a quarter of all the calves assigned. Carcass data showed significant differences between sires for carcass weight, yield grade, marbling score, fat thickness, grid value, and value per carcass. Breed association EPDs for these young bulls were low accuracy, about 0.05 for carcass traits and 0.3 for ultrasound carcass traits and some were poorly correlated with observed carcass measurements: reflecting the shortcomings of the low accuracy EPDs that are associated with young sires. The average carcass value of steers from different sires varied by as much as $160. The total value of steer calves produced showed large variation between sires, ranging from $0 to $21,098. Calf output was more important than average carcass value in determining the gross income per sire. The five most prolific bulls contributed half of the total income from the steer calf crop. This work demonstrates the need for accurate tools for early decisions regarding bull selection.

**Key Words:** parentage, bull, evaluation, EPD

## Introduction

Many beef cattle are produced in multi-sire breeding pastures limiting opportunities for visual sire identification and genetic evaluation. Single bull breeding pastures can provide paternity but are not typically used due to extensive land use, inadequate fences, risk of bull failure, and the increased labor costs associated with pasture subdivisions. DNA-testing offers an approach to assign parentage without changing production practices (Dodds et al., 2005), and provided the tests are sufficiently powerful they can even be used to assign paternity in large multi-sire groups (Van Eenennaam et al., 2007). Multi-sire breeding pastures have shown large variation in calf output (number of calves) by individual sires (DeNise, 1999; Holroyd et al., 2002; Van Eenennaam et al., 2007). Age of bull, breed, fertility-associated antigen status, sperm motility, and morphology, and social dominance have been associated with variation in calf output in multi-sired herds (Whitworth et al., 2003). In the absence of progeny information, genetic evaluation of herd bulls has been limited to that derived from their pedigree. Although breed-based EPDs are the most effective selection tool currently available for yearling bulls, the low accuracy of young sire evaluations means that considerable genetic variation remains among bulls with similar EPD profiles. DNA-testing allows progeny testing to be undertaken on commercial bulls and offers an approach to develop on-ranch or "commercial ranch" genetic evaluations of herd bulls based on the performance of their offspring under field conditions. Such evaluations may help producers identify bulls whose progeny perform well in their ranch environment. Tracking the performance of individual offspring through processing and grading further presents an opportunity to improve the accuracy of carcass trait genetic evaluations for herd bulls. Genetic evaluations for beef carcass traits have not been reported from multi-sire breeding herds. The objective of this work was to use parentage assigned from DNA markers to determine calf output, progeny carcass trait performance, and gross carcass value derived from commercial sires in multi-sired breeding pastures. Additionally, commercial carcass trait genetic evaluations were developed and compared to the low accuracy pedigree-based genetic evaluations typically associated with yearling bulls.

## Material and Methods

*Animals and ranch operation.* This study was conducted on a commercial farm using animals that were owned by the cooperator and standard animal husbandry practices were employed. Nineteen bulls registered with the American Angus Association (**AAA**) were randomly assigned to three multi-sire breeding pastures with mature cows. All bulls were in good body condition and had passed a breeding soundness examination (**BSE**) by a licensed veterinarian prior to the breeding season. Bulls were assigned to achieve approximately a 25:1 female:bull ratio. Injuries and fighting, as well as changes in body condition of bulls, contributed to management decisions to move bulls among breeding groups to maintain an approximate 25:1 ratio throughout the breeding season. A single Angus AI sire selected for calving ease was used to breed a group of first-calf heifers. Calves were born between 1/4/06-3/3/06 and steers were shipped and fed together on a commercial

feedlot in California. Steers were on feed for 149 days and group performance was 1.29 kg/d. Individual carcass data was obtained by a USDA grader. Gross income was derived for individual carcasses based on weight and a $2.87/kg carcass value adjusted by the AAA grid. Partial assumptions of this grid are: Choice-Select spread $11.00, Prime premium $8.00, Standard discount $-15.00, YG 1 premium $3.00, YG 2 premium $1.50, YG 4 & 5 discount -$25.00.

*DNA collection.* Tail hair samples were obtained from the 19 natural service sires and their steer progeny to obtain DNA for parentage determination. A semen straw was used to obtain DNA from the one AI sire. No DNA was obtained from the dams. Microsatellite analysis and sire/calf matching was conducted at the UC Davis Veterinary Genetics Laboratory as described by Van Eenennaam et al. (2007).

*Data analysis.* Carcass traits and values were analyzed by ANOVA as a randomized design with sire as the class variable. Carcass weight was used as a covariate. Contrasts were used to separate groups of bulls for mean comparisons. All statistical procedures were conducted in the General Linear Model module of Systat 11. Genetic evaluation of carcass traits from the steer progeny of 17 herd bulls and the AI sire was carried out using a sire model equation y = m+Zu+e where y represented the dependent variable, m was the mean, u was a vector of direct sire progeny differences, Z was an incidence matrix relating calves to their sires, and e was a vector of residuals. The resulting mixed model equations were solved directly, and BIF accuracies were computed from diagonal elements of the inverse coefficient matrix assuming a heritability ($h^2$) of 0.25. Spearman's rank correlation was used to compare commercial ranch and AAA EPD rank.

## Results and Discussion

Calves were either assigned to a single sire (n=180) or were excluded from all sire candidates (n=25). Large differences in steer calf output (Figure 1), ranging from 0 to 22 steer calves per bull, were observed. Age was associated ($P$ <0.01) with prolificacy, and no progeny were assigned to the two yearling bulls involved in this study.

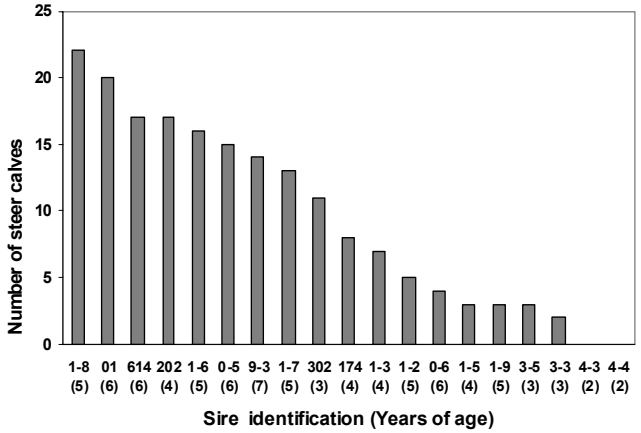


**Figure 1.** Number of steer calves assigned to each herd sire present in multi-sire breeding pastures as determined by microsatellite analysis of DNA from progeny and sires.

Sire differences (Table 1) were found for mean carcass weight, yield grade, fat thickness, marbling score, grid value, and mean value per carcass, but not for ribeye area ($P$=0.22). Carcass weight was a significant covariate for all carcass traits except grid value. When adjusted for carcass weight, sire remained significant for value per carcass, fat thickness, marbling score, yield grade and grid value. There was a sire effect ($P$<0.05) on mean carcass value which ranged from $798 to $958, a difference of $160 per head (Figure 2). None of the 16 progeny of the sire with the highest mean carcass value ('1-6') had a marbling grade of Slight or less, whereas 85% (17/20) of the progeny of sire '01' graded Slight or less. This information is not typically relayed to commercial cow-calf producers, and although it impacts the profitability of the feedlot sector, it is only of direct economic relevance to producers who retain ownership of their cattle or receive some premium for product quality.

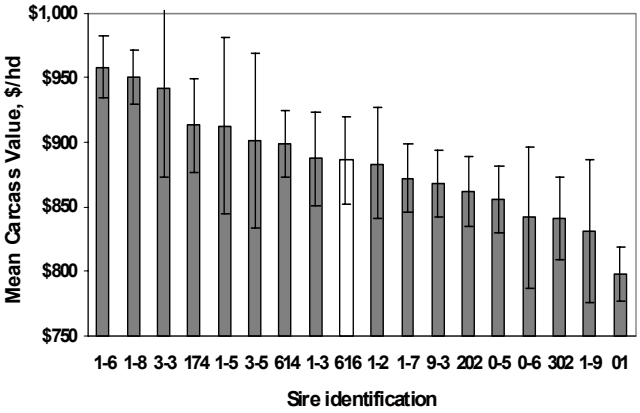


**Figure 2.** Mean value of carcasses (SEM) derived from steer calves assigned to herd sires in multi-sire breeding pastures. The white bar indicates the AI bull.

The total gross value of steer carcasses produced showed large variation between sires, ranging from $0 to $21,098 (Figure 3). In aggregate, the top five bulls produced nearly 50 percent of the gross income derived from the steer calf crop. The bull with the lowest mean carcass value ('01') was ranked second in terms of gross carcass value due to his high calf output.

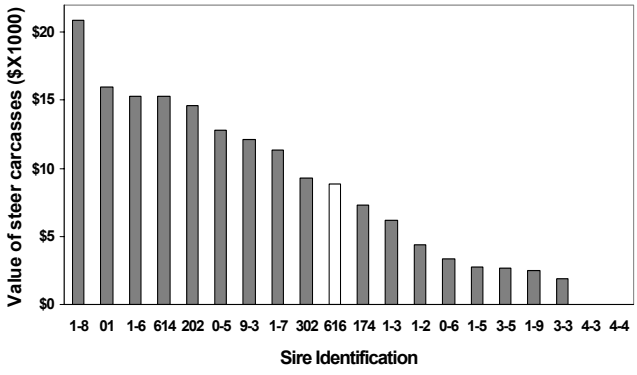


**Figure 3.** Total gross value of carcasses from steer calves assigned to herd sires in multi-sire breeding pastures. The white bar indicates the AI bull.

Commercial ranch EPDs were calculated for carcass weight (CARCASS), marbling score (MARBLING), ribeye area (REA), rib fat thickness measurement (FAT), and AAA grid value (AAAGRID) for the 17 herd bulls that had carcass data (Table 2). The BIF accuracy for the commercial ranch EPDs ranged from 0.06 for bulls with only 2 phenotyped progeny, to 0.35 for the bull with 21 phenotyped progeny.

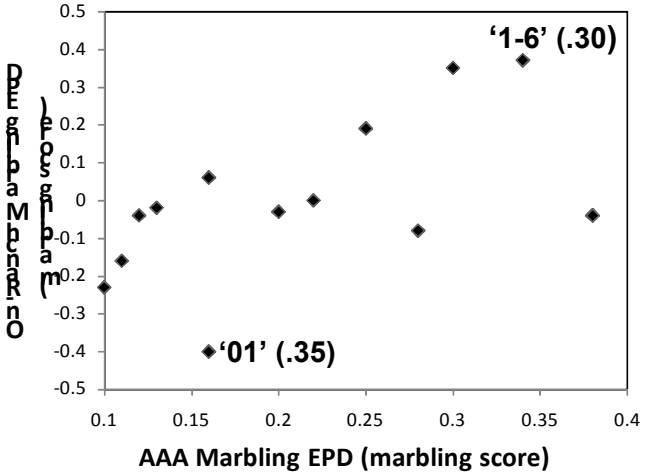


**Figure 4.** Commercial ranch marbling EPD versus American Angus Association (AAA) EPD for commercial Angus sires. The bulls with the highest and lowest commercial ranch EPDs had 16 and 20 phenotyped steer progeny, respectively. The BIF accuracy of the commercial ranch EPD for these two bulls is shown in brackets (assuming heritability=0.25).

The rank order of the bulls using the commercial ranch EPDs was compared to those based on AAA EPDs. Rank order correlations of carcass traits ranged between 0.37 and 0.47 except for carcass weight which was lower (0.10), and fat thickness which had a negative correlation (Table 3).

**Table 3.** Spearman rank order correlations between AAA and commercial ranch EPDs

| Commercial ranch EPD: AAA EPD | n | Spearman Rank Order Correlation |
|---|---|---|
| CARCASS: CARCASS | 14 | 0.10 |
| MARBLING:MARBLING | 14 | 0.43 |
| MARBLING:IMF | 17 | 0.37 |
| REA:REA | 14 | 0.47 |
| REA:ultrasoundREA | 17 | 0.38 |
| FAT:FAT | 14 | - 0.20 |
| FAT:ultrasoundFAT | 17 | 0.47 |
| AAAGRID:AAAGRID | 14 | 0.37 |

Most natural service bull-buying decisions are made using relatively low accuracy EPDs. The use of DNA-tests to assign parentage gives commercial cow-calf producers the opportunity to develop commercial ranch EPDs for any trait that is routinely measured on their calves. Culling decisions can then be informed by both realized calf output and EPDs developed under individual commercial ranch conditions.

Large variation in bull output was observed in this study and greatly affected the gross income derived from each bull. Other studies have shown bull output to be moderately repeatable across years providing the composition of bull mating groups remain relatively similar (DeNise, 1999; Holroyd et al., 2002). Van Eenennaam et al. (2007) reported that young bulls often sired no progeny when run in a multi-sire group with mature bulls. Given the obvious importance of calf output on bull profitability, and the fact that years of service has a significant effect on the return to investment of developing commercial ranch EPDs (Weaber, 2004), using fewer total bulls while managing young bulls as a separate breeding group would seem to be a common-sense practice with high potential for economic return.

If cow-calf producers retain ownership of their cattle or receive product quality premiums, then carcass attributes become economically relevant and should factor into selection decisions. The commercial EPDs developed in this project ranked some bulls quite differently to the rank derived from low accuracy carcass trait EPDs based on pedigree indices. Further economic analysis is needed to determine if the benefit derived from making improved selection decisions based on commercial ranch EPDs, outweighs the additional expenses involved with genotyping and collection of progeny performance data.

**Acknowledgements**

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**Table 1.** Mean value and standard deviations of carcass traits of progeny groups for herd sires

| Sire | n | Carcass weight (kg) | SD | YG | SD | Marbling score [a] | SD | Rib eye area ($cm^2$) | SD | Fat Depth (mm) | SD | Grid Value ($) | SD | Value per carcass ($) | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-6 | 16 | 339 | 7 | 2.9 | 0.2 | 5.7 | 0.2 | 83.9 | 1.9 | 13.4 | 1.1 | -1.86 | 1.84 | 958 | 24 |
| 1-8 | 21 | 344 | 6 | 3.0 | 0.1 | 5.5 | 0.2 | 82.7 | 1.6 | 13.1 | 1.0 | -4.86 | 1.60 | 951 | 21 |
| 3-3 | 2 | 324 | 21 | 2.3 | 0.4 | 5.5 | 0.6 | 84.2 | 5.3 | 9.1 | 3.2 | 1.78 | 5.20 | 941 | 68 |
| 174 | 7 | 320 | 11 | 3.0 | 0.2 | 6.1 | 0.3 | 75.4 | 2.9 | 11.8 | 1.7 | -0.76 | 2.78 | 913 | 36 |
| 1-5 | 2 | 360 | 21 | 3.5 | 0.4 | 4.6 | 0.6 | 85.5 | 5.3 | 18.3 | 3.2 | -15.20 | 5.20 | 913 | 68 |
| 3-5 | 2 | 326 | 21 | 2.0 | 0.4 | 4.7 | 0.6 | 84.2 | 5.3 | 6.1 | 3.2 | -5.15 | 5.20 | 901 | 68 |
| 614 | 14 | 329 | 8 | 2.4 | 0.2 | 5.0 | 0.2 | 79.5 | 2.0 | 7.6 | 1.2 | -6.16 | 1.96 | 899 | 26 |
| 1-3 | 7 | 342 | 11 | 2.8 | 0.2 | 4.9 | 0.3 | 80.6 | 2.9 | 11.5 | 1.7 | -11.57 | 2.78 | 887 | 36 |
| 616[b] | 8 | 326 | 10 | 3.1 | 0.2 | 5.5 | 0.3 | 79.9 | 2.7 | 14.5 | 1.6 | -7.16 | 2.60 | 886 | 34 |
| 1-2 | 5 | 323 | 13 | 3.0 | 0.3 | 4.9 | 0.4 | 78.1 | 3.4 | 12.4 | 2.0 | -6.11 | 3.29 | 884 | 43 |
| 1-7 | 13 | 321 | 8 | 2.7 | 0.2 | 5.0 | 0.2 | 78.8 | 2.1 | 11.1 | 1.2 | -6.76 | 2.04 | 872 | 27 |
| 9-3 | 14 | 340 | 8 | 3.2 | 0.2 | 4.7 | 0.2 | 78.0 | 2.0 | 12.9 | 1.2 | -14.38 | 1.96 | 868 | 26 |
| 202 | 12 | 326 | 8 | 2.9 | 0.2 | 4.7 | 0.2 | 78.6 | 2.2 | 11.9 | 1.3 | -10.04 | 2.12 | 862 | 28 |
| 0-5 | 14 | 329 | 8 | 3.1 | 0.2 | 4.5 | 0.2 | 77.2 | 2.0 | 12.9 | 1.2 | -12.00 | 1.96 | 856 | 26 |
| 0-6 | 3 | 340 | 17 | 3.2 | 0.3 | 4.7 | 0.5 | 78.5 | 4.4 | 13.2 | 2.6 | -17.98 | 4.24 | 842 | 55 |
| 302 | 9 | 306 | 10 | 2.8 | 0.2 | 4.9 | 0.3 | 74.1 | 2.5 | 11.1 | 1.5 | -5.42 | 2.45 | 841 | 32 |
| 1-9 | 3 | 308 | 17 | 2.2 | 0.3 | 4.5 | 0.5 | 76.8 | 4.4 | 6.8 | 2.6 | -7.67 | 4.24 | 831 | 55 |
| 01 | 20 | 304 | 7 | 2.1 | 0.1 | 4.3 | 0.2 | 78.6 | 1.7 | 6.8 | 1.0 | -10.70 | 1.64 | 798 | 21 |
| *P* [c] | | 0.006 | | <.0001 | | <.0001 | | 0.22 | | <.0001 | | <.0001 | | 0.0013 | |

[a] Marbling score: 4.0=Slight- (Select-), 5.0=Small- (Choice-), 6.0=Modest- (Choice).
[b] A.I. sire.
[c] Test of sire effect within column.

**Table 2.** Commercial ranch and EPDs and BIF accuracies (ACC) for 17 registered Angus herd bulls derived from carcass records on a single contemporary group containing 2 to 21 steer progeny per sire, and American Angus Association carcass EPDs and ACC.

| | Commercial ranch | | | | American Angus Association | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | EPD | | ACC | | EPD | | | ACC | | | |
| | min | max | min | max | min | max | mean | min | max | mean | Percentile[a] |
| CARCASS | - 30 | 13 | 0.06 | 0.35 | -2 | 13 | 4.29 | 0.05 | 0.05 | 0.05 | 50 |
| MARBLING | -0.4 | 0.37 | 0.06 | 0.35 | 0.06 | 0.38 | 0.21 | 0.05 | 0.05 | 0.05 | 40 |
| REA | -0.2 | 0.35 | 0.06 | 0.35 | 0.02 | 0.36 | 0.16 | 0.05 | 0.05 | 0.05 | 47 |
| FAT | -0.1 | 0.5 | 0.06 | 0.35 | -0.02 | 0.03 | 0 | 0.05 | 0.05 | 0.05 | 56 |
| AAA GRID | -3.16 | 3.08 | 0.06 | 0.35 | 12.3 | 25.2 | 17.8 | -- | -- | -- | 31 |
| ultrasoundREA | -- | -- | | | -0.1 | 0.52 | 0.18 | 0.19 | 0.37 | 0.32 | 60 |
| IMF | -- | -- | | | 0.01 | 0.31 | 0.18 | 0.17 | 0.38 | 0.30 | 30 |
| ultrasoundFAT | -- | -- | | | -0.02 | 0.02 | 0 | 0.20 | 0.39 | 0.33 | 48 |

[a] Average EPD percentile compared to current Angus bulls (http://www.angussiresearch.com/brekdown.html?epd_parent_ct=1).

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# FACTORS INFLUENCING PRICE OF NORTH DAKOTA, SOUTH DAKOTA, AND MONTANA FEEDER CALVES

**J. L. Leupp[1], G. P. Lardy[1], R. Daly[2], C. L. Wright[3], and J. A. Paterson[4]**
[1]Department of Animal Sciences, North Dakota State University, Fargo, ND 58105
Department of Veterinary Science, South Dakota State University, Brookings, SD 57007
[3]Department of Animal and Range Sciences, South Dakota State University, Brookings, SD 57007
[4]Department of Animal and Range Sciences, Montana State University, Bozeman, MT 59717

**ABSTRACT:** Our objective was to determine factors influencing sale price of feeder calves from ND, SD, and MT auction markets. Data were collected at 3 auction markets in ND and 2 auction markets in both SD and MT in fall 2006 (68,475 calves; avg wt = 236 ± 57 kg; 3 sales per market) and winter 2007 (30,106 calves; avg wt = 294 ± 78 kg; 3 sales per market). Data were collected during the same wk in each state to reduce confounding effects of fluctuations in market patterns. The following data were collected for each lot of calves sold and used as independent variables in the MIXED procedures of SAS: lot size, sex, wt, breed description, vaccination history, implant status, and natural qualified. In the fall, lot sizes ≥ 21 head received greater ($P$ = 0.04; \$114.74 vs. \$111.38/45.45 kg) prices when compared with lots of ≤ 20 head. Lot sizes of 11–20 and 6–10 head were priced similarly ($P$ = 0.92; \$112.85 and \$112.76/45.45 kg, respectively) but were greater than ($P$ < 0.001) lots of ≤ 5 head (\$108.54/45.45 kg). Price for steers were \$9.78/45.45 kg greater ($P$ < 0.001) than heifers. Price for black cattle was greater ($P$ = 0.002) than prices received for mixed, red, and white cattle which were priced similarly ($P$ ≥ 0.28). Vaccinations (7-way clostridial, 4-way viral, and Pasteurella) increased ($P$ ≤ 0.04) sale price in the fall compared with calves with no vaccination history. In winter, price for lot sizes of ≥ 21 head was greater ($P$ < 0.03; \$99.42 vs. \$97.02/45.45kg) than lots of ≤ 20 head. Lot sizes of 11–20 and 6–10 head were priced similarly ($P$ = 0.38; \$98.47 and \$98.11/45.45 kg, respectively) but were greater than ($P$ < 0.001) lots of ≤ 5 head (\$94.47/45.45 kg). Price for steers were \$8.40/45.45 kg greater ($P$ < 0.001) than heifers. Price for both black and white cattle was greater ($P$ ≤ 0.04) than mixed and red cattle (\$98.43 vs. \$96.82/45.45 kg, respectively). Vaccination history did not affect ($P$ = 0.71) sale price in winter. Data suggest feeder calf price is dependent on multiple factors. Selling calves in larger lot sizes is economically advantageous; value of vaccination history varied depending on marketing time.
Key Words: auction markets, feeder calves, prices

## Introduction

Feeder calf prices are dependent on multiple factors. Many of these factors are affected by environmental conditions such as feed and cattle prices and/or weather conditions (Schroeder et al., 1988). Other factors can be can be controlled by the producer when calves are marketed such as lot sizes, BW, vaccination programs, and season (Schroeder et al., 1988; King and Seeger, 2004).

Data has effectively demonstrated calves marketed in larger lot sizes receive premiums compared with calves sold in smaller lot sizes (Schroeder et al., 1988; Barham and Troxel, 2007). Premiums may be paid for larger lot sizes because they facilitate filling truckloads and cattle originate from 1 source (Faminow and Gum, 1986).

Calves in value-added calf programs sell for greater prices, compared with unweaned, unvaccinated calves (King and Seeger, 2004; Corah et al., 2006). The price advantage for calves in value-added calf programs has been increasing in recent years (King and Seeger, 2004). Additional factors influencing sale price in these studies were region of the country, sex, breed description, horns, weight variation, lot size, flesh and frame score.

Little quantitative information exists on factors influencing price of North Dakota, South Dakota, and Montana feeder calves. Because prior management may affect calf prices received in the marketplace it is important to inform producers of these factors in order for them to make informed decisions. Therefore, the objectives of this study were to determine factors influencing sale price of feeder calves from ND, SD, and MT auction markets.

## Materials and Methods

*Data Collection*

Data were collected from 3 sale barns in ND and 2 sale barns in SD and MT during the weeks of October 23, October 30, and November 6, 2006 (**fall**), when most calves sold were freshly weaned. The 3 auction markets in ND included Napoleon Livestock, Napoleon; Kist Livestock, Mandan; and Stockmen's Livestock, Dickinson. The 2 auction markets in SD were Faith Livestock, Faith and Philip Livestock Auction, Philip. The 2 auction markets in MT were PAYS Auction Yard, Billings and Miles City Livestock Commission Company, Miles City. Data again were collected at the same auction markets during the weeks of January 15, January 29, and February 12, 2007 (**winter**). University representatives were present at each sale and collected the following for each lot of calves sold: lot size, sex, BW, hair color, health programs, vaccination history, use of deworming products, implant status, natural qualified, source and age verification status, and beef quality assurance (**BQA**) status.

Lot sizes were categorized into groups of ≥ 21 calves, 11–20 calves, 6–10 calves, and ≤ 5 calves. Lots of

calves sold were split into 4 hide color categories. Categories used for hide color were black-hided, red-hided, white-hided, and mixed-colored pens. Lots were categorized based on 75% of one lot having a predominant color. For example, a lot having 4 black-hided calves and 1 red-hided calf would be categorized as a black-hided lot. A lot having 2 black-hided calves and 3 red-hided calves would be categorized as a mixed-colored lot. Three categories for vaccination status were used. Categories included 1) calves receiving a 7-way clostridial plus 4-way viral plus *Pasteurella* (**741 vaccination program**), 2) 4-way viral only, or 3) calves with no vaccination history or only having a 7-way viral.

*Statistical Analysis*

Data were analyzed using the MIXED procedures of SAS. Lot was used as the experiment unit. A separate analysis was conducted for the fall and winter data sets. Statistical analysis was based on methods described by King et al. (2006).

Price was the dependent variable and independent variables used in the model included lot size, sex, BW hair color, vaccinations, implant status, and natural qualified. In order to prevent multicollinearity, a quadratic term for base weight was included in the model. Base weight is individual lot BW subtracted from mean BW of all the lots. The frequency procedure of SAS was used to determine number of lots in each category.

During the fall and winter, there were insufficient lots of calves marketed as source and age verified or BQA certified. Therefore, no data is presented for these factors. Similarly, in the winter there were insufficient lots of calves marketed as implanted or natural qualified, therefore these factors were not included in the data.

## Results

Data are presented as fall sales (October and November, 2006; Table 1) and winter sales (January and February, 2007; Table 2).

*Fall Sales*

During fall, 2006 there were 68,475 beef calves sold in 6251 lots (Table 1). The average BW was 236 kg with a price slide of \$8.60/45.45 kg.

Lot size affected ($P < 0.001$) calf price. Calves sold in lot sizes ≥ 21 calves were worth \$114.74/45.45 kg which was greater ($P = 0.04$) than lot sizes of ≤ 11 head. Calves sold in lot sizes of 11–20 and 6–20 were priced similarly ($P = 0.92$; \$112.81/45.45 kg). Calves sold in lot sizes of ≤ 5 sold for less ($P < 0.001$) than larger lot sizes (\$108.54/45.45 kg).

As expected, calf sex influenced ($P < 0.001$) sale price. Prices for steer calves were greater ($P < 0.001$) than prices for heifer calves (\$117.11/45.45 kg vs. \$107.33/45.45 kg for steers and heifers, respectively).

An effect ($P \leq 0.001$) of color was observed in the fall. Black-hided cattle sold for \$114.40/45.45 kg which was more than ($P < 0.002$) the other cattle. Pens of mixed-colored, red, and white-hided cattle were priced similarly ($P \geq 0.28$) and averaged \$111.50/45.45 kg.

An effect ($P < 0.001$) of vaccinations was observed for calves sold in the fall. Calves vaccinated with a 4-way viral only or 741 vaccination program were priced similarly ($P = 0.11$; \$112.85/45.45 kg) and were priced greater ($P = 0.04$) than calves sold with no vaccination history (\$110.96/45.45 kg).

In the fall there was a small premium for calves which qualified for a natural program ($P = 0.04$). Producers received \$1.55/45.45 kg premium when calves were marketed as natural qualified.

Implant status did not have an effect ($P = 0.18$) on sale price of calves. Insufficient lots sold as source and age verified and BQA certified in the fall prevented any determination of the value of those attributes.

*Winter Sales*

In the winter, 2007 there were 30,106 calves sold in 2698 lots (Table 2). The average BW was 294 kg with a price slide of \$5.50/45.45 kg.

Lot size had an effect ($P < 0.001$) on calf price during the winter. Calves sold in large lot sizes (≥ 21 calves) received a premium ($P = 0.03$) compared to calves sold in lot sizes of ≤ 11 head. Calves sold in lot sizes of 11–20 and 6–10 were priced similarly ($P = 0.38$) and sold for \$98.29/45.45 kg. Calves sold in small lot sizes of ≤ 5 sold for less ($P < 0.001$) than all other lots of calves (\$94.47/45.45 kg).

Calf sex had an effect ($P < 0.001$) on price. Steer calves sold for \$8.40/45.45 kg more ($P < 0.001$) than heifer calves (\$101.82/45.45 kg vs. \$93.42/45.45 kg for steers and heifers, respectively).

Color influenced ($P < 0.001$) calf sale price in the winter months. Black-hided cattle were priced similarly ($P = 0.12$) to white-hided cattle and sold for \$98.43/45.45 kg. Black-hided and white-hided cattle received greater ($P \leq 0.04$) prices compared to mixed-colored and red-hided calves. Mixed-colored cattle and red-hided cattle were priced similarly ($P = 0.80$) and sold for \$96.82/45.45 kg, respectively).

## Discussion

As BW increases feeder calf price decreases (Faminow and Gum, 1986; Schroeder et al., 1988; Barham and Troxel, 2007). Our data demonstrated this effect with a price slide of \$8.60 and \$5.50/45.45 kg for the fall and winter, respectively.

Our results indicate selling cattle in larger lots has a financial advantage over selling in small lot sizes. This was demonstrated in both the fall and the winter. Barham and Troxel (2007) indicated price producers received in Arkansas for calves sold in lot sizes ≥ 6 was more than calves sold in lots sizes of 2–5 which was also greater that calves sold as individuals. Similarly, Schroeder et al. (1988) determined lot size had an effect on price producers received in the market place. Greater premiums were received for calves sold in truckload lot sizes.

Sex effect on selling price was evident in both the fall and winter as steers received greater prices than heifers. The price slide observed may be do to lower production efficiencies associated with feeding heifers (Garber et al.,

1990; Kreikemeier and Unruh, 1993) and the cost of pregnancy in feedlot heifers (Kreikemeier and Unruh, 1993).

Coat color of calves affects prices received in the auction market. This agrees with studies conducted in Kansas, Oklahoma, and Arkansas, (Schroeder et al., 1988; Smith et al., 2000; Barham and Troxel, 2007). Similar to our winter data, King and Seeger (2004) reported black-hided and white-hided cattle sold for greater prices than mixed-colored and red-hided cattle. Color may affect buyer's perception of growth, performance, and carcass characteristics (Smith et al., 2000).

In the fall, producers that marketed calves with a 4-way viral or 741 health program received premiums compared with calves marketed with no vaccination history. However, in the winter this premium was not observed. This may be due to the perception by buyers that calves purchased in the winter have less risk of illness as buyers may assume (correctly or incorrectly) that the calves are weaned and vaccinated. King and Seeger (2004) reported vaccinated calves marketed through an auction barn in Missouri sold for greater prices than calves with no vaccination history. Evidence to support this has also been demonstrated with calves sold in video auctions (King and Seeger, 2004).

These data suggest that the price received for feeder calves in ND, SD, and MT is dependent on multiple factors. Selling vaccinated calves in larger lot sizes seems to be economically advantageous and the value of vaccination history varied depending on marketing time.

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Table 1. Factors influencing price of ND, MT, and SD calves during fall 2006

| Factor | Number of lots | Lot price | Price Premium[a] | *P*-value |
|---|---|---|---|---|
| Lot size | | | | <0.001 |
| ≥ 21 | 911 | 114.74[a] | 6.20 | |
| 11–20 | 885 | 112.85[b] | 4.31 | |
| 6–10 | 1113 | 112.76[b] | 4.22 | |
| ≤ 5 | 3342 | 108.54[c] | 0.00 | |
| Calf sex | | | | <0.001 |
| Steers | 3440 | 117.11[a] | 9.78 | |
| Heifers | 2805 | 107.33[b] | 0.00 | |
| Color | | | | <0.001 |
| Black, BWF[b] | 3831 | 114.40[a] | 3.48 | |
| Mixed | 968 | 112.15[b] | 1.23 | |
| Red, RWF[c] | 983 | 111.42[b] | 0.50 | |
| White | 450 | 110.92[b] | 0.00 | |
| Vaccinations | | | | <0.001 |
| 4-way viral | 1191 | 113.46[a] | 2.50 | |
| 741[d] | 1559 | 112.24[a] | 1.28 | |
| No vac[e] | 3502 | 110.96[b] | 0.00 | |
| Natural | | | | 0.04 |
| Yes | 898 | 113.00[a] | 1.55 | |
| No | 5354 | 111.45[b] | 0.00 | |
| Implants | | | | 0.18 |
| Yes | 286 | 113.05 | 1.66 | |
| No | 5966 | 111.39 | 0.00 | |
| Base weight[f] | 6251 | | -0.086 | <0.001 |
| Base weight (quadractic) | 6251 | | 0.0001 | <0.001 |

[a]Price/45.45 kg
[b]BWF = black white face
[c]RWF = red white face
[d]741 = 7-way clostridial plus 4-way viral plus *Pasteurella*
[e]No vaccination history, but may have 7-way clostridial
[f]Mean base weight of all lots (236 kg) – base weight of each lot

Table 2. Factors influencing price of ND, MT, and SD calves during winter 2007

| Factor | Number of lots | Lot price | Price Premium[a] | *P*-value |
|---|---|---|---|---|
| Lot size | | | | <0.001 |
| ≥ 21 | 406 | 99.42[a] | 4.95 | |
| 11–20 | 442 | 98.47[b] | 4.00 | |
| 6–10 | 494 | 98.11[b] | 3.64 | |
| ≤ 5 | 1356 | 94.47[c] | 0.00 | |
| Calf sex | | | | <0.001 |
| Steers | 1416 | 101.82[a] | 8.40 | |
| Heifers | 1282 | 93.42[b] | 0.00 | |
| Color | | | | <0.001 |
| Black, BWF[b] | 1594 | 98.84[a] | 2.08 | |
| White | 162 | 98.02[a] | 1.26 | |
| Mixed | 545 | 96.87[b] | 0.11 | |
| Red, RWF[c] | 396 | 96.76[b] | 0.00 | |
| Vaccinations | | | | 0.71 |
| 4-way viral | 622 | 97.78 | 0.29 | |
| 741[d] | 1332 | 97.59 | 0.10 | |
| No vac[e] | 744 | 97.49 | 0.00 | |
| Base weight[f] | 2698 | | -0.055 | <0.001 |
| Base weight (quadractic) | 2698 | | 0.0001 | <0.001 |

[a]Price/45.45 kg
[b]BWF = black white face
[c]RWF = red white face
[d]741 = 7-way clostridial plus 4-way viral plus *Pasteurella*
[e]No vaccination history, but may have 7-way clostridial
[f]Mean base weight of all lots (294 kg) – base weight of each lot

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# CSUBEEF.COM AS A WEB RESOURCE FOR DISSEMINATING BEEF CATTLE EDUCATIONAL INFORMATION AND FOR ON-LINE ACCESS TO THE CATTLE PRODUCER'S LIBRARY

**J.C. Whittier[1]*, R.W. Ellis[1], D. Colburn[1], M. Fisher[1], M. Jarosz[1], S.P. Porter[1] and the Western Beef Resource Committee[2]**

[1]Colorado State University, Fort Collins, CO and [2]Extension Specialists from 12 Western States

**ABSTRACT:** The Cow-Calf Management Guide & Cattle Producer's Library (**CL**) is an educational resource for cattle producers and educators prepared by the Western Beef Resource Committee (**WBRC**) which consists of extension specialists in 12 western states. Historically, the CL has only been distributed in printed form. In 1999 a CD-ROM version was added. The CL contains approximately 250 factsheets in sections on quality assurance, nutrition, reproduction, range and pasture, animal health, management, marketing, finance, genetics, and drought and other natural disasters. The CL is revised annually by WBRC. In 2005, Colorado State University (CSU) was charged by WBRC to develop a site for on-line electronic access to the CL. Concurrent with this, CSU was enhancing their web delivery of beef cattle information and secured the CSUBeef.Com Uniform Resource Locator (**URL**). The CL was added to CSUBeef.Com as a menu item and requires users to register a user name and password to access the CL. This on-line CL is in Adobe PDF format and registered users can download and print factsheets individually. The site contains a search function to find terms and phrases in the CL. The on-line CL factsheets are not currently located using Google or similar search engines. During calendar year 2007, there were 4,697 visits by 3,037 people to CSUBeef.Com and 21,663 page views. The average user spent 3m2s on the site and viewed 4.6 pages per visit. Of those who visited the site, 63% were new visits and 41% bounced from this site to another page or a site without visiting any other pages. Site traffic consisted of 56% from referring sites, 23% direct traffic and 21% from search engines. By February 2008 there were 399 registered CL users with on-line access and 11,117 total factsheet downloads. The CL index was the most frequent download. This demonstrates that the internet provides an effective tool for disseminating information about beef cattle production and management topics.

**Keywords:** Cattle Producer's Library, CSUBeef.Com, Internet, Web Delivery, Beef Cattle

## Introduction

Delivery methods for educational material have undergone dramatic changes in the past decade. This is spurred primarily by the increased use of computer technologies throughout society. Not the least of these is the World Wide Web (**WWW**) or internet. It is commonplace for educational groups (academic and private) to host websites for user access. These websites typically provide current information and updates and serve as an avenue for downloading and printing educational materials. Academic websites typically do not require a fee for use of the materials.

Dairy Extension Specialists have incorporated and expanded use of the Internet into educational programs. Discussion groups, subject matter courses, and searchable databases are examples of Internet use by dairy educators (Chase et al., 2006).

Vergot et al. (2005) used a survey method to determine sources and channels of information used by beef producers sampled in 2003 in Northwest Florida. They reported that approximately 30% of the 264 beef producers who responded to the survey stated that university and/or extension websites were their preferred channel of information. Although more recent data could not be found, it is likely that the use of the internet has increased markedly among beef producers since 2003 when the survey was done.

Colorado State University (**CSU**) Animal Science Department, like many universities, has a departmental home page (http://ansci.colostate.edu/) designed to provide internet users with information about the department. In 2004, CSU enhanced their web delivery of beef cattle information and secured the CSUBeef.Com Uniform Resource Locator (**URL**). The URL for CSUBeef.Com was set as a tab on the departmental home page titled Extension Sites: Beef. CSUBeef.Com is managed by the CSU Beef Team to provide targeted information and useful resources for cattle producers. In 2005, CSU was charged by the Western Beef Resource Committee (**WBRC**) to

develop a trial site for the CL to evaluate on-line access and use.

The Cow-Calf Management Guide and Cattle Producer's Library (**CL**) was first published in November 1980 with a second edition published in October 1992. The printed version of the second edition is bound in a large 3-ring yellow-colored notebook and is frequently referred to as "The Yellow Book". The CL is a result of research and practical experience of members of the WBRC. The WBRC is a collaboration of state and county extension faculty from Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming. The CL remains current because of the commitment of the WBRC, which meets annually to review handbook contents and either add, revise, or delete papers after science-based, peer review scrutiny. In total, more than 10,000 copies of the handbook have been purchased by users in at least 42 states and eight countries (Glaze et al., 2006).

A Spanish translation of the CL is near completion. The index and nutrition section were the first to be translated and loaded to the on-line version of the CL; to-date most of the factsheets have been translated and are available on-line. This translation was spearheaded by Extension personnel at New Mexico State University.

The objective of this paper is to report the use of CSUBeef.Com and the on-line access to the CL during the calendar year 2007.

## Materials and Methods

In 2004 CSU Animal Science Department recognized the need to expand and refine the department's webpage. This process included enhancements of topics and reference information, as well as specific tabs for Animal Science, Equine Science, Western Center for Integrated Resource Management (**WCIRM**), access to the CSU student application materials, and Extension sites for Equine and Beef. The Beef Extension page is known as CSUBeef.Com.

Concurrent with the enhancement of the department's webpage, CSU Beef Extension proposed to the WBRC that the CL be loaded to CSU webpage. The pdf files of the factsheets in the CL were already developed for the CD version of CL. WBRC granted approval to place the files on the CSU site in exchange for CSU developing the on-line access process to the factsheets. The process for accessing the CL on-line became operational in 2006.

Access to the CL at CSUBeef.Com requires users to register a user name and password. Registration is free and allows tracking of downloads and other access to the on-line CL. Once users have registered on CSUBeef.Com they establish a user name and password that opens a menu link to the CL. Users can then use a keyword search application on this page to identify factsheets with information of interest to them. Or, the user can scroll through the Table of Contents which contains a direct link to the pdf file for each factsheet. To read and print the pdf files contained in the library, a user must have the Adobe® Acrobat® Reader installed on their computer.

Google Analytics (www.google.com/analytics) was used to track and summarize site usage of CSUBeef.Com during the calendar year 2007. Google Analytics is a free service offered by Google, Inc. that generates detailed statistics about the visitors to a website. This tool allows a webmaster to determine such things as where the website visitors came from, how long they stayed on the website and their geographical position (Wikipedia, 2008).

## Results and Discussion

Table 1 shows the data for site usage for CSUBeef.Com during 2007. Visitors to CSUBeef.Com may have come for access to various types of information, not solely to access the CL. The most viewed page was the CSU Beef Team homepage (29.9%), followed by the Beef Resources page (5.3%), the Beef News page (4.1%), and CSU BeefCast (3.1%).

Table 1. Site usage for CSUBeef.Com during calendar year 2007.

| Item | Outcome |
|---|---|
| Visits | 4,695 |
| Absolute unique visitors | 3,037 |
| Page views (total) | 21,663 |
| Page views (unique) | 13,863 |
| Average page views per visit | 4.61 |
| Time on site | 3 min 2 sec |
| Bounce rate* | 40.8% |
| New visits | 63.4% |

*A bounce occurs when a website visitor leaves a page or a site without visiting any other pages before a specified session-timeout occurs. This may occur most frequently if the page or site is used as the homepage for the user's browser.

Over 3,000 unique visitors viewed the CSUBeef.Com during 2007. This represented 64.7% of the visits. Visitors viewed a total of 21,663 pages at the site, with 13,863 (64.0%) unique page views.

The primary sources of webpage traffic to CSUBeef.Com were 1) referring sites (56.3%), 2) direct traffic (23.3%) and when found from search engines (20.4%). Referral traffic came primarily (42.8%) from the CSU Animal Science homepage by clicking the "beef" tab. Direct traffic users typed the URL "CSUBeef.Com" directly into their browser locator. Google searches using the keyword "csubeef" accounted for 16.3% of the webpage traffic.

Table 2 shows the visits for CSUBeef.Com by country. Users in the United States visited the site most frequently, followed by Canada and the Netherlands. Although most of the traffic was from the U.S., this information shows the international aspect of internet opportunities for beef education.

Table 2. Site usage by country for CSUBeef.Com during calendar year 2007.

| Country | Visits | % New Visits | % Bounce Rate |
|---|---|---|---|
| United States | 4,093 | 61.7 | 40.4 |
| Canada | 132 | 67.4 | 44.7 |
| Netherlands | 60 | 35.0 | 33.3 |
| United Kingdom | 38 | 68.4 | 52.6 |
| Mexico | 36 | 72.2 | 36.1 |
| Australia | 33 | 90.9 | 60.6 |
| Brazil | 25 | 88.0 | 40.0 |
| Argentina | 17 | 82.4 | 29.4 |
| New Zealand | 13 | 69.2 | 38.5 |
| Uruguay | 13 | 69.2 | 53.9 |

Table 3 outlines the connection speeds at which users connected to CSUBeef.Com. This information indicates that high-speed access is apparently readily available to most users. Conversely, it may also indicate that users with slower connection speeds (e.g. dialup) use the internet for this type of information less frequently.

Table 3. Connection speeds used to access CSUBeef.Com during 2007.

| Connection Speed | Visits | % Visits |
|---|---|---|
| Unknown | 1,679 | 35.8 |
| T1 | 1,526 | 32.5 |
| DSL | 678 | 14.4 |
| Cable | 548 | 11.7 |
| Dialup | 208 | 4.4 |

Table 4 lists the 10 factsheets that were downloaded most frequently from the time the CL became available in 2006 until January 23, 2008. There were a total of 11,117 factsheets downloaded indicating a high level of use of this information. It appeared that every factsheet in the library was downloaded at least one time.

Table 4. Top ten most frequently downloaded CL factsheets from CSUBeef.Com.

| Rank | Title of Factsheet Downloaded | No. of Downloads |
|---|---|---|
| 1-2 | Index (Spanish version) | 143 |
| 1-2 | Index (English version) | 143 |
| 3 | Identifying the Functional Bull: Bull Soundness and Management | 141 |
| 4 | Beef Cattle Implants | 127 |
| 5 | Castration of Bulls | 125 |
| 6 | Alfalfa for Beef Cows | 124 |
| 7 | Grafting Calves | 121 |
| 8 | Artificial Insemination of Beef Cattle | 112 |
| 9 | Creep Feeding Beef Calves | 110 |
| 10 | Coccidiosis in Beef Cattle | 106 |
| | Total downloads from inception to January 23, 2008 | 11,117 |

It appears that the Spanish translation of the factsheets was received well by users (see Table 5). Of the top 165 factsheets downloaded, 12 were Spanish translations. This finding indicates that there is an opportunity to expand the use of the CL into Spanish speaking audiences.

Table 5. Title and number of Spanish version factsheets downloaded from CSUBeef.Com from the inception of the on-line version through January 23, 2008.

| Title of Spanish Version of Factsheet | Times Downloaded |
|---|---|
| Cattlemen's Library Index | 143 |
| Analysis of Water Quality for Livestock | 104 |
| Nutrition of Mature Beef Cows | 82 |
| Evaluating Your Cattle Herd's Need for Supplemental Nutrients | 75 |
| Identifying the Functional Bull: Bull Soundness and Management | 74 |
| Abortion in Cattle | 73 |
| Cash-Flow Budgeting | 52 |
| Use of Water and Other Tools for Improving Grazing Management | 46 |
| Management to Minimize Hay Waste | 39 |
| Commercial Beef Sire Selection | 35 |
| Partial Budgeting | 34 |
| Genetics of Reproduction | 31 |

Glaze et al., (2006) reported the primary users of the printed for of the CL to be producers (53%), followed by Extension Faculty (30%). The majority of handbook users reported that they used the handbook monthly (42%) or quarterly (37%). Of those surveyed by Glaze et al., (2006) the majority reported that they preferred the book (74%) compared to the CD-ROM version (24%). The survey by Glaze et al., (2006) was completed before the on-line version of the CL was available. However, respondents were asked about their willingness to pay a user fee to access an on-line version of the handbook and 31% reported that they would be willing to pay a fee.

To-date there has not been an aggressive marketing campaign for the on-line version of the CL by either CSU or WBRC. Rather, most marketing has been by word-of-mouth and by mention in extension newsletters or during producer education meetings. It was determined by WBRC that a test period for the on-line version was needed before a wide-spread marketing initiative was undertaken. In addition, use of the on-line version may be in competition with sales of the printed or CD-ROM versions, particularly if there is not a fee to access the on-line CL. Further discussion and review of this question is planned by WBRC.

## Implications

The summary of use of internet educational resources reported in this manuscript indicates that a strong potential exists for delivery of information using this media. On-line access of the CL at the CSUBeef.Com website has been well received with limited marketing and demonstrates that this system is capable of delivering educational material to computer users throughout the world. The use of the CL factsheets by Spanish speaking users illustrates that potential exists to expand in this audience.

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# THE EFFECTS OF AGE AND SOURCE VERIFICATION OF CALVES ON VALUE RECEIVED ON SUPERIOR LIVESTOCK VIDEO AUCTIONS

**A. Kellom, J. Paterson, J. Vanek, M. Watts, M. Harbac**
Montana State University, Bozeman, MT

**ABSTRACT:** Third party age and source verification has become part of a marketing plan for cattle producers during the last four years. This research project was designed to determine if a premium was being paid for source and age verified calves sold via video auction. Data provided by Superior Livestock Video on 68,665 head of Montana calves marketed in two sales during June and July of 2007 were evaluated. A hedonic model, OLS as the estimator, was used to evaluate the economic benefits of production practices such as age and source verification vs. no verification, weaned calves vs. non-weaned calves, sex of the calf, vaccinated vs. not vaccinated calves and calves sold during the June vs. July sales. Average sale weight of all calves was 265 kg and the average sale price was $2.58/kg with an average lot size of 116 head. Thirty-one percent of all calves sold were age and source verified, 60% of the calves were steers, 15% were weaned from their dam, and 88% were vaccinated prior to shipment (VAC 34 or VAC 45 protocols). When calculated for a 272 kg calf, the premium received for source and age verification was $12.83. Other premiums received for this weight of calf were: vaccinated $14.81 per head, weaned $17.64 per head, and steers $52.43 per head more than heifers. Results of this study indicate that when calves were age and source verified, weaned, and/or followed a vaccination protocol, additional dollars were received when marketed via a video auction.

**Key Words:** Age and source verification, Traceability, Auction market

## Introduction

Cow calf producers have many value added options available to them as they choose a marketing route for their product. Many in the western United States choose to market in load lots on the various video auctions. King and Seeger (2005) reported that over a ten year period from 1995 through 2004, calves following vaccination and or weaning protocols received premiums ranging from $0.99 per 45.36kg to $7.91 per 45.36kg when marketed on Superior Livestock Auction. Another analysis of calves sold in Iowa auction markets indicated that third party certification of vaccination and weaning added $6.15 per 45.36kg to the price of calves sold (Bulut et al., 2006). A survey of three Kansas auction markets found that one of three markets reported buyers paying premiums for RFID tagged and preconditioned calves (Bolte 2007).

In recent years, third party source and age verification has become another value added characteristic that producers can use to market feeder calves. Limited data is available to demonstrate the economic benefit of doing so. Citing research conducted by Colorado State University and Pfizer Animal Health, Jim Kelley (business manager of Superior Livestock Auction) reported in a web interview that source and age verified calves brought a premium of $1.77 per 45.36kg in the summer sales of 2006 (CattleNetwork 2007). The objective of this study was to evaluate the effects of source and age verification as well as vaccination strategy, gender, and weaning on the price received for Montana feeder calves sold on Superior Livestock Auction in June and July of 2007.

## Materials and Methods

These data were collected from Superior Livestock Video Auction following the June and July 2007 feeder calf sales. Data were received in electronic format and contained approximately 68,665 observations. The total observations were sub-categorized into 590 individual lots of calves.

Each "sale lot" of feeder calves was described by various variables. The variables evaluated in this study included: gender, weight, vaccination protocol, age and source verification, and weaning protocol. Each of these variables contributed their own value to the final price of the "sale lot". Buyers made decisions based on the variables of each sale lot and the summation of implicit values of each variable.

A hedonic model was used to statistically estimate the implicit values of the lot attributes. The hedonic model was developed by Sherwin Rosen (1976) in consumer and production economics. The hedonic model is used to estimate the marginal value of an attribute to the overall price of the product. Hedonic models assume the final good is comprised of a quantity of individual attributes and it is the summation of the value of the attributes that ultimately determines the value of the final product.

Ladd and Martin (1976) used the hedonic model to estimate the implicit prices associated with individual corn grades. The model has frequently been used to estimate the implicit prices of bull attributes on sale prices. Vanek (2007) completed a study using the hedonic model to estimate the value of various EPDs on purebred bull prices.

For this analysis the hedonic model is:

$$P = \sum_i p_i z_i$$

Where:
P = Price per kg of the "sale lot"
$p_i$ = Implicit price of the attribute i
$z_i$ = Quantity of attribute i

## Results

Table 1 presents a summary of the ranchers and calves sold on June and July Superior Livestock Video sales. Average weight of the calves was 265 kg and the average price received was $2.58 per kg. The average sale lot size was 116 head. Thirty-one percent of the calves were sold as age and source verified, 15 percent were weaned prior to shipment, 88 percent followed vaccination protocols, and 60 percent were steers.

When calculated for a 272 kg calf, the premium received for third party independent source and age verification was $12.83 (Table 2). Other premiums received for this weight of calf were; vaccination protocol $14.81 per head, weaned $17.64 per head, and steers $52.43 per head more than heifers. Finally, calves that sold in the July sale received $16.51 per head more than calves which sold in the June sale.

In order to receive the benefit of value added practices, cow-calf producers must find the market that is willing to reward them. In a 2007 interview with BEEF Magazine, Dr. Bill Mies encouraged producers to first find a marketing channel that rewards these production practices and then produce the calf. It doesn't make sense to produce a calf and send it to a market that does not reward for these practices.

## Implications

Many source and age verification programs cost around $3 per head to enroll. Therefore, the net return to the producer was $9.86 using data from these two video sales. Other value added attributes such as following a vaccination and or weaning protocol were also rewarded with higher prices when marketed through Superior Livestock Auction.

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Table 1. Summary averages for 68,665 Montana calves sold in either June or July Superior Livestock Video auctions.

| Variable Measured | Average |
|---|---|
| Weaning weight | 265 kg |
| Price received | $2.58 per kg |
| Number of calves that a Montana rancher sold | 116 |
| Percentage of the calves that were steers | 60% |
| Percentage of the calves that were weaned | 15% |
| Percentage of the calves that were source and age verified | 31% |
| Percentage of calves sold in the July sale vs. June sale | 62% |
| Percentage of calves sold as VAC 34 (vaccinated) | 76% |
| Percentage of calves sold as VAC 45 (weaned and vaccinated) | 12% |

Table 2. Additional value received for various characteristics of Montana feeder calves sold in the June and July 2007 Superior Livestock Video Auctions

| Variable | $/kg | $/272 kg |
|---|---|---|
| Steer vs. heifer | .193 | 52.43 |
| Weaned vs. not weaned | .065 | 17.64 |
| Source and age verified vs. not verified | .047 | 12.83 |
| Vaccinated vs. not vaccinated | .054 | 14.81 |
| July vs. June sale | .061 | 16.51 |

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# MANAGEMENT SIMULATION TOOL FOR ESTIMATING VALUE OF INDIVIDUAL IDENTIFICATION OF BEEF CATTLE

**J. W. Oltjen[1], D.J. Drake[1], A.B. Ahmadi[1], A.J. Romera[2], S.J.R. Woodward[3], L.N. Bennett[4], F. Haque[4], L.J. Butler[4]**

[1]Animal Science, University of California, Davis, California
[2]Whole Farm Modelling Team, DairyNZ, Hamilton, New Zealand
[3]Supply Chain Systems Group, Lincoln Ventures Limited, Hamilton, New Zealand
[4]Agricultural and Resource Economics, University of California, Davis, California

**ABSTRACT:** One difficulty in cost/benefit analysis of animal identification systems is estimation of benefits from improved herd management. Animal identification makes possible decisions based on individual records, particularly selection of animals for culling, mating, sale or rearing as replacements. Because the productive life of some cows is over ten years, management policies can significantly alter the distribution of cow ages and thus productivity within a herd. To capture the delayed and indirect effects of proposed management actions, therefore, herd dynamics must be simulated over long time horizons. Without a long term dynamic tool these consequences are impossible or difficult to project. Our objective is to develop tools for outreach purposes that will permit ranch managers to see how they might benefit when individual animal identification is used. A cowherd simulation model has been developed based on an existing object-oriented system. The model is deterministic, but each animal inherits certain genetic attributes from its parents, plus or minus a random variation. There is also climatic variation driving pasture growth using actual or randomly generated weather sequences from 129 weather stations in California. These two elements generate results over time, with a distribution of values for each output variable that can then be analyzed for significant effects using standard statistical methods. To drive this model we have also developed 62 decision rules to represent real management changes/options that a rancher could adopt if he had individual animal identification. Rules are grouped into those for breeding, weaning, culling, saving replacements, moving between pastures, and supplemental feeding. A long term goal is to embed our model into a decision support tool with an interface allowing different management changes to be adopted for the user input ranch. Outputs will show the advantages and disadvantages of identification assisted management.

**KEY WORDS:** Cost-benefit analysis, Decision support system, Simulation model.

## Introduction

One difficulty in cost/benefit analysis of animal identification systems is estimation of benefits from improved herd management. Animal identification makes possible decisions based on individual records, particularly selection of animals for culling, mating, sale or rearing as replacements. Because the productive life of some cows is over ten years, management policies can significantly alter the distribution of cow ages and thus productivity within a herd. To capture the delayed and indirect effects of proposed management actions, therefore, herd dynamics must be simulated over long time horizons. Without a long term dynamic tool these consequences are impossible or difficult to project. Our objective is to develop tools for outreach purposes that will permit ranch managers to see how they might benefit when individual animal identification is used.

## Model Overview

PC Ranch is a cowherd simulation model based on an existing object-oriented system (Romera et al., 2004) The model is deterministic, but each animal inherits certain genetic attributes from its parents, plus or minus a random variation. There is also climatic variation driving pasture growth using actual or randomly generated weather sequences from 129 weather stations in California. These two elements generate results over time, with a distribution of values for each output variable that can then be analyzed for significant effects using standard statistical methods. To drive this model we have also developed 62 decision rules to represent real management changes/options that a rancher could adopt if he had individual animal identification. Rules are grouped into those for breeding, weaning, culling, saving replacements, moving between pastures, and supplemental feeding.

## Computer Program Description

PCRANCH is written in the C++ programming language and runs under the Windows operating system. PCRANCH consists of three components. The first component is the input interface which consists of range (physical characteristics of the farm), herd (animal numbers and type), block (land allocation), weather (climate data), and rules (management) dialog boxes. The user enters all input data in these dialog boxes. The second component is the run interface. It runs the simulation engine, CCFARM, which is written in the Java programming language as a separate program and gets its input as a series of text files from PCRANCH. The third component is the output interface which consists of a series of reports and graphs,

generated from the output files of the CCFARM simulation engine.

## Discussion

One difficulty for most research-oriented simulation systems is that their user interfaces are written to be used by the researcher and not by ranchers. The PCRANCH software package addresses this problem by decoupling CCFARM from its research-oriented user interface and coupling it with a more friendly rancher-oriented user interface without reducing its capabilities. This approach allows the simulation program written in a different programming language to be used by researchers with its original user interface, and at the same time ranchers can use the new user interface written in another programming language by a separate team of programmers. This method has great potential for other research-oriented simulation programs to make them accessible. A long term goal is to embed our model into a decision support tool with an interface allowing different management changes to be adopted for the user input ranch. Outputs will show the advantages and disadvantages of identification assisted management.

The benefit of the model is, at minimum, producer exposure to the potential benefits of identification and record keeping, and more likely, an enhanced sense of appreciation of how animal identification can be used to improve ranch management. Livestock producers are demanding this information to address their highest level of concerns with the proposed national animal identification system and assist them with important business decisions. The uncertain feelings expressed by producers serve to frustrate and deter them from identification. Typically, many livestock producers are conservative decision makers and it appears many producers are taking a "wait and see" approach to the national animal identification system because they report insufficient information on the costs for implementation. The more aggressive producers with management and marketing goals are already using the technology.

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# MONTANA'S BVD-PI SCREENING PROJECT

**Mo Harbac*[1], C. Peck[1], J. Paterson[1], B. Hoffman[2]**
Montana State University, Bozeman, MT USA[1]; API, Portland, OR, USA[2]

**ABSTRACT:** The Montana bovine viral diarrhea (BVD) persistent infection (PI) Herd Screening Project was initiated to improve the overall health of Montana's cattle herd and add value to the state's calf crop. The project continues efforts begun in 2006 by providing technical assistance and testing supplies to Montana ranchers who want to screen their herds for the BVD virus. The focus of this project is to assist ranchers in adopting biosecurity practices that will prevent transmission of the BVD virus from PI animals to cattle breeding herds. This project is designed to:1) gauge the incidence of BVD-PI in Montana, 2) demonstrate overall livestock biosecurity practices, 3) demonstrate innovative disease screening/diagnostic techniques, 4) investigate the economics of BVD-PI elimination on a herd-by-herd basis and 5) develop templates for BVD virus exposure risk at the ranch level. The Animal Profiling International (API) laboratory was a collaborating partner in the research. Recent innovations in diagnostics allows cattle herds to be screened at a relatively minimal cost through the use of reverse transcriptase polymerase chain reaction (PCR) technology using pooled animal tissue samples. A reverse transcriptase–PCR assay is a sensitive and specific method of screening cattle for the BVD virus. Diagnosis, coupled with an animal identification system, ensures the producer can remove the positive animal(s) from the herd. During 2007, 106,660 head of Montana cattle were tested for the virus from 403 operations. One hundred ten cattle were positive for BVD-PI (.1%). These cattle were from 31 (7.6%) of the operations that participated in the program. A 24-question survey was mailed to the 2007 participants. Three hundred (75%) responded. Ninety percent of the respondents took part in the program to enable them to gauge the prevalence of BVD-PI in their herd, while 16% did it based on past herd health issues. Seventy three percent stated that they did not suspect their herd to be at risk for the BVD virus infection. When asked if they had vaccinated their cows/heifers for BVD in 2007, 90% responded yes. Ninety percent of the producers vaccinated their calves. Sixty two percent vaccinated at a combination of branding and pre-conditioning and 87% use a modified live vaccine. Eighty one percent stated that they plan to now incorporate BVD screening as a normal part of their herd biosecurity program.

Keywords: Bovine Viral Diarrhea, Persistent Infection, Biosecurity

## Introduction

The Montana BVD-PI Herd Screening Project was initiated to improve the overall health of Montana's cattle herd and add value to the state's calf crop. The project continues efforts begun in 2006 by providing technical assistance and limited financial support to Montana ranchers who want to screen their herds for persistent infection (PI) with bovine viral diarrhea (BVD) virus. The focus of this project is to assist ranchers in adopting an array of biosecurity practices that will prevent transmission of the BVD virus from PI animals to cattle breeding herds.

Management and control of the BVD virus in cattle herds must consider two ways the virus passes from one animal to another. The first is *horizontal transmission* – when a transiently (temporarily) infected animal releases the virus in its nasal and other secretions and the virus enters a susceptible animal through the mouth or respiratory tract. The second is *vertical transmission* of BVD virus from an infected dam's bloodstream to her fetus during pregnancy.

Subsequent fetal infection can lead to fetal death, the birth of a normal calf, or the birth of a PI calf – meaning that the infection lasts the entire life of the animal. It's important to note that PI females of breeding age not only are a source of horizontal transfer of BVD virus, but will always produce a PI calf themselves.

The primary source of BVD virus is PI cattle; with transiently infected cattle considered a less important source. The cost of the presence of at least one PI animal in a beef herd has been reported to range from $14.85-$24.84 per cow/year (Larson, 2006). The economic value of screening for PI animals in cow-calf herds is influenced by the likelihood of finding at least one PI animal in the herd, the negative production effects when PI animals are present, the cost of inputs and the value of animals sold.

Because of the low prevalence of PI animals, not all producers can justify diagnostic screening for PI cattle. However, if ranch history, a significant breech in biosecurity or changes in production practices increases the risk of PI cattle being present in the herd, a protocol to screen the herd can be defended based on the likelihood to improve economic return.

A survey was conducted of ranchers who participated in the statewide 2007 BVD-PI Herd Screening Program to measure the participant's management practices and to determine what educational needs are required in the future.

## Materials and Methods

The survey was developed by stakeholders (beef producers, veterinarians, breed organization, Montana Stockgrowers Association) who wanted to gain information on producer/ranch profiles, what participants herd health practices were and the effectiveness of the program to help develop future educational needs related to biosecurity

issues.Twenty four questions for this survey were grouped into three categories: program and producer profile, ranch health practices, and program evaluation. The survey was peer reviewed by individuals familiar with survey design and the beef industry within the state. The resulting survey instrument was mailed to the 403 MT operations that participated in the 2007 Montana BVD-PI Herd Screening project.

Surveys were mailed to producers on October 13, 2007. Respondents were given a deadline of November 21, 2007 to return the surveys. Follow-up phone calls were made to respondents when clarification was required for an answer. These comments were then noted on each survey. Surveys were analyzed during the first ten days of December, 2007. Surveys received after this date were not included in the data set. Summary statistics were used to analyze the data.

## Results

### *Program and Producer Profile*

Of the 403 surveys mailed to 2007 participants, 301 were returned;a 75% response rate.The data in Table 1 summarizes the program and producer profile for the program participants. More producers (41%) learned about the BVD-PI Herd Screening project from MSU Extension publications and personnel than from newspapers or magazines (30%) or a member of the BVD-PI Herd Screening Project (21%)

The majority of the people (90%) took part in the program to determine if they had BVD-PI animals in their herd. Twenty seven percent of the respondents indicated that they suspected that their herd was at risk for the BVD virus. When asked to estimate the degree of difficulty in obtaining the tissue samples and submitting them to the lab, 65% stated it was not difficult to obtain the samples and zero percent indicated that it was too difficult to do again.

When producers were asked if they purchased replacement heifers in the last year, 103 of the 301 respondents answered yes. Of this pool of 103 respondents, 38% indicated that these heifers were screened for BVD virus prior to arrival at the ranch. At the same time, 243 of the 301 respondents indicated that they had purchased bulls in the last year. Seventy percent of this pool of 243 stated the bulls were screened for BVD virus prior to arriving at the ranch; 30% did not purchase screened bulls.

### *Ranch Health Practices*

Table 2 presents the ranch health practices of the respondents. Seventy seven percent of the respondents had not screened for BVD-PI virus prior to 2007. Only 2% had screened as early as 2003. Seventy eight percent of the survey participants did the BVD-PI sampling at branding. A majority of the respondents indicated that they had vaccinated their calves for BVD virus in 2007. Sixty two percent vaccinated twice; a combination vaccinating at branding and pre-conditioning.

### *Program Evaluation*

The third table shows the summary of producersevaluation of the testing program. A majority of the respondents (67%) did not have a quarantine program in place when new animals were introducedinto the herd. When asked to explain how participation in this program influenced the future management of their herd, 81% responded that they became more aware of how to test for, and control BVD virus in their herds.

Respondents were given the opportunity to comment on any aspect of the program. Most of the comments were of a general nature. Like many other issues associated with herd health management, labor is an issue. It was often stated that it took an additional person at branding to do the sampling. Participants were pleased with the short turnaround time from the lab which gave them the ability to manage those animals that tested positive for the virus. Other comments were seeking clarification on "what next" for them to maintain their BVD-PI free status.

## Implications

The overarching implication of the project is to use it as a template for assessing livestock ranch biosecurity and vulnerability to infection and transmission of a host of livestock disease threats. Ranchers and cattle feeders are using the process of assessing biosecurity risks and employing biosecurity practices for the BVD virus as demonstrated in this project to measure risk of other common diseases. Eradication or control of diseases that range from complex calf scours to catastrophic foreign animal diseases like foot-and-mouth disease is the goal. The components of a livestock biosecurity system including risk assessment, vaccination, screening for organisms, animal traceability, record keeping, and prudent livestock movement and handling can be holistically applied as management to decrease or eliminate any livestock disease.

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**Table 1.** Program and Producer Profile for Montana BVD-PI project

| Question | % |
|---|---|
| **How did you hear about the MT BVD project?** | |
| Newspaper, magazine, newsletter | 30 |
| A member of the BVD-PI herd screening team | 21 |
| MSU extension publication/personnel | 41 |
| Other | 27 |
| **Why did you choose to participate in this project?** | |
| Cost-share | 75 |
| Increase market value of my calves | 67 |
| Wanted to see if I had BVD-PIs | 90 |
| Past herd health issues | 16 |
| **Did you suspect your herd was at risk for the BVD virus?** | |
| Yes | 27 |
| No | 73 |

**Table 2.** Ranch health practices from the Montana BVD-PI project

| Question | % |
|---|---|
| **In what year did you first screen for BVD?** | |
| 2003 | 2 |
| 2004 | 4 |
| 2005 | 34 |
| 2006 | 13 |
| No screening prior to 2007 | 77 |
| **When did you take tissue samples to test for BVD?** | |
| Birth | 12 |
| Branding | 78 |
| Pre-conditioning | 5 |
| Weaning | 7 |
| **Did you vaccinate calves for BVD in 2007?** | |
| Yes | 87 |
| No | 13 |
| **If you vaccinated your calves in 2007, when did you vaccinate?** | |
| Branding | 19 |
| Pre-conditioning | 19 |
| Combination of branding and pre-conditioning | 62 |

**Table 3.** Program Evaluation of the Montana BVD-PI project

| Question | % |
|---|---|
| **When introducing new animals to your herd, do you have a quarantine program in place?** | |
| Yes | 33 |
| No | 67 |
| **Knowing what you now know about BVD, IF you have a BVD animal identified on your operation, who would you seek for help in handling the problem. Mark all that apply.** | |
| Attending vet | 87 |
| Member of the team from the project's laboratory | 16 |
| MSU Extension agent | 22 |
| MT Beef Extension specialist | 25 |
| Member of the BVD-PI herd screening project team | 50 |
| **Explain how your participation in this BVD program has influenced the current/future management of your herd? Mark all that apply.** | |
| More aware of BVD and the types of BVD infections | 77 |
| More aware of how to test for/control BVD | 81 |
| More aware of the economical and herd health consequences of BVD | 65 |
| More aware of herd biosecurity practices | 56 |

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# NAIS FIELD TRIALS: STRENGTHENING IDENTIFICATION of HIGH-RISK ANIMALS USING a NOVEL IDENTIFICATION APPROACH

S.P. Porter[+]*, C.Smith†, A. Clark†, J.C. Whittier[+]
[+]Colorado State University, Fort Collins, CO, USA
†Optibrand Ltd. LLC, Fort Collins, CO, USA

ABSTRACT: The need for animal disease surveillance is a subject of constant discussion within the U.S. Choosing an appropriate method of identification (**ID**) and traceback that coincides with commerce is of the utmost importance. The production cycle of the imported feeder animal is fairly well defined: animals enter the U.S., are either sent to stocker or to feedlot operations, and after finishing are sent to the abattoir. The lifecycle of the imported roping animal is not clearly defined; the animals enter the U.S. and eventually the food chain, with limited knowledge of movements between importation and harvest. A novel form of animal identification (**AID**) utilizing retinal imaging was tested to maintain AID during the production cycle of both Mexican roping steers and spayed feedlot heifers. This secure and reliable method of ID combines GPS capabilities with the vascular pattern of the ocular fundus. Incorporation of this technology into a lifetime traceback system for high risk animals has the capability to follow animals through their production cycle to the abattoir when other forms of ID are lost. To test the ability of this technology to maintain AID 102 spayed feeder heifers were retinal imaged at the time of importation. The feeder heifers were verified at the feedlot and again at harvest. 935 recreational animals were retinal imaged at the time of importation. Recreational animals typically have a lifespan longer than feeder cattle, and due to the scope of the research, were only followed for the first 90-120 days in the U.S. Recreational animals have been pinpointed as a high-risk source of cattle tuberculosis (*Mycobacterium bovis*) within the US, and maintaining AID through out the production chain would benefit the U.S. in the event of an animal disease outbreak. This technology has the capability to bridge the gap between a Mexican ID system that allows herd of origin traceback and the U.S.

**Keywords:** Disease surveillance, Imported cattle, Retinal images

## Introduction

In 2003, the United States Department of Agriculture (**USDA**) set out to design a national animal identification system. This system would be the source of producer contact information in the event of an animal health crisis. The goals of this program would allow animal health officials to identify animals involved in a disease outbreak, know where the infected animals are currently located and identify other animals that may have been exposed to the disease (USDA,1).

With this program, USDA set standards to evaluate technologies that would potentially provide a uniform system for animal identification. With this search for a way of tracing animals that would satisfy the needs of most livestock producers, the USDA funded a series of field trials to evaluate the technologies available.

Retinal Imaging has emerged as a technology worthy of further research. This technology produced by Optibrand, Inc. LLC, allows individuals to take an image of the vascular pattern of the ocular fundus. This vascular pattern is unique to each animal as well as unique to each retina. This biometric marker is unique to the individual and is completely

secure. With the OptiReader® technology users are able to link visual identifiers (ear tags, breed characteristics, sex characteristics) with the retinal image as well as the GPS coordinates for the location where animals are being scanned.

The New Mexico Livestock Board and Colorado State University evaluated the functional use of the OptiReader® at the international port at Santa Teresa, NM. This port crosses over 300,000 head of cattle each year, representing one-third of the U.S. imports (Border Authority). It is a state of the art import facility with 10,000 head capacity located just miles east of El Paso, Texas.

Each year the United States imports between 800,000 – 1,000,000 head of Mexican cattle through the ten ports along the U.S. – Mexico border (Skaggs, 2001). The USDA has pinpointed imported cattle as an animal health concern. In 1993, the United States and Mexico formed U.S.-Mexico Bi-National Tuberculosis and Brucellosis Eradication committee. The committee meets to improve traceback efforts, monitor progress of the eradication programs, and evaluate laws and regulations applying to areas of animal health concern (Johnson). With these efforts, disease eradication has made large improvements

*Mexican Identification System*

The majority of cattle that cross at the Santa Teresa port are from the Mexican state of Chihuahua. The Union Ganadera de Chihuahua – the cattlemen's union for the Mexican state of Chihuahua in cooperation with the state and federal governments, have a tightly regulated and largely successful animal identification system which essentially ensures herd of origin identification and tuberculin testing proof through a series of ear tags. Cattle enter the US with ear tags, a spayed heifer tag if appropriate, a Mexican state brand and an M or M*x* brand to designate Mexican steers and heifers, respectively.

The Mexican cattle enter the US with these ear tags in tact, but much of the information is lost through production systems in the US. Ear tags are often removed or lost in the feedlot or removed before the animals are used for roping or rodeo-type events. Loss of ear tags complicates any trace back should an animal test positive for a disease of concern at any time in the production cycle.

The OptiReader® technology has the potential to bridge the information vacuum that currently exists at the border, allowing the Mexican identification data to be carried over into the US.

*Research*

102 spayed feeder heifers were retinal imaged in June 2007 at the border crossing. Heifers were transported to a feedlot in north eastern Texas for finishing. In August 2007, the heifers were re-implanted and the animal's identification was verified at the feedlot processing facility. The heifers continued on feed until late February and early April depending on their initial weights. Identification was again verified at the abattoir in Amarillo, TX.

935 recreational animals (those animal imported with the intention to be used for roping or rodeo-type events) were retinal imaged at the Santa Teresa crossing on two different dates (January and February) 2008. These animals were imported by one owner and sold to approximately six buyers throughout the western US. The production cycle of the recreational animal is longer than a feeder animal, as most stay in recreational use for upwards of three years before being harvested. The animals were tracked for their first 90 to 120 days in the US. With producer cooperation, animal identification was verified at each new owner's location, and will be monitored for future movements. Limited knowledge of the typical production cycle of the recreational animal, led to the examination of the creational market via brokers, buyers and producers of recreational animals. Skaggs et al stated that little is know about the final destinations of the cattle imported from Mexico (2004).

*Recreational Cattle Cycle*

Recreational-*Corriente* type animals traditionally are raised in the mountainous areas of Mexico, and as animals are bought for exportation they are gathered in increasingly larger groups until they begin the processes for exportation. The age of recreational cattle upon entrance to the US is not typically known, but

brokers estimate the average age of the imported animal to be at minimum two years of age. With advancing age, the chance of disease exposure increases. The animals enter the US as part of a large group and are split off into smaller groups and are sold the first time immediately after importation. These new owners utilize the animals for recreational events for one or two seasons, depending on the size and performance of the animals. At this time, the animals typically are sold to another owner for a different recreational use, for a different type of recreational user or are sent to a feedlot for finishing. With these production characteristics the average age of the recreational animal at entry to the food chain is at minimum three years of age.

Recreational animals are very transient. They are comingled in different groups as the animals change ownership. Arriving at a premise the animals are sorted in various ways for different events – often based on speed, weight and the specifications of the event many times in their useful lifespan.

As the animals begin their trek into the US, there is no uniform stand for bringing animals out of New Mexico (the only mandatory brand state in the Union) to their intended new home. The animals leave the New Mexico port of entry with brand inspection papers written out to the intended new state of premise. However, many states do not have brand laws – or are on a completely voluntary basis. Some states require mandatory tuberculosis testing within 60d of entry to the new state and others no requirement at all. This lack of uniformity leaves the population of recreational animals at high risk, as well as any animals they come in contact with.

**Implications**

Further discussion and research is warranted to find the best fit identification system to link a strong Chihuahua identification system with the United States. The challenge will arise in finding a system that will meet the needs of commerce and provide the opportunity to re-establish identity in the loss of all other information.

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# EXTENSION PROGRAMMING IN MANURE MANAGEMENT

**J.G. Davis*, A.L. Elliott, and K.C. Doesken**
Colorado State University, Fort Collins, CO

**ABSTRACT**: Manure management is a critical link in sustainable livestock production, protecting water, air, and soil quality. However, the 4-H livestock curriculum does not include manure management at all. There are over 150,000 4-H youth with livestock projects in the western U.S. with no manure management training. The addition of manure management into 4-H livestock curricula has the potential to have a significant environmental impact in both the short-term and the long-term as these future leaders and business people move into the workforce. We have developed a 4-H Manure Management curriculum that includes the following topics: Livestock and the Community, Animal and Human Health, Protecting Water Quality, Protecting Air Quality, Composting, Manure Utilization, and Economics of Manure Management. In addition, we have begun a hands-on, week-long composting school called the Rocky Mountain Compost School. The intended audience for this school is large-scale professional composters of all kinds of solid waste including animal manures and mortalities. The Rocky Mountain Compost School offers the growing compost industry a solid technical background in biological principles governing the compost process, environmental concerns, compost quality in relationship to its proposed end use, regulatory issues, and recent research findings that pertain to composting. These two educational programs target different audiences with the overall intention of improving manure and environmental management in the West.

**Key Words:** Manure, Environment, Composting

## Introduction

*4-H Manure Management Curriculum.* According to EPA regulations, almost every 4-H livestock project could be classified as an Animal Feeding Operation (AFO). Although we don't expect EPA to inspect 4-H projects, these projects do present an opportunity for education of future livestock producers in the essential practice of manure management.

Manure management practices are related to water quality degradation, air quality problems (such as odor and dust), and improved soil quality. Therefore, manure management is a critical link in sustainable livestock production. Use of manure on-farm as a soil amendment and fertilizer also improves the integration of agricultural enterprises and restores nutrient cycles.

Manure management is an integral part of livestock and equine operations. Regulations are in place to ensure that the environment is not adversely affected by the high numbers of stock often associated with modern dairies, feedlots, and horse boarding and training facilities. To keep up with the current state of animal production, 4-H curricula need a manure management component. Teaching the concepts of Best Management Practices for manure utilization can begin early and be incorporated into the curriculum so that 4-H youth will fully understand the environmental principles behind the regulations, as well as, how to apply them to their own operations when they need to make decisions about farming and animal production.

In Colorado alone, there are more than 13,000 youth involved in 4-H livestock projects. The western states have more than 150,000 youth involved in 4-H livestock projects. Unfortunately, at this time, the 4-H livestock curriculum does not include manure management at all. Therefore, many 4-H youth may believe that manure management doesn't matter, since it is possible to be an excellent 4-H livestock project participant without knowing the first thing about manure management and environmental impacts of livestock.

*Rocky Mountain Compost School.* Composting animal manure transforms an odorous, heterogeneous by-product into an earthy-smelling, uniform, fine-textured soil amendment. Converting manure into high-quality compost opens up markets and an additional income stream to livestock producers, while simultaneously reducing manure disposal problems. In addition, the high temperatures achieved during the compost process kill most weed seeds and pathogens present in manures. However, these outcomes are dependent on knowledgeable composters who understand the principles at work and what measurable qualities the finished compost should have.

Composting is both a science and a practical art. Biological principles govern the composting process; therefore, there are many variables encountered by composters that must be evaluated in light of the basic biological principles at work. Developing the ability to apply biological principles to the situation at hand is a complex process. Opportunities for obtaining the technical information and skills to produce good quality compost from agricultural by-products are limited. We delivered the Rocky Mountain Compost School for the first time in May 2007 to provide compost training for large-scale manure composters working in conjunction with livestock producers. Participants came from several western states. Our goal is for this school to become the place to go for training for agricultural composters in the Inter-Mountain West and Great Plains states. The objective of this school is to train professional composters in the principles and practices of composting and in using the latest research results to improve their management and compost quality.

## Materials and Methods

*4-H Manure Management Curriculum.* The 4-H program emphasizes "learning by doing" with a focus on hands-on, experiential education. In every 4-H lesson, there are three components: do, reflect, and apply. We are using the same approach in our manure management curriculum so that youth can learn by doing. We developed a manure management curriculum that includes the following topics:

- Livestock and the Community
- Animal and Human Health
- Protecting Water Quality
- Protecting Air Quality
- Composting
- Manure Utilization
- Economics of Manure Management

The curriculum was presented to the CSU Extension Front Range 4-H Staff for input, and following detailed review by leading 4-H livestock agents, we made revisions to improve it. In addition, we pilot tested the curriculum at the Colorado State 4-H Conference in June 2006. We produced CDs of the curriculum which were distributed among all western states, and the curriculum is widely available on our website (www.manuremanagement.info).

*Rocky Mountain Compost School.* The Rocky Mountain Compost School is an annual, weeklong school (www.rockymountaincompostschool.info) designed to provide scientific and technical information to commercial composters who agricultural residuals. The Rocky Mountain Compost School has drawn professional composters from Idaho, Washington, and Wyoming, in addition to Colorado. We currently have a research composting facility at CSU's Agricultural Research, Development and Education Center, located on I-25 a few miles north of Fort Collins. We have classroom, laboratory and field facilities at this site to have a maximum of 20 participants in each school session. This school supports the existing composting industry and provides the necessary training and education to composters so that the industry can grow in size and technical expertise, producing more and better quality composts.

The Rocky Mountain Compost School offers the growing compost industry a solid technical background. With this in place, there is a mechanism for composters to become knowledgeable about biological principles governing the compost process, environmental concerns, compost quality in relationship to its proposed end use, regulatory issues, and recent research findings that pertain to composting.

The format of the school includes classroom teaching, laboratory exercises that are designed to learn about composting processes, hands-on composting exercises at the composting research site, and field trips to commercial compost sites. In adult education, it is critical to use hands-on learning techniques and a variety of teaching methods to reach different types of learners. With that in mind, the Rocky Mountain Compost School is taking a creative and innovative approach to compost education. We are focusing on experiential learning techniques in both field (making compost) and lab (evaluating compost) exercises. We also use both individual and group exercises to enhance the learning experience.

The Rocky Mountain Compost School came out of a partnership between the Rocky Mountain Organics Council (a group of producers and providers of compost and soil amendments) and Colorado State University. These connections with the composters themselves provide us at CSU with a much better understanding of the needs and priorities within the composting industry. We aim to expand our partnerships with professional composters throughout the region.

## Expected Outcomes

*4-H Manure Management Curriculum.* In the short-term, we are enhancing awareness of environmental issues related to livestock production and increasing knowledge of manure management practices among extension 4-H agents and volunteer leaders. In the medium-term, we aim to improve the skills of 4-H agents and leaders, in particular, to increase their ability to provide educational programs in manure management to 4-H youth.

In the long-term, we anticipate that 4-H agents and leaders will use the curriculum and encourage youth participating in 4-H livestock programs to complete the manure management training. We expect that agents and leaders will be able to document behavioral changes in participating youth. Behavioral changes may include an increased number of youth who are composting manure, changes in the corrals to prevent manure contaminated runoff, and utilization of manure at agronomic rates.

When the principles of good nutrient management are introduced early in a youth's life, they are more likely to practice these principles in their adult years. Livestock producers who utilize methods for handling manure that do not reflect Best Management Practices may choose not to change their management techniques in order to protect the environment. However, 4-H youth are more likely to practice what they have learned from reliable sources such as 4-H curricula, and may influence their elders as well. The inclusion of manure management in livestock 4-H curricula has the potential to have a significant environmental impact as these future leaders and business people move into the workforce.

*Rocky Mountain Compost School.* The outputs of this project include: the delivery of an annual weeklong, hands-on workshop for professional, large-scale composters, and partnership with the Rocky Mountain Organics Council and composters throughout the region. The outcomes of this project range from short-term to long-term outcomes. In the short-term, we are increasing both knowledge and skills of composters, so that they have the necessary foundation and motivation to optimize their composting practices. The short-term impacts are assessed using pre and post testing of the participants in order to quantify the knowledge gained. This also helps us to evaluate and improve our teaching for the next school.

The medium-term outcomes are changes in what the participants actually do, for example, increased amounts of composting, greater diversion of wastes of a wide variety, changes in management to optimize compost quality, and changes in policy to encourage manure composting. These action outcomes are assessed through email and phone follow-up contacts 6 months after each compost school.

The long-term impacts will be environmental, economic, and social. More manure will be composted and sold as a soil amendment. Increasing soil organic matter content increases water use efficiency and reduces irrigation requirements. As composting becomes a more common aspect of our culture, social values will change, too, leading to increased recycling of other types of wastes, as well.

**Acknowledgments**

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# A SURVEY EVALUATION OF CALIFORNIA BEEF PRODUCERS' BEST MANAGEMENT PRACTICES AND PERCEPTIONS OF A NATIONAL ANIMAL IDENTIFICATION SYSTEM[1]

**S. P. Doyle[2], K. L. Cooprider[2], J. L. Evans[3], and V. J. Velez[3]**
[2]College of Agriculture, California State University, Chico 95929
[3]California Department of Food and Agriculture – Animal Health Branch, Sacramento 95814

**ABSTRACT**: The objectives of this study were to evaluate California beef producers' perceptions of a National Animal Identification System (NAIS) and to survey current Best Management Practices (BMP) employed in the production of beef. The survey contained four primary sections: demographic information, ranching/farming operation description, management and marketing practices, and NAIS. Survey data were collected over a period of two years. The first year's data were collected at four separate locations, including the 2006 California Cattlemen's Association Annual Convention. The second year's data were collected at the California State University, Chico's Beef Field Day and through producer participation in an online survey. Data were initially analyzed across commercial and purebred producers using Chi-Square; one sample proportion tests were used to evaluate BMP responses (Statistix8, 2003). There were a total of 63 respondents, primarily from northern California. Data included 13 purebred beef operations and 45 commercial beef operations. A significant proportion of beef producers surveyed (89.7%, $P < 0.01$) reported following Beef Quality Assurance (BQA) guidelines; however, only 56.9% have attended a BQA certification. Nearly all respondents (98.3%, $P < 0.01$) vaccinate cattle using subcutaneous methods when available. Furthermore, 72.4% ($P < 0.01$) reported consulting with their veterinarian in developing a whole herd health program. When asked to rate their understanding of the current NAIS, 24% of commercial cattlemen reported a lower understanding ($P < 0.05$) compared to purebred producers who reported a fairly good understanding. Furthermore, a significant number of producers (72%, $P < 0.01$) believe the implementation of NAIS is a live animal traceability program, suggesting the appropriate message is being disseminated through California's outreach programs.

Key Words: Beef Cattle, Survey, Animal Identification

## Introduction

California's total agricultural cash receipts for 2006 reached $31.4 billion, keeping it ranked number one among the nation's states (USDA, 2007a). The top commodities include milk and cream followed by all varieties of grapes. Cash receipts from cattle and calves rank sixth among California's commodities, reaching $1.68 billion (5.4% of the total) in 2006. California's 11,500 beef cow-calf operations (USDA, 2007a) are major contributors to the economy. Events and government policy affecting California's cattle producers ultimately affect California's bottom line.

Recent food safety scares, international trade barriers, and animal health concerns such as bovine spongiform encephalopathy (BSE) are major driving forces behind policies and efforts in food animal and food product tracking and source verification (Smith et al., 2005). Domestic and international consumers are asking more about their food, where it came from, how it was produced, and how safe it is. Government agencies at all levels and the nation's cattle producers are re-examining current legislation and production practices to answer these concerns. Two such efforts include the proposed, voluntary National Animal Identification System (USDA, 2007b) and the beef industry's Beef Quality Assurance producer programs (Cattlemen's Beef Board, 2007). The former is a government initiative to protect animal health and achieve animal traceability in the event of an animal disease outbreak such as foot and mouth disease.

Producer perceptions, knowledge of, and commitment to such programs will determine the future success of the nation's beef industry. Thus, the objectives of this study were to evaluate California beef producers' perceptions of a National Animal Identification System (NAIS) and to survey current Best Management Practices (BMP) employed in the production of beef.

## Materials and Methods

Research approval was ascertained from California State University, Chico's Human Subjects and Animal Care Committee prior to the start of the project. The survey contained four primary sections: demographic information, ranching/farming operation description, management and marketing practices, and NAIS. Demographic information and ranching/farming operation descriptions were ascertained through categorical, multiple choice and fill-in-the blank questions. Questions in this section included gender, age, education, type of operation, physical location, sources of income, primary livestock species, number of primary livestock species, and land ownership. The section on

[1]*Funded in part by the United States Department of Food and Agriculture and the California Department of Food and Agriculture – Animal Health Branch through the NAIS cooperative agreement.*

management and marketing practices were primarily yes/no questions. NAIS perceptions were gathered through Likert-scale questions. Questions in the NAIS section dealt with knowledge and understanding of NAIS, perceptions of value of NAIS beyond safeguarding animal health, and primary producer concerns regarding NAIS and its components.

Survey data were collected over a period of two years. Locations were selected based on targeted event audience along with physical location and event date. The first year's data were collected at four separate locations, including the 2006 California Cattlemen's Association Annual Convention. The second year's data were collected at the California State University, Chico's Beef Field Day and through producer participation in an online survey.

Data were initially analyzed across commercial and purebred producers using Chi-Square; one sample proportion tests (Ho: $\mu$=.5) were used to evaluate BMP responses and rank data when no significant differences were found among commercial and purebred producers' responses (Statistix8, 2003).

## Results and Discussion

Year and venue were not significant sources of variation. There were a total of 63 respondents across seven venues, primarily from northern California. Survey response was low. Pennings et al. (2002) reported low response rates are typical for surveys involving farmers and dependent upon incentive to do survey, perceived length, and organization distributing survey. Participants were primarily male (n=39; females, n = 23) and represented varying age groups (18 yr to 65+ yr) and education levels (high school to Ph.D.). Data included 13 purebred beef operations and 45 commercial beef operations with complete survey data of varying herd size (Table 1) and land base (Table 2). Commercial operators reporting large herd sizes also reported that the operation represented their primary source of income (100 or less, 9.5%; 101-499, 43.8%; 500+, 88.9%; $P < 0.05$); no such trend was observed among purebred operators and may be due to the small sample size. The majority of participants ranch deeded parcels supplemented with private leases.

A significant proportion of beef producers surveyed (89.7%, $P < 0.01$) reported following Beef Quality Assurance (BQA) guidelines; however, only 56.9% have attended a BQA certification (Table 3). Nearly all respondents (98.3%, $P < 0.01$) vaccinate cattle using subcutaneous methods when available. Furthermore, 72.4% ($P < 0.01$) reported consulting with their veterinarian in developing a whole herd health program. Other practices common among survey participants reflecting best management practices include deworming cattle, supplementing cowherd with mineral, and castrating non-breeding stock.

While California beef producers surveyed appear to following BQA guidelines in many areas, production practices surrounding preventative animal health and disease management may require heightened emphasis in future educational efforts. For example, approximately half of all producers surveyed precondition calves (55.2%), quarantine livestock (56.9%), control visitor access (58.6%, Table 3), or control wildlife from contaminating water and feed (50.0%). Even fewer clean equipment between feeding and cleaning activities (43.1%). Promising is the number that reported changing needles and (or) palpation sleeves between animals (61.3%; $P = 0.15$).

In order to assess the California Department of Food and Agriculture (CDFA) NAIS message, several questions were developed to evaluate current perceptions among California's beef producers. When asked to rate their understanding of the current NAIS, 24% of commercial cattlemen reported a lower understanding ($P < 0.05$) compared to purebred producers who reported a fairly good understanding. Vander Mey et al. (2005) reported more than half of survey respondents reported being somewhat to very familiar with NAIS. Bolte et al. (2008) reported similar results among Kansas livestock market operators. A significant number of producers (72%, $P < 0.01$) believe the implementation of NAIS is a live animal traceability program, suggesting the appropriate message is being disseminated through California's outreach programs. However, approximately half of all producers surveyed also believe the NAIS is a Homeland Security program (51.7%), a food safety program (58.6%) or will improve consumer confidence in the nation's beef supply (58.6%). These results require attention in future NAIS educational efforts in California.

Questions were developed to evaluate producer perceptions beyond NAIS' purpose of safeguarding animal health. As with any technology or new production practice, incentive is often required before adoption occurs. For example, economic signals are required. Thus, producers were asked to evaluate NAIS' value in improving the economic viability of the beef industry, increasing marketing opportunities, and increasing export markets. While 41.4% of survey participants are still undecided as to the necessity of an animal identification program for the economic viability of animal agriculture, a significant proportion of beef producers do believe NAIS can increase marketing opportunities, including those abroad (69.0% and 72.4%, respectively; $P < 0.05$). Results are in agreement with those reported by Breiner et al. (2007) where cow-calf producers rated NAIS important to regaining foreign markets and increasing consumer confidence but were undecided on its importance relative to increased profitability. Of note in the current study is the fact that while many report the value of NAIS to improve market opportunities, including exports, few of those surveyed participate in a Processed Verified Program (PVP) or Quality Systems Assessment (QSA) program (22.4% and 12.1%, respectively; Table 3).

Simplicity, cost of identification/technology, confidentiality, private control of data, and data security were important to beef producers surveyed, regardless of production sector (Table 4). Breiner et al. (2007) reported similar results among the nation's cow-calf producers. Cost, confidentiality of information, reliability of the technology, and liability topped producer lists of concerns regarding the implementation of NAIS. All will need to be addressed in future educational efforts involving NAIS. Furthermore, the majority of beef producers believe being notified of a

contagious disease outbreak in the area is important and that this is an important aspect of NAIS. It appears that California beef producers see the value of NAIS; however, the concerns lie in the security of the data produced.

## Implications

Many of California beef producers appear to follow BQA guidelines in many areas; however, production practices surrounding preventative animal health and disease management may require heightened emphasis in future Cattlemen's Beef Board and California Department of Food and Agriculture grassroots educational efforts.

CDFA's message regarding NAIS appears to be reaching beef producers through California's outreach programs. Future efforts will need to outline more clearly the program's intent regarding ensuring food safety and increasing consumer confidence.

It appears that California beef producers see the value of NAIS. However, cost, confidentiality of information, simplicity, and data security and control topped producer lists of concerns regarding the implementation of NAIS. All will need to be addressed in future educational efforts involving NAIS.

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Table 1. Survey numbers across beef operation type, categorized by number of beef cattle.

| Operation | Number | Small (100 or less hd) | Medium (101-499 hd) | Large (500 + hd) |
|---|---|---|---|---|
| Commercial[1] | 47 | 21 | 16 | 9 |
| Purebred | 13 | 9 | 3 | 1 |
| Other[2] | 3 | 1 | 0 | 1 |
| Total | 63 | 31 | 19 | 11 |

[1]47 surveys were returned by commercial cattlemen; only 45 were complete.
[2]3 surveys were returned representing other types of beef operations such as research herd and youth projects; only two were complete.

Table 2. Average (SD)[1] acres of deed and leased land reported by California beef producers, categorized by herd size.

| | | | Lease | | |
|---|---|---|---|---|---|
| Operation[2] | Number | Deeded (ac) | Private (ac) | State (ac) | Federal (ac) |
| Commercial | | | | | |
| Small | 21 | 435.9 (493.2) | 429.5 (431.0) | 0 | 200 |
| Medium | 16 | 1155.8 (2698.9) | 2470.0 (2439.1) | 160 | 200 |
| Large | 9 | 7742.2 (3746.4) | 2575.0 (1738.5) | 2800 | 50,000 |
| Purebred | | | | | |
| Small | 9 | 232.6 (429.4) | 43.3 (23.1) | 0 | 0 |
| Medium | 3 | 1876.7 (1859.7) | 2075 (2015.3) | 0 | 0 |
| Large | 1 | 800 | 2500 | 22 | 0 |

[1]Average without SD represents a single response within herd size category.
[2]Small herd size (100 hd or less); Medium herd size (101 – 499 hd); Large herd size (500 + hd)

Table 3. Best Management Practices (BMP) among California's commercial and purebred beef producers.

| Management, Biosecurity and Marketing Practices. | Percent Answered "Yes" | Ho: μ=.5; *P*-value |
|---|---|---|
| Implant cattle with growth promoting hormones. | 9.0% | 0.00 |
| Feed antibiotics. | 9.0% | 0.00 |
| Participate in BQA training or a best management practices workshop. | 56.9% | 0.36 |
| Follow Beef Quality Assurance guidelines. | 89.7% | 0.00 |
| Vaccinate livestock. | 98.3% | 0.00 |
| Use subcutaneous injections when available. | 96.6% | 0.00 |
| Deworm livestock. | 96.6% | 0.00 |
| Use pour-on parasite control. | 84.5% | 0.00 |
| Use insecticide ear tags. | 36.2% | 0.05 |
| Supplement livestock with mineral. | 93.1% | 0.00 |
| Work with a veterinarian to develop an animal health program. | 72.4% | 0.00 |
| Castrate livestock. | 96.6% | 0.00 |
| Precondition calves, lambs, etc. | 55.2% | 0.51 |
| Use individual animal identification. | 81.0% | 0.00 |
| Body condition score your livestock. | 51.7% | 0.90 |
| Market livestock through a "Quality Systems Assessment" Program. | 12.1% | 0.00 |
| Market livestock through a "Processed Verified Program". | 22.4% | 0.00 |
| Isolate sick livestock. | 86.2% | 0.00 |
| Control access of visitors and service people. | 58.6% | 0.24 |
| Quarantine new livestock. | 56.9% | 0.36 |
| Clean equipment between feeding and cleaning activities and/or use separate equipment. | 43.1% | 0.36 |
| Change needles and/or palpation sleeves between animals. | 61.3% | 0.15 |
| Control outside animals (i.e. wildlife) from contaminating feed and water. | 50.0% | 1.00 |

Table 4. Important aspects of the National Animal Identification System

| Aspect | % Rating Important - Very Important | Ho: μ=.5; *P*-value |
|---|---|---|
| Simplicity | 75.9% | 0.00 |
| Cost of identification/technology | 77.6% | 0.00 |
| Confidentiality of data | 79.3% | 0.00 |
| Private control of data | 77.6% | 0.00 |
| Data security | 82.8% | 0.00 |

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# DEVELOPMENT OF AMP-ACTIVATED PROTEIN KINASE FLUORESCENCE CONSTRUCTS FOR MONITORING PLURIPOTENTIAL CELL DIFFERENTIATION

J.M. Kimzey[1], R. J. McCormick[1], M. M. Stayton[2] and M. Du[1]
Department of Animal Science[1] and Department of Molecular Biology[2], University of Wyoming, Laramie, WY 82071

**ABSTRACT:** Fetal stage is crucial for skeletal muscle development because there is no increase in muscle fiber number after birth. Fetal muscle development is characterized by both myogenesis and adipogenesis from mesenchymal stem cells (pluripotent progenitor cells). The underlying mechanisms regulating commitment of mesenchymal stem cells remain poorly studied, largely due to the lack of a method to track the differentiation of mesenchymal stem cells *in vivo*. The objective of this study is to develop a novel approach for monitoring pluripotent cell differentiation *in vivo* through the development of fluorescence constructs containing either the constitutively active (pAMPK+) or constitutively inactive (pAMPK-) AMPK catalytic subunit. The AMPK α2 subunit was cloned, purified, and sequenced. AMPK cDNA was mutated at Thr 172 into constitutively active and constitutively inactive states. pAMPK+ and pAMPK- cDNAs were cloned into Clontech's piRES2-DSRed2 (fluoresces red) piRES2-AcGFP1 (fluoresces green) vectors, respectively. Vectors were transformed into *E. coli* and subsequently purified and sequenced (Genbank accession number: EU131097). Plasmids containing mutated DNA insert were then transfected into CH3 10T½ mesenchymal stem cells and checked for fluorescence. Stably transfected cells were selected using G418. Fluorescence microscopy confirmed fluorescence in both constantly active and inactive constructs. During selection, cells containing the pAMPK+ gene grew very slowly and experienced massive die-off whereas cells containing the AMPK pAMPK- gene thrived. Due to the fact cells with constantly active and constantly inactive constructs fluoresce differently, these cells can be used for tracking mesenchymal stem cell differentiation *in vivo*. This model will be crucial for understanding the role of AMPK in the proliferation and differentiation of cells and for studying intracellular signaling pathways which affect differentiation of pluripotent cells in fetal muscle.

Key words: AMPK, Thr172, AMPKα2, maternal obesity, fetal programming, pluripotent stem cells

## Introduction

Type II diabetes and obesity are closely linked metabolic complications, both of which are increasing at alarming rates, especially in teenagers and children (Petersen, et. al., 2005). The increasing prevalence of overweight and obese women of childbearing age is also a growing public health concern (Siega-Riz et. al., 2006). Maternal obesity represents a special problem that can result in lifelong health complications in offspring, including a pre-disposition to obesity and diabetes (Morino, et. al. 2005, Barker et al. 2002). Unfortunately, mechanisms linking maternal obesity and over-nutrition to obesity and diabetes of offspring remain poorly defined.

Skeletal muscle is the main periphery tissue responsive to insulin stimulated uptake of glucose and fatty acids, and its conversion to insulin resistance is the crucial step in the development of type II diabetes (Lowell, et. al. 2005). In fetal muscle up to late gestation, there are a large number of mesenchymal stem cells (pluripotent proginator cells). Their proliferation and lineage commitment directly affect the number and size of muscle fibers developed. The commitment of pluripotent cells to adipogenesis instead of myogenesis will increase the number of intramuscular adipocytes, an event associated with insulin resistance in skeletal muscle. However, molecular mechanisms controlling proliferation and differentiation of pluripotent cells in fetal muscle *in vivo* remain poorly defined.

AMP-activated protein kinase (AMPK) is a master controller of energy metabolism in skeletal muscle (Hardie, D. G. 2004, 2005). AMPK sensitizes insulin signaling through phosphorylation of insulin receptor substrate-1 (IRS-1) at Ser 789 (Jakobsen et. al., 2001). On the other hand, Akt, a key mediator of insulin signaling, phosphorylates AMPK at Ser 485/491, which inhibits AMPK activation (Kocacic et. al., 2003, Soltys et. al., 2006). In addition, AMPK is involved in the control of lipid synthesis and adipogenesis (Woods, et. al., 2000, Blau et. al., 1995), both of which are linked to insulin resistance in skeletal muscle. Our preliminary data showed that maternal obesity and over-nutrition down-regulate AMPK activity in fetal muscle, which impairs insulin signaling and fetal muscle development, and increases lipid accumulation and adipogenesis in fetal muscle, leading to insulin resistance of skeletal muscle of offspring. Indeed, our *in vitro* studies showed that AMPK activation improved sensitivity of insulin signaling and inhibited adipogenesis and lipid accumulation. However, due to the lack of a proper experimental model *in vivo*, the role of AMPK in

fetal muscle development *in vivo* remains untested. Therefore, establishing an *in vivo* model will greatly facilitate our studies. This model will also be useful for other studies in muscle development.

## Objective

To establish an experimental model which can track the proliferation and differentiation of pluripotent stem cells in fetal muscle *in vivo*, and to use this model to test the role of AMPK in fetal muscle development.

## Hypothesis

We hypothesize that mesenchymal stem cells transfected with fluorescence constructs will differentiate into myogenic, adipogenic and other cells when injected into fetal muscle.

## Materials and Methods

RNA was isolated from ovine placentome using Tri Reagent and cDNA was created using Promega's ImProm-II Reverse Transcription System. The alpha2 subunit of AMPK was amplified using BD Advantage 2 High Fidelity PCR Kit by Clontech using oligos based on a bovine model. DNA bands were purified from a 1% agarose/1x TAE gel using Qiagen's Qiaquick gel extraction kit.

The AMPK gene was cloned using Invitrogen's Topo Cloning Kit and purified using Qiagen's miniprep kit and sequenced by Retrogen, Inc. The wild-type AMPK sequence was subjected to site-directed mutagenesis (AC 514, 515 to GA, Thr 172 Asp) and (A 514 to G, Thr 172 Ala) to form constitutively active (pAMPK+) and constitutively inactive (pAMPK-) forms, respectively, using Statagene's QuikChange mutagenesis kit. The mutated DNA was transfected into XL1-Blue supercompetent cells and purified and sequenced as before. The mutated AMPK constitutively active and constitutively inactive DNA was digested with EcoR1 and excised from a 1% agarose/1x TAE gel using Qiagen's Qiaquick gel extraction kit.

Mutated DNA was subcloned into Clontech's piRES2-AcGFP1 and piRES2-DSRed2 vectors using Promega's TSAP and LigaFast Rapid DNA ligation system. Vectors were transformed into DH5α *E. coli* and purified using Stratagene's DNA miniprep kit and subsequently sequenced. Plasmids were then transfected into CH3 10T½ cells which were grown to 80% confluence in DMEM containing 5% fetal bovine serum with 1% antibiotic at 37ºC with 5% humidity. Fluorescence was verified using fluorescent microscopy.

## Results

The full length of AMPK alpha2 subunit cDNA was cloned and sequenced (Genbank accession number: EU131097), which were used to generate constitutively active and negative AMPK constructs.

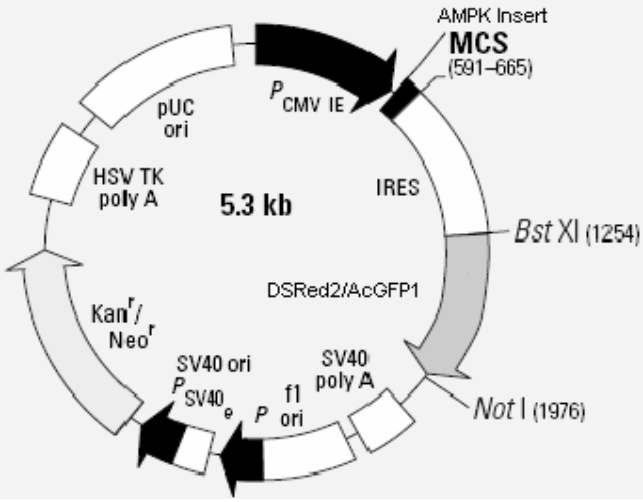


Figure 1: DSRed/AcGFP1 construct design

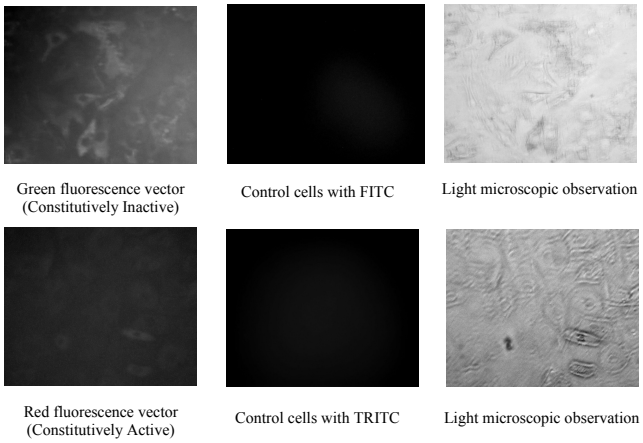


Figure 2: CH3 10T1/2 mesenchymal stem cells transfected with constantly active or constantly inactive plasmid. Positively transfected cells fluoresce.

The pAMPK+ mutant contains a mutation from Thr to Asp, mimicking the Thr 172 phosphorylation, while the pAMPK- mutant contains a mutation from Thr to Ala, preventing phosphorylation. Since phosphorylation at Thr 172 is correlated with its activity, pAMPK+ is predicted to show protein kinase activity in the presence or absence of its effector, AMP. In contrast, the pAMPK- mutant should be enzymatically inactive regardless of AMP concentration. Both the constitutively active and inactive constructs were successfully transfected into CH3 10T1/2 pluripotent stem cells and successfully expressed fluorescence *in vivo*, indicating successful transfection.

Stable colonies were established of both cell lines and it was noted that the constitutively inactive cells grew much more vigorously than the wild type or the constitutively active form. The constitutively active cells grew very slowly, but were not killed by the mutation. It was also observed that the constitutively active cells appear morphologically different than the constitutively inactive cells. Further testing will be required to gain a better understanding of this phenomenon.

```
           ++++++++++++++..++++++++++++++++++
Wild-type  GGTGAATTTCTGCGAACTAGCTGCGGATCCCCAAAT
Active     GGTGAATTTCTGCGAGATAGCTGCGGATCCCCAAAT
Inactive   GGTGAATTTCTGCGAGCTAGCTGCGGATCCCCAAAT
```

Figure 3: Multiple alignment of wild-type, pAMPK+ (active) and pAMPK- (inactive) sequences.

## Discussion and Conclusion

Intramuscular injection of cells is a relatively new technique. In several previous studies, primary muscle cells have been cultured, transferred with foreign genes, and injected into cardiac muscle to replace damaged tissues (Blau and Springer, 1995). Myoblast transplantation has been used to cure diseases like muscular dystrophy (Cossu and Mavilio, 2000). In a previous study, muscle progenitor cells were cultured *in vitro* and injected into the muscle of nude mice. These injected cells were retained in mouse muscle, proliferated and fused with myofibers. In addition, portion of these injected cells retained in muscle as satellite cells (Montarras et al., 2005). Cells expressing the green fluorescent protein reporter gene have been used to track the destination of myoblasts in skeletal muscle (Relaix et al., 2005). These studies strongly suggest that it is feasible to inject cells into muscle and to study the proliferation and differentiation of these cells during muscle development.

The C3H 10T1/2 mesenchymal stem cell line is well characterized and widely used. These cells are not tumorogenic and, therefore are fit to inject into animals *in vivo*. In this study, C3H 10T1/2 mesenchymal stem cells were stably transfected with either constitutively active or inactive AMPK fluoresce constructs. In the very near future, we plan to inject both fluorescence colors simultaneously into muscle *in vivo*. Due to the difference in AMPK activity, we expect cells with red and green fluorescence will differentiate into myogenic cells or adipogenic cells at different rates, establishing a very useful model for studying muscle development.

## Acknowledgement

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# AMP-ACTIVATED PROTEIN KINASE (AMPK) MEDIATES PHOSPHORYLATION OF FOXO TRANSCRIPTION FACTORS INDEPENDENTLY OF INSULIN-LIKE GROWTH FACTOR-1 (IGF-1)/PKB PATHWAY IN C2C12 MYOTUBES

**J. F. Tong, K. R. Underwood, X. Yan, M. J. Zhu, and M. Du**
Department of Animal Science, University of Wyoming, Laramie, WY 82071

**ABSTRACT:** The forkhead type transcription factors (FOXO) regulate the expression of two newly identified muscle-specific E3 ubiquitin ligases, MAFbx and MuRF1, which are crucial mediators of myofibrillar protein breakdown. AMP-activated protein kinase (AMPK) regulates protein synthesis in skeletal muscle, but its role in myofibrillar protein degradation is unclear. The insulin-like growth factor-1 (IGF-1)/PKB pathway induces muscle hypertrophy partially through phosphorylation of FOXO which inhibits its function by translocation and nuclear exclusion. The role of AMPK in FOXO phosphorylation is unclear. C2C12 myotubes are commonly used for studying skeletal muscle growth. The objective was to examine the effects of AMPK and its interaction with IGF-1 on FOXO phosphorylation in C2C12 myotubes. C2C12 cells were incubated in DMEM medium with 10% fetal bovine serum. Fusion was induced by 2% horse serum. Myotubes were treated with 5-Aminoimidazole-4- carboxamide-1-β-D-ribonucleoside (AICAR, 0, 0.1, 0.3 and 1.0 mM), and/or IGF-1 (50 ng/ml). After 24 h incubation, myotubes were collected for immunoblotting and immunofluorescence analyses. As expected, IGF-1 activated PKB, which enhanced phosphorylation of FOXO1 at Thr 24 and FOXO3 at Thr 32. Activation of AMPK by 0.1mM, 0.3mM, 1.0mM AICAR, a specific AMPK activator, synergized the activation of PKB by IGF-1. However, the phosphorylation of FOXO was not enhanced but reduced dose-dependently by AMPK activation, which indicates that AMPK mediates the phosphorlytion of FOXO through a mechanism independent of PKB. Immunofluorescence assay showed IGF-1 treatment induced higher FOXO exclusion from the nuclei while 1.0 mM AICAR treatment promoted FOXO nuclear relocation. In conclusion, in addition to regulating IGF-1/PKB signaling pathway and the subsequent protein synthesis in skeletal muscle, AMPK also mediates skeletal muscle protein degradation through regulation of FOXO phosphorylation. These data indicate that AMPK may be a crucial molecular target for enhancing lean growth in livestock.

**Key Words:** AMP-activated protein kinase, C2C12, FOXO, Degradation, Skeletal muscle.

## Introduction

The maintenance of skeletal muscle depends upon the dynamic balance of anabolic and catabolic reactions to determine the level of muscle protein (McKinnell and Rudnicki, 2004), but the key molecular mediators of hypertrophy and atrophy have only begun to be elucidated (Stitt, 2004 ). Induction of hypertrophy in skeletal muscle by increased load is accompanied by the increased expression of insulin-like growth factor 1 (IGF-1) (Stitt, 2004, DeVol et al., 1990). When released, IGF-1 binds to the IGF receptor and activates the IGF/protein kinase B (PKB) signaling cascade which further phosphorylates mammalian target of rapamycin (mTOR) and glycogen synthase kinase-3 (GSK3) and increases protein synthesis (Glass, 2003). Atrophy is associated with increased rate of protein breakdown which has been correlated with the activation of cellular proteases, most notably the ATP-dependent ubiquitin proteasome system. Evidence in multiple experimental models suggests that MAFbx (muscle atrophy F box), also called atrogin-1 and MuRF1 (muscle RING finger 1), two muscle specific E3 ubiquitin ligases, play a pivotal role in muscle atrophy (Glass, 2003, Chan et al., 2005, Doucet et al., 2007). Recent evidence suggests FOXO transcription factors (FOXO1, FOXO3a, and FOXO4), a subfamily of the forkhead type transcription factors, induce the E3 ubiquitin ligase and cause skeletal muscle atrophy (Sandri et al., 2004), but the regulation and function of FOXO in skeletal muscle remain poorly understood.

AMP-activated protein kinase (AMPK) is a serine–threonine heterotrimeric kinase which acts as a sensor of cellular energy status that is conserved in all eukaryotic cells. More recently, it has been realized that hormones and other extracellular signals have acquired the ability to modulate the AMPK system (Towler and Hardie, 2007). It has been shown that pharmacological activation of AMPK results in inhibition of protein synthesis (Bolster et al., 2002; Chan and Dyck, 2005; Reiter et al., 2005). AMPK regulates protein synthesis in skeletal muscle, but little is known regarding its role in myofibrillar protein degradation. The objective of this study was to examine the effect of AMPK and its interaction with IGF-1 on FOXO phosphorylation in C2C12 myotubes.

## Materials and Methods

*Chemicals and Antibodies.* AICAR was purchased from Sigma Aldrich (St. Louis, MO). All chemicals for cell culture were bought from Sigma-Aldrich (St Louis, MO). Antibodies against phospho-AMPK at Thr 172, phospho-Akt at Ser 473, phosphor-mTOR at Ser 2448, phospho-FOXO1(Thr 24)/FOXO3a (Thr 32) and horseradish peroxidase linked secondary antibody were purchased from Cell Signaling (Danvers, MA). Anti-β-actin antibody was obtained from Developmental Studies Hybridoma Bank (DSHB, Iowa City, IA).

*C2C12 cell culture*. C2C12 myoblasts were obtained from the American Type Culture Collection (ATCC; Manassas. VA) and grown in Dulbecco's modified Eagle medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS), 100 units/ml of penicillin, and 100 µg/ml of streptomycin in a humidified atmosphere of 95% air and 5% $CO_2$ at 37°C. Fusion was induced by 2% horse serum for 3 d. Then, C2C12 myotubes in 6-well plates were incubated with AICAR (0, 0.1, 0.3 and 1.0 mM) with or without 50 ng/ml IGF-1for 24 h in DMEM containing 2% horse serum.

*Immunoblotting Analysis.* C2C12 myotubes were washed with PBS and lysed in buffer containing 137 mM NaCl, 50 mM HEPES (pH 7.4), 2% SDS, 1% NP-40, 10% glycerol, 2 mM phenylmethylsulfonyl fluoride, 10 mM sodium pyrophosphate, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 2 mM $Na_3VO_4$, and 100 mM NaF. Soluble proteins were recovered after a 10-min centrifugation (10,000 x g), and their concentrations were determined according to the Bradford method (Bio-rad Laboratories, Hercules, CA) (Zhu et al., 2006). Proteins in cell lysates were separated by SDS-PAGE and transferred to a nitrocellulose membrane. Subsequently, the membranes were treated with blocking buffer (5% nonfat dry milk in TBS/T buffer containing 150 mM NaCl, 10 mM Tris pH8.0 and 0.1% Tween 20) for 1 h. The blocked membranes were probed with primary antibodies and further incubated with a secondary antibody conjugated with horseradish peroxidase. Membranes were visualized using Enhanced Chemiluminescence (ECL) Western blotting reagents (Amersham Bioscience, Piscataway, NJ) and exposure to film (MR, Kodak, Rochester, NY). Density of bands was quantified by using Imager Scanner II and ImageQuant TL software (Amersham Bioscience). Band density was normalized according to the β-actin content (Zhu et al., 2004).

*Immunofluorescence assay.* C2C12 Cells were seeded on coverslips, and cultured and treated as described above. Cells were fixed for 15 min in phosphate-buffered saline (PBS) containing 4% paraformaldehyde and permeabilized for 15 min in PBS containing 0.4% Triton X-100. After incubation for 60 min in blocking buffer (2% goat serum in PBS and 1% bovine serum albumin and 0.1% Tween 20), coverslips were incubated overnight at 4 °C in a rabbit anti-FOXO1 antibody (1:50 dilution) prepared in blocking buffer. Following washes with PBS, samples were incubated for 60 min in a horse anti-rabbit Texas red antibody (1:100 dilution) (Jackson Laboratories, Bar Harbor, Maine). After washes with PBS, coverslips were mounted in Vectashield (Vector Laboratories, Burlingame, CA) and visualized with Olympus BX 51 microscope attaching to a fluorescence illuminator (BX-URA2).

*Statistical Analysis.* Data were analyzed by GLM procedure (SAS Inst., Inc., Cary, NC). Statistical significances were determined by the Tukey's Studentized Range test. $P < 0.05$ was considered significant.

## Results

*AICAR induced phosphorylation of AMPK.* AICAR is an adenosine analog taken up by muscle and phosphorylated to form 5-aminoimidazole- 4-carboxamide- 1-β-D-ribofuranosyl -5monophosphate (ZMP), which stimulates AMPK activity (Sakoda et al., 2002). Western blot analysis of extracts from C2C12 myotubes treated with 1.0mM AICAR revealed increased phosphorylation of AMPK (Figure 1), while IGF-1 treatment alone had no effect on AMPK activity ($P > 0.05$). When combining IGF-1 with 1.0mM AICAR, the AMPK phosphorylation levels increased, providing evidence that they may act synergistically.

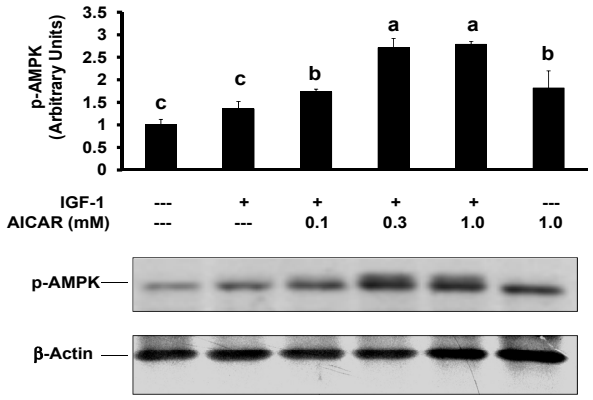


Figure 1. Effects of AICAR and IGF-1 treatment on phosphorylation of AMPK in C2C12 Myotubes. C2C12 cells were treated with various concentrations of AICAR (0.1, 0.3, and 1.0mM) with or without 50 ng/ml IGF-1 for 24 h. Bars depict means ± SE of 3 independent experiments. Within treatments, means lacking a common letter differ, $P < 0.05$.

*AICAR enhanced IGF-1 induced phosphorylation of PKB but reduced the phosphorylation of mTOR.* There is accumulating evidence that the IGF-1/PI3K/PKB pathway is a crucial intracellular signaling mechanism underlying muscle hypertrophy (Glass, 2003). Activated PKB stimulates the phosphorylation of its substrate mTOR. It is suggested that AMPK can inhibit mTOR signaling through the phosphorylatin of Tuberous sclerosis 2 (TSC2), the upstream regulator of mTOR (Inoki et al., 2003). Our results for the first time showed that activation of AMPK by AICAR synergized the activation of PKB induced by IGF-1 (Figure 2A) ($P < 0.05$). Elevated PKB activation is supposed to promote the phosphorylation of mTOR, but as shown in Figure 2B, it decreased significantly with increased concentration of AICAR added, showing that AMPK activation inhibited mTOR, surpassing the activation by IGF-1/PKB pathway.

*AICAR reduced p-FOXO1, p-FOXO3a levels.* IGF-1 enhanced phosphorylation of FOXO1 at Thr 24 and FOXO3a at Thr 32 in C2C12 myotubes without changing total FOXO1 concentration (Figure 3). It was observed that AICAR incubation in C2C12 myotubes for 6 h stimulated the level of FOXO transcription factors mRNA and protein expression (Nakashima and Yakabe, 2007). Our results didn't show the same effect after 24 h of AICAR incubation, but instead the total FOXO1 decreased as AICAR

concentration increased (Figure 3A). Treatment of AICAR induced a dose-dependent reduction in FOXO1/FOXO3a phosphorylation (Figure 3B), suggesting that AMPK may inhibit FOXO phosphorylation, thereby causing their nuclear retention and up-regulating specific genes including E3 ubiquitin ligases expression.

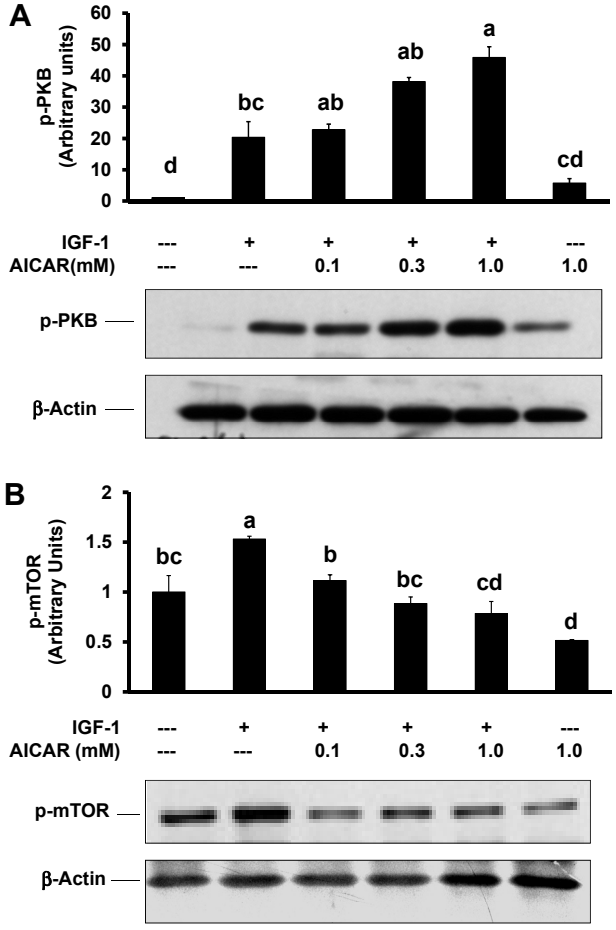


Figure 2. Effect of AICAR and IGF-1 treatment on phosphorylation of PKB and mTOR in C2C12 Myotubes. Bars depict means± SE of 3 independent experiments. Within treatments, means lacking a common letter differ, $p < 0.05$.

*Effects of AICAR and IGF-1 treatment on subcellular localization of FOXO1*. Immunofluorescence assay showed IGF-1 treatment induced higher FOXO1 exclusion from nuclei while 1.0 mM AICAR treatment promoted FOXO1 nuclear relocation in C2C12 myotubes (Figure 4).

## Discussion

It has been shown that IGF-1 can block the transcriptional up-regulation of key mediators of skeletal muscle atrophy, the ubiquitin-ligases MuRF1 and MAFbx, through the PI3K/PKB pathway by inhibiting FOXO transcription factors (Glass, 2003). In response to IGF-1, PKB phosphorylates FOXO transcription factors on multiple sites, leading to the exclusion of phosphorylated FOXO proteins from the nuclei and inhibition of the transcriptional functions (Brunet et al., 1999). As expected, our results showed that IGF-1 activated PKB, which enhanced phosphorlytion of FOXO1 at Thr 24 and FOXO3 at Thr 32. Activation of AMPK by 0.1 mM, 0.3 mM and 1.0 mM AICAR, synergized the activation of PKB by IGF-1. However, the phosphorylation of FOXO was not enhanced but reduced dose-dependently by AMPK activation, which indicates that AMPK may mediate the phosphorlytion of FOXO through a mechanism independent of PKB. It has been indicated that in hepatocytes, kinases distinct from PKB phosphorylate FOXO1 at Thr 24 (Nakae et al., 2001). AMPK can directly phosphorylate FOXO3 at residues that are different from the residues regulated by PKB, and AMPK phosphorylation of FOXO3 appears to enhance the ability of these transcription factors to up-regulate specific target genes expression (Greer et al., 2007). In addition, we detected the down-regulation of mTOR phosphorylation by AMPK, which may also contribute to the up-regulation of ubiquitin ligases. Inhibition of mTOR increases the expression of ubiquitin ligases independently of FOXO (Latres et al., 2005).

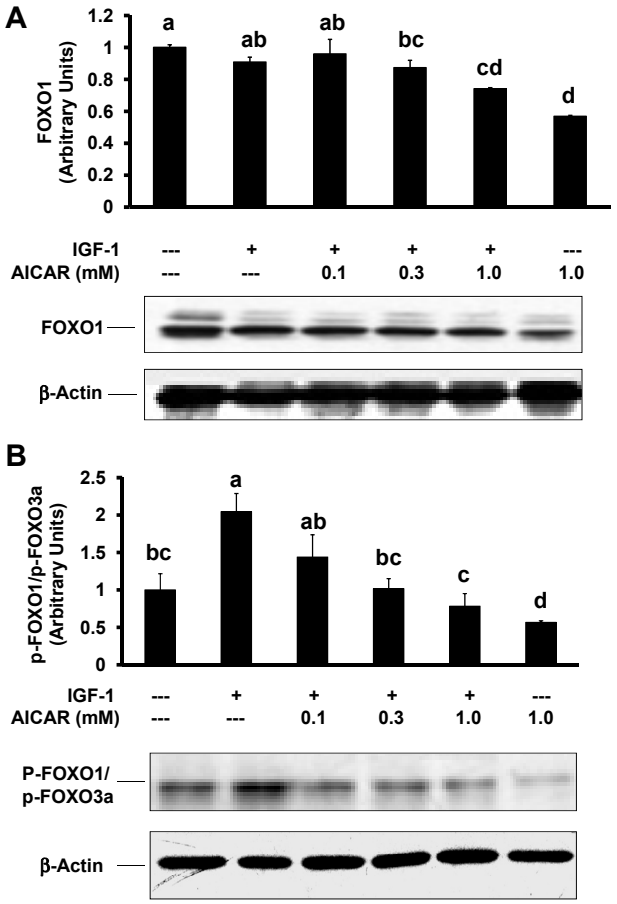


Figure 3. Effect of AICAR and IGF-1 treatment on total FOXO1, p-FOXO1/p-FOXO3a in C2C12 myotubes. Bars depict means ± SE of 3 independent experiments. Within treatments, means lacking a common letter differ, $p < 0.05$.

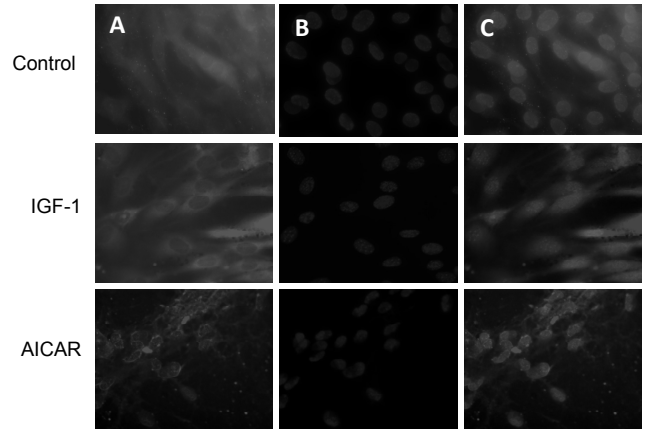


Figure 4. Effect of AICAR and IGF-1 treatments on subcellular localization of FOXO1. A. TRITC staining; B. DAPI staining; C. merged. Magnification, x 100.

In conclusion, in addition to regulating IGF-1/PKB/mTOR signaling pathway and the subsequent protein synthesis in skeletal muscle, AMPK mediates skeletal muscle protein degradation through interaction with FOXO transcription factors, which regulate the expression of muscle specific E3 ubiquitin ligases. These data indicate that AMPK may be a crucial molecular target for enhancing lean growth in livestock. The recent discovery that Rendement Napole gene in pigs, characterized by superior growth performance, is due to a mutation in AMPK, strongly suggests the important role of AMPK in regulating muscle growth and development.

## Acknowledgement

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# EFFECT OF SEPIOLITE SUPPLEMENTATION ON BROILER GROWTH PERFORMANCES AND CARCASS YIELD

**M. Hadj Ayed*[1], I. Zghal[1], B. Rekik[2]**
Institut Supérieur Agronomique, Chott Mariem, Sousse, Tunisia[1], Ecole Supérieure d'Agriculture, Mateur, Tunisia[2]

**ABSTRACT**: The effects of incorporating sepiolite into diet on growth performance were studied in broilers. A total of 800 one day-old Hubbard JV chicks of both sexes were divided into 16 litter pens (50 chicks per pen). Pens were allotted randomly to four diets: diet 0 (D0), diet 0.5 (D0.5), diet 1 (D1), and diet 2 (D2) (with 4 replications x 50 birds/diet). Dietary treatments were achieved by incorporating 0%, 0.5%, 1%, and 2%, respectively, of sepiolite into rations based on corn and soybean meal, for starter, grower, and finisher periods. Growth performance, mortality rate (MR), feed intake (FI), and feed conversion ratio (FCR) were evaluated weekly. At 36 days of age, twelve male and female broilers from each pen were slaughtered to evaluate hot carcass yield (HCY). Results showed that overall growth performances were significantly enhanced by increasing the percentage of sepiolite in the diet and the best results were obtained for the lot fed D2. Live body weight at 36 days of age and daily gain were improved ($P < 0.05$) by about 11%, while overall FI and FCR were reduced ($P < 0.05$) by 6% and 15%, respectively. The effect of sepiolite on chicks' performances was more important ($P < 0.01$) between 1 and 21 days of age than in the rest of the experimental period. Furthermore, the dose of sepiolite in the diet had no effect ($P = 0.23$) on either MR (1.4%) or HCY (73%). Incorporating sepiolite into broiler diets improved growth performances and feed efficiency particularly in starter and grower periods, and a 2% dose seemed to generate the best results. However, economic aspects for using optimal sepiolite doses in broiler diet formulation should be addressed.
**Keywords:** Broiler, sepiolite, feed efficiency

## Introduction

Sepiolite is a natural ingredient; a hydrated magnesium silicate used often as a binder in pelleted feeds. The addition of sepiolite facilitates the transport and conditioning of feeds, and improves the durability and hardness of pellets, especially in broiler rations rich in fat (Melcion, 1995; Angulo et al., 1995). Sepiolite also enhances physical stability of concentrates, reduces dust losses, and decreases bacterial development (Pontes Pontes and Castello LLobet, 1995). Furthermore, this additive may replace growth factors, antibiotics, and anticoccidians such as avoparcin, monensin, and tylosin in rations of monogastric animals. The use of sepiolite in poultry and swine diets improved growth performances and carcass quality (Castaing, 1994; Parisini, 1993). In Tunisia, the use of sepiolite in animals' nutrition is still limited. The objective of this trial was to assess the effects of incorporating sepiolite in broilers diets on growth performances and carcass yield.

## Materials and Methods

The trial was conducted in a controlled environment on 800 Hubbard JV one day old chicks. Birds were divided into 16 groups of 50 chicks each. They were logged in 1.9 m x1.7 m pens (15-16 birds/$m^2$) and received 24 hours light. All birds were vaccinated against Marek, Newcastle, Infectious Bronchitis and Gomboro diseases. Four treatments (diets) were used by incorporating into starter, grower, and finisher concentrates for broilers 0 (D0), 0.5 (D0.5), 1 (D1), or 2% (D2) of sepiolite. Composition and forms of aliments are given in Table 1. Each treatment was randomly assigned to four groups of birds fed starter, grower, and finisher concentrates during 1-13, 14-23, and 24-36 days of age, respectively. Growth performance, mortality rate (MR), feed intake (FI), and feed conversion ratio (FCR) were evaluated weekly. At 36 days of age (end of the trial), twelve male and female broilers from each pen were slaughtered to evaluate hot carcass yield (HCY). Treatment means for growth performances, MR, FI, FCR, and HCY were compared by TUKEY test following a one way ANOVA (SAS, 1989). Variations of daily gains and feed efficiency with the dose of sepiolite were also studied by linear regression at 22 and 36 days of ages.

Table 1. Composition (in %) and forms of aliments

| Aliment | Maize | Soya | Minerals and vitamins | Form |
|---|---|---|---|---|
| Starter | 62 | 34 | 4 | Crumbed |
| Grower | 64 | 32 | 4 | Crumbed |
| Finisher | 67 | 29 | 4 | Pelleted |

## Results and Discussion

Live weights and weight daily gains of birds are given in Table 2. Average daily feed intake and feed conversion ratio are shown in Table 3. Growth performance was enhanced by incorporating sepiolite into chicks' rations. At the end of the trial (36 days), the live body weight of birds receiving sepiolite was significantly higher ($P < 0.015$) than that of birds receiving the control diet (D0). D2 birds were around 200 g heavier than D0 birds at 36 days of age. Furthermore, D2 resulted in heavier birds compared to those receiving D0.5 and D1. D2 birds had heavier weights by the end of the trial because they made the highest daily gains among all birds between 1 and 21 days (Table 2). Daily gains of birds receiving D2 were around 11% higher ($P < 0.05$) than control (D0) birds. It seems that the effect

Table 2. Live weights (g) and weight daily gains (g/d) of chicks fed rations with 0, 0.5, 1, and 2% of sepiolite

| Sepiolite in feed (%) | Live weight[1], (N=4) | | | | | |
|---|---|---|---|---|---|---|
| | 1 day | 8 days | 15 days | 22 days | 29 days | 36 days |
| 0 | 38.87 | 171.40[a] | 425.58[a] | 756.87[d] | 1233.47[c] | 1866.13[c] |
| 0.5 | 38.76 | 175.96[ab] | 441.85[b] | 803.80[c] | 1268.73[b] | 1951.33[bc] |
| 1 | 38.84 | 175.94[ab] | 465.20[a] | 841.33[b] | 1299.33[b] | 1988.47[ab] |
| 2 | 38.82 | 181.10[b] | 469.49[a] | 872.87[a] | 1358.60[a] | 2068.13 [a] |
| MSE[2] | 0.399 | 2.0124 | 2.980 | 6.015 | 8.718 | 28.29 |
| Probability | 0.998 | 0.100 | 0.0001 | 0.0001 | 0.0001 | 0.015 |
| | Daily gain[1], (N=4) | | | | | |
| Sepiolite in feed (%) | 1-8 d | 9-15 d | 16-22 d | 23-29 d | 30-36 d | Aggregate |
| 0 | 18.9 | 36.3[b] | 47.3[c] | 68.1 | 90.4 | 52.2[c] |
| 0.5 | 19.6 | 38.0[b] | 51.7[b] | 66.4 | 97.5 | 54.6[bc] |
| 1 | 19.6 | 41.3[a] | 53.7[b] | 65.4 | 98.4 | 55.7[ab] |
| 2 | 20.3 | 41.2[a] | 57.6[a] | 69.4 | 101.4 | 58[a] |
| MSE[2] | 0.322 | 0.615 | 0.8545 | 1.3337 | 3.4987 | 0.9411 |
| Probability | 0.087 | 0.001 | 0.001 | 0.237 | 0.23 | 0.015 |

[1] Means in the same column with different superscripts are significantly different at $P = 0.05$
[2] Standard error of the mean.

Table 3. Average feed intake (g) and feed conversion ratio for chicks fed rations with 0, 0.5, 1, and 2% of sepiolite

| Sepiolite in feed (%) | Average feed intake[1], (N=4) | | | | | |
|---|---|---|---|---|---|---|
| | 0-8 d | 9-15 d | 16-22 d | 23-29 d | 30-36 d | Global |
| 0 | 27,8 | 57,1[a] | 96,6[a] | 120,9[a] | 207,7 | 102,0[a] |
| 0,5 | 26,6 | 58,9[a] | 94,1[ab] | 114,3[ab] | 199,0 | 98,6[b] |
| 1 | 26,2 | 52,7[b] | 93,5[ab] | 115,9[ab] | 197,3 | 97,1[b] |
| 2 | 25,4 | 56,9[a] | 91,4[b] | 109,4[b] | 195,0 | 95,6[b] |
| MSE[2] | 0,61 | 0,81 | 0,97 | 1,96 | 3,14 | 0,89 |
| Probability | 0,17 | 0,006 | 0,06 | 0,04 | 0,117 | 0,011 |
| | Feed conversion ratio[1],(N=4) | | | | | |
| Sepiolite in feed (%) | 0-8 d | 9-15 d | 16-22 d | 23-29 d | 30-36 d | Global |
| 0 | 1,47[a] | 1,57[a] | 2,09[a] | 1,78 | 2,32 | 1,96[a] |
| 0,5 | 1,36[ab] | 1,55[a] | 1,85[b] | 1,72 | 2,04 | 1,80[ab] |
| 1 | 1,34[b] | 1,27[c] | 1,75[c] | 1,77 | 2,01 | 1,74[b] |
| 2 | 1,27[b] | 1,36[b] | 1,59[d] | 1,56 | 1,96 | 1,65[b] |
| MSE[2] | 0,031 | 0,023 | 0,024 | 0,052 | 0,113 | 0,050 |
| Probability | 0,025 | 0,001 | 0,001 | 0,118 | 0,209 | 0,028 |

[1] Means in the same column with different superscripts are significantly different at $P = 0.05$
[2] Standard error of the mean.

of sepiolite on chicks' performances was more important ($P < 0.01$) between 1 and 21 days of age than in the rest of the fattening period. Plots of body weights for the four diets (D0, D0.5, D1, and D2) at 22 and 36 days of ages are shown in figure 1. And variations of global daily gains and feed conversion ratios with the percentage of sepiolite in the ration are illustrated by figure 2.

Overall FI and FCR were reduced ($P < 0.05$) by 6% and 15%, respectively (Table 3). Although D2 birds had the highest body weights, they converted feed better than other

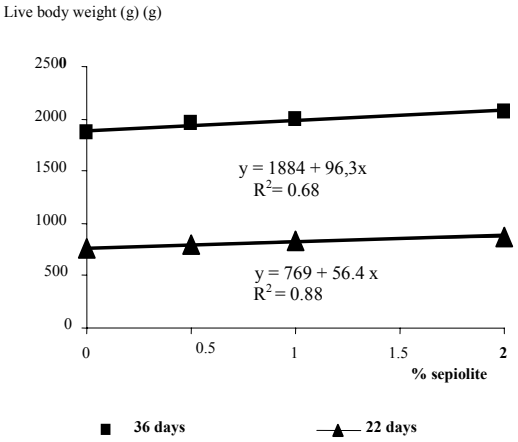


Figure 1. Variations of live body weights of Hubbard JV chicks with sepiolite percentage in the ration.

diet birds, mainly the control ones (Table 3, Figure 2). The FCR was 1.96 for the D0 birds but only 1.65 for the D2 birds (Table 3, Figure 2). Those of D0.5 and D1 birds were intermediate. FCR for all bird groups were the highest in the last week of the trial compared to other experimental periods. This increase was obviously the result from mainly increased feed intake and relatively reduced weight gains (Table 3).

Improving effects of sepiolite on growth performances in poultry and swine were reported by Pontes Pontes and Castello LLobet (1995), Ouhida et al. (2000), Castaing (1994), and Parisini et al. (1993). These authors reported similar results to those found in this study with respect to weight gains and feed efficiency. Increased gains and reduced feed intake by animals fed rations with sepiolite might be explained by the fact that the specific physical structure of sepiolite may reduce the by pass of nutrients and consequently improve their absorption. Lengthened transit of nutrients enhances digestibility and mineral absorption.

The dose of sepiolite in the diet had no effect ($P = 0.23$) on either MR or HCY (MSE = 0.40). The overall MR was low (1.4%) and HCY was between 72.1 (D0) and 73.9% (D2)

## Conclusion

Incorporating sepiolite into broiler diets improved growth performances and feed efficiency. Although relations of weight gains and feed conversion ratios with the percentage of sepiolite in rations seemed linear, better performances were obtained in starter and grower periods. Adding a 2% dose seemed to generate the best results. The use of sepiolite may be limited to the starter and the beginning of grower periods. However, economic aspects for using optimal sepiolite doses in broiler diet formulation should be addressed.

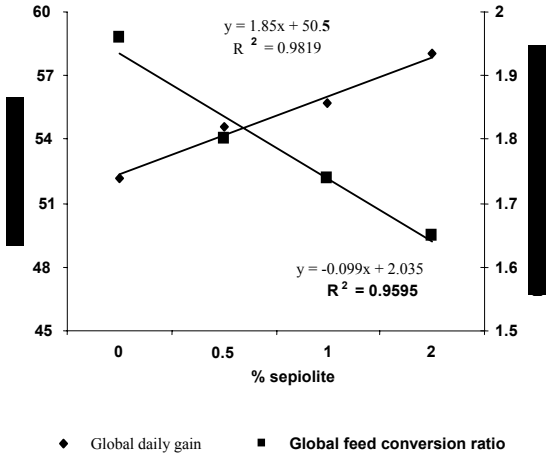


Figure 2. Variations of global daily gains and feed conversion ratios of Hubbard JV chicks with sepiolite percentage in the ration.

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# EFFECT OF THE CHROMIUM ADDITION AT THE DIET ON REPRODUCTIVE PERFORMANCE OF SOWS SERVED DURING THE SUMMER SEASON

**J. A. Romo[1*], R. Barajas [1], H. R. Guemez[1, 2], J. M. Romo [2, 1], and F. Juarez[1]**
[1]Facultad de Medicina Veterinaria y Zootecnia de la Universidad Universidad Autonoma de Sinaloa
[2]Granja Porcina "La Huerta"
E-mail: romo60@uas.uasnet.mx

**ABSTRACT:** With the objective of determine the effect of chromium methionine supplementation on reproductive performance of sows served during the summer season, 199 multiparous hybrid sows were used in a reproductive performance experiment. In a complete randomized experiment design, sows were assigned to receive or not supplemental chromium in to the diet fed during complete lactating period and first seven days after weaning. Chromium supplementation provides 0.4 ppm of Cr from chromium methionine (Microplex® Zinpro Co.). All sows were served in the hot and humid period from June to September, 2006. Chromium supplementation increased ($P < .01$) in 14.7% total pig birth, improved ($P = .02$) in 14% pig birth alive, and enhanced ($P < .01$) 17% litter weight at birth. Farrowing rate and weaning-to-estrus interval were similar ($P > .35$) across treatments. This result indicates that chromium methionine supplementation could be an effective tool to improve the liter size and weight litter at born on sows served during the critical summer season.

Key Words: Chromium, Reproductive performance, Sows.

## Introduction

Reproductive performance of sows usually decreases during summer season. Heat stressed sows reduces its voluntary food intake, increases breathing and drinking water intake in an effort to cope with adverse environmental condition. Stressful condition induces an increment in chromium losses via urine (Anderson et al. 1991). Actually is known that chromium is integrant of the system that amplifies insulin signal (Vincent, 2000), and its deficiency could impairs well working of metabolic routes for energy usage (Anderson, 1981). Energy status is important for restarting ovary activity after weaning and for support pregnancy. In this way, is hypothesized that restoring tissue chromium pool, could help to facilitate reproductive function of sows during high stress summer season. Chromium picolinate supplementation has shown be effective to increases litter size (Trottier and Wilson, 1998; Lindermann, 2000; Hagen et al., 2000).

Campbell (1996), supplementing chromium picolinate in to the diet, found enhancement on farrowing rate and interval weaning to estrous. Romo et al. (2005a, 2005b), fed chromium methionine, both to young and multiparous sows found an improvement on farrowing rate and weaning to estrous interval.

The objective of current study was to determine the effect of the chromium addition in the diet, supply to sow during the lactating period and a week after weaning, on its reproductive performance during the summer season.

Table 1. Composition of the lactating diet used in the experiment.

| Ingredients | Treatments | |
|---|---|---|
| | Control | Cr |
| Ground corn (8% CP) | 637 | 636.6 |
| Soybean meal (47% CP) | 248 | 248 |
| Wheat bran | 50 | 68 |
| Soybean oil | 29 | 29 |
| Mic. Lactancia LP [1] | 36 | 36 |
| Microplex [2] | 0.0 | 0.4 |
| Total, kg | 1000 | 1000 |
| | Calculated | Analyses [3] |
| Crude protein , % | 17.98 | 17.97 |
| Calcium, % | 0.920 | 0.920 |
| Phosphorous, % | 0.741 | 0.741 |
| Lysine, % | 1.08 | 1.08 |
| Supplementary Cr, ppm | 0.0 | 0.4 |
| ME, kcal/kg | 3,352 | 3,352 |

[1]Mic. Lactancia LP Vimifos S.A. de C.V. [2] Microplex [MR] (Zinpro, Co.) chromium methionine premix containing 1 g of Cr per kg, [3] Calculated from published values (NRC, 1998)

## Material and methods

The experiment was realized during the months of June 2006 to January 2007, with the services of the commercial pork farm "La Huerta" localized in Culiacan, Sinaloa, in the Northwest of Mexico (24º45' N; 107º 31' W; 23 m over mean sea level). Hundred ninety nine hybrid sows were used in a completely randomly design experimental. Sows were assigned to one of two treatments that consisted on: 1) control group (CG; n = 101) received a corn-soybean meal lactating diet during the lactating period and one week after weaning; 2) the test group (MetCr; n = 98) received during same phase a

similar diet to control but with addition of 0.4 ppm of chromium from chromium methionine (Microplex®, Zinpro Co.). During the lactating period the diet was supply in agreement to appetite of the sow with multiple served during the day and free access to food seven days after weaning. Sows having permanent access to water drink. Sows were served during the months of June to September 2006 by means of A.I. with fresh semen. The average temperature for this period was 29.35 °C, with an average maxim temperature of 39.9 °C. The births occurred between October of 2006 and January of 2007. Information of weaning to estrous interval (WEI), total pig birth (TPB), pig birth alive (PBA), litter weight at birth (LWB) and farrowing rate at first service after weaning (FR), of one reproductive cycle were registered.

*Statistical analysis.* The data of WEI, TPB, PBA, LWB, were analyzed as a design experimental randomized completely (Steel y Torrie, 1985), utilized the procedure ANOVA/COV for generals lineal models of the program Satistix ® 8 (Analytical Software; Tallahassee, FL). Data of FR its applied the test of $X^2$ used tables of contingence 2 x 2 that program Satistix ® 8.

**Results and Discussion**

The effect of chromium supplementation on litter size and litter weight is shown in Table 1. Chromium supplementation in the diet supplied sows during the lactating (21 days) and after weaning (7 days) period, improved ($P < .05$) in 14.7% the total pig birth. Chromium supplementation increased ($P < .05$) in 1.19 (14%) pig by litter the number of pigs birth alive. This result is agreement with the increment in 2.1 pig by litter observed for Lindemann et al. (1995) as consequence of chromium picolynate supplemented to gilts. In other experiment chromium supplementation has been increased the litter size (Trottier and Wilson, 1998; Hagen et al. 2000; Lindemann et al., 2000). The chromium supplementation could be associated with the presence of healthier ovules at estrus time and more embryonic survival, due to its participation in the insulin activity and cellular Hazeleger et al., 2005). Hence producing a good quality oocyte is essential for embryo survival and the maintenance of litter size in pigs (Webb et al., 2007).
Evock-Clover et al. (1993) demonstrated by the blood sampling results, that exist a large decrease in the insuin:glucose ratio as result of chromium supplementation, that can be considered as a crude index of tissue sensitivity to insulin. Considering that chromium is part of auto amplification system of insulin signal (Vincent, 2000), then chromium enhancing insulin activity promote its anabolic functions.
Cr supplementation to improve litter size is logical considering previous research related to the metabolic effects of Cr on glucose and insulin. Cox et al. (1987) demonstrated that insulin injection of gilts immediately preceding estrus increased ovulation rate. Whitley et al. (1998) also noted that insulin injection to primiparous sows for 5 d after weaning increased follicular estradiol and progesterone levels. Ramirez et al. (1997) demonstrated that the effects of insulin administration after weaning and before breeding could still be seen at the next farrowing, where increases of up to one pig/litter were observed with some of the insulin treatments. Whitley et al. (2002) demonstrated that the insulin administration for that 4 or 5-d period could increase litter size by as much as two pigs. Also, it was noted in the initial study of Lindemann et al. (1995) that the insulin:glucose ratio of midgestation females was lower, implying greater efficiency of insulin action across all gestation period.
Litter weight was enhanced in 17% ($P < .05$) by additional chromium treatment. This increment was mainly due to augment in the number of pig birth alive more than by increment on individual piglet weight, which was similar ($P > .20$) between treatments with values of 1.351 ± 0.180 kg and. 1.372 ±0.210 kg for control and chromium treatments, respectively.

Table 1. Effect chromium addition on total pig birth, pig birth alive and litter weight at birth.

| Items | Treatments | | SEM | P-value |
|---|---|---|---|---|
| | Control | MetCr | | |
| Sows, n | 62 | 59 | | |
| TPB, n [1] | 9.68 | 11.10 | .27 | .009 |
| PBA, n [2] | 8.47 | 9.66 | .26 | .02 |
| LWB, kg [3] | 11.25 | 13.21 | .35 | .005 |

[1] TPB = total pig birth
[2] PBA = pig birth alive
[3] LWB = litter weight at birth

In the Table 2, is shown the effect of methionine chromium addition at the lactating diet on the weaning to estrous interval.

Table 2. Effect Chromium addition on weaning to estrous interval.

| Items | Treatments | | SEM | P-value |
|---|---|---|---|---|
| | Control | MetCr | | |
| Sows, n | 101 | 98 | | |
| WEI, days [1] | 6.0 | 5.4 | .34 | .38 |

[1] WEI = Weaning to estrus interval

Chromium supplementation no modified ($P$ = .38) the WEI. At difference of this result, in previous experiments, chromium supplementation usually has been reduced observed the weaning to estrus interval (Campbell, 1996; Hagen et al., 1998; Romo et al., 2005a; 2005b). The absence of effect of chromium supplementation on the interval, could be due to extreme heat weather conditions in that was performed the actual research. The effect of chromium addition on farrowing rate at first service after weaning is showed in Table 3.

Table 3. Effect chromium addition on farrowing rate at first service after weaning

| Items | Treatments [1] | |
|---|---|---|
| | Control | MetCr |
| Sows, n | 89 | 89 |
| Sows farrowed, n | 62 | 58 |
| Sows no farrowed, n | 27 | 31 |
| Farrowing rate, % | 69.7 | 65 |

[1] Was not observed difference between treatments (P = .52)

Farrowing rate was not modified (P = .52) by chromium addition. The 67% of farrowing rate of experiment appears to be low, but this value is common during summer season in commercial farms localized close to parallel 24 in the Northwest of Mexico. During this experiment the mean temperature was 29.35 °C, and average maxim temperature reach of 39.9 °C and relative humidity up of 80%.

Others authors (Campbell, 1996; Hagen et al., 1998; Romo et al., 2005a; 2005b), has been appreciate an improvement in farrowing rate by organic chromium supplementation, but those data were obtained under moderate weather.

Under heat-stress condition, like gilts in this experiment, LH secretion could be compromised (van den Brand et al., 2000), and LH is the key to restarting ovary cycle in the sows (King and Martin, 1989). Heat-stress reduced feed intake and both gilts under-fed (Koketsu et al., 1998) or exposed to high environmental temperatures (Armstrong et al., 1984), declines its LH production. Consequently all reproductive performance is affected.

Also, it is proposed that the failure of sows to maintain pregnancy in summer-autumn results from disruption of maternal recognition of pregnancy causing regression of the corpora lutea, loss of pregnancy and return of the sow to estrous (Love et al., 1993). This is partially caused by gonadotropin insufficiency (Goossens and van den Berg, 1979).

## Implications

During the hard time of summer in the Northwest of Mexico, chromium methionine supplementation contributes to alleviate partially the adverse effects of weather on reproductive performance improving the litter size

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# USE OF THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM (CNCPS) FOR PREDICTING BEEF CATTLE COW-CALF PRODUCTION IN HAWAII

**K. Buck[1], J. R. Carpenter*[1], and B. W. Mathews[2]**
[1]College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa
[2]College of Agriculture, Forestry and Natural Resource Management, University of Hawaii at Hilo

**ABSTRACT:**
Computer models intended to predict animal performance on a given diet are commonly used. Little research has been undertaken to validate these models for beef production in tropical regions. The objective of this research was to evaluate the CNCPS version 5.0 Model for use in Hawaii's kikuyugrass (KG) pasture systems. The cow/calf model (using 3-breed cross) was run using a ration consisting of four different nutrient profiles of KG (<12%, 12-15%, 15-18%, and >18% CP), KG with 10 and 20% legume, and varying animal weights (408.2, 498.9, or 589.6 kg), and body condition (3, 5 or 7 on 9 point system). Mean bias and mean square prediction error (MSPE) were calculated and analysis of regression was performed to determine the accuracy of the model in predicting observed results. Both animal size and protein level of the KG have an impact on ADG and DMI with animal size having a more dramatic impact than nutrient profile of the forage. Animals fed low protein KG see a more dramatic increase in intake (about 0.603 kg w/10% legume) than animals that are fed a high protein kikuyu (about 0.0036 kg w/20% legume). The level of protein has a much greater impact on ADG than it does on DMI. An increase in CP of 11.09% (9.48 to 20.57%) corresponds to an increase in ADG of 0.408-0.499 kg, depending on the size of the animal, with larger animals showing a greater increase in ADG as protein increases. There is also an impact of adding legume to the diet, but only in animals fed a diet containing kikuyu with CP less than 15%. Increasing BCS decreased ADG for animals fed a high-protein KG, whereas it increased ADG for animals fed a low-protein KG, and it had no effect on DMI, although there was a trend for increased intake with increasing protein levels. If the common forages used in Hawaii were more accurately characterized, including bioavailability and rates of digestion of nutrients, the CNCPS would likely be an adequate tool for predicting animal performance. Further investigations need to occur to evaluate actual grazing systems to determine accuracy of DMI and performance predictions.

**Key words:** CNCPS Model, Kikuyugrass pastures, Predicted intakes & gains

## Introduction

Computer models intended to predict animal performance on a given diet have been around for some time, particularly for cattle. Currently, these models are an integral component of many operations, in both temperate and tropical regions. However, little research has been undertaken to validate these models for beef production in tropical regions. Poorer production by cattle in tropical regions, even in developed countries has been well-documented (Minson and McLeod, 1970; Stobbs, 1971). Tropical regions differ from temperate regions in that the forages present are often lower in soluble carbohydrates and higher in cell wall and lignin components (Van Soest, 1994). Because of this increase in lignin and cell wall, tropical grasses are on average about 15% less digestible than temperate forages (Van Soest, 1994). The decrease in digestibility, coupled with a decrease in intake (INRA, 1989), can lead to a decrease in nutrients available for the animal.

The CNCPS is a mechanistic, deterministic, static model based on the principles of rumen function, microbial growth, feed digestion and passage, and animal physiology that was developed to predict nutrient requirements, efficiency of feed utilization, and nutrient excretion for dairy and beef cattle. The system was first presented in a series of four papers (Fox et al., 1992; Russell et al., 1992; Sniffen et al., 1992; O'Connor et al., 1993) and has since been continually refined (Ainslie et al., 1993; Tylutki et al., 1994; Fox et al., 1995, 1999; Pitt et al., 1996; Tedeschi et al., 2000a, 2000b, 2001, 2002a, 2002b, 2002c, 2003). Version 4.0 of the CNCPS was used as the structure for the most recent NRC beef (2000) and dairy (2001) cattle models (Fox et al., 2003). As the CNCPS is Windows-based, rather than DOS-based as the NRC models, the program is more flexible and easier to use.

There have been several evaluations of the CNCPS model with animal performance data. Kolver et al. (1998) used a total mixed ration (TMR) and grazing dairy cows to evaluate the performance of the CNCPS and found the CNCPS underpredicted energy allowable milk production by 2.5% in the animals fed TMR and by 6.8% in the animals fed pasture. Dairy replacement heifers have also been used to evaluate the predictions of the CNCPS. Van Amburgh and coworkers (1998) used both the CNCPS and the 1989 Dairy NRC with 273 Holstein replacement heifers. The CNCPS ME allowable ADG accounted for 86% of the actual ADG.

The CNCPS has also been evaluated for tropical cattle production. Lanna et al. (1996) measured energy and protein content of empty body weight gain of growing animals and milk production of dual purpose lactating cows. Using actual dry matter intake (DMI) of feeds that were characterized for carbohydrate content and protein

fractions, as well as digestion rates, the CNCPS accounted for 72% of the variation in live weight gain and 71% of the variation in milk production. Molina et al. (2004) found similar results in predicting DMI of dual-purpose cattle in Mexico. Juarez Lagunes et al. (1999) found that when feeds were properly characterized, an increase in NDF or a decrease in the rate of digestion of NDF, affected the prediction of milk production of dual-purpose cows by CNCPS accordingly.

The objectives of the research presented here were to: 1. evaluate the Cornell Net Carbohydrate and Protein System for use in Hawaii beef production; and 2. evaluate how changing body size, body condition, level of intake, forage composition, and legume incorporation in the diet affects the predictions of the CNCPS prediction model.

**Materials/Methods**

The CNCPS version 5.0 was used in this research. "Theoretical" trials were run, using standardized weights and rations, in order to evaluate what effect increasing animal weight, body condition score, increasing age of regrowth of kikuyugrass, and increasing legume content of the diet have on the model predictions.

Parameters and Inputs

Inputs were in English units on a dry matter basis using calories, and at Level 1 aggregation, since amino acid profiles of the feeds were not available. Inputs for animal description, production, and management/environment can be found in Table 1. A full accounting of the choices available for each of the options is available upon request.

A series of simulations were conducted using "theoretical" animals. The descriptive, management, and environmental characteristics were the same as for the evaluation of the model above, with the exception that body weight was set to either 408.2, 498.9, or 589.6 kg, and body condition scores of 3, 5 or 7 (9 point system). The model was run using a ration consisting of four different nutrient profiles of kikuyu grass (KG), KG + 10% legume, or Kg + 20% legume. The CNCPS model was also used to predicted DMI, and was also used to establish the level of intake.

Feeds

The nutrient profiles of KG used were representative of changes normally seen with varying ages of regrowth. Nutrient profiles for the kikuyu grass and legume were determined by sorting based on CP and NDF, then averaging the nutrient profiles of 46 samples of kikuyu grass and 16 samples of legumes grown in Hawai'i, respectively. As it was mentioned above, the kikuyu was divided into four age levels, based on CP content (<12%, 12-15%, 15-18%, and >18%) of the grass. Nutrient profiles of these forages can be found in Table 2. A more detailed description of the nutrient and mineral composition of these grasses and legumes are available upon request.

Statistics

Mean bias and mean square prediction error (MSPE) were calculated as described by Tedeschi et al. (2000b). Analysis of regression was performed to determine the accuracy of the model in predicting observed results. The model prediction was the x-variate, while observed values were the y-variate. The regression statistic, $r^2$, the slope, and intercept confidence intervals were used to evaluate the model. Statistical analyses were conducted using SAS 9.1 (SAS Institute, Inc., 2004).

**Results**

Predictions of daily change in weight and DMI of 408.2, 498.9, or 589.6 kg animals representative of the beef herd at the Mealani Research Station on the Big Island of Hawaii can be found in Table 3. As can be seen, both animal size and protein level of the kikuyu grass have an impact on daily change in weight and DMI. As far as intake is concerned, animal size has a far more dramatic impact than does the nutrient profile of the forage. There is a mild increase in intake as the forage increases from low protein to high protein, with the greatest increase being 0.644 kg. The effect of weight on DMI, however, is much more pronounced; 2.757 kg of increased DMI as animal weight increased, in the most dramatic example. The amount of legume in the diet also has an impact on intake, although this impact depends on the amount of protein in the KG. Animals fed KG with a low level of protein see a more dramatic increase in intake (about 0.603 kg w 10% legume) than animals that are fed a high protein kikuyu (about 0.0036 kg w 20% legume).

On the other hand, the level of protein in the kikuyu has a much greater impact on daily change in weight than it does on DMI. An increase in percent CP of 11.09% (9.48 to 20.57% CP) corresponds to an increase in ADG of 0.408-0.499 kg, depending on the size of the animal, with larger animals showing a greater increase in daily change in weight as protein increases. Here, again, there is an impact of size on ADG. An increase of 90.70 kg in animal body weight corresponds to an increase in daily change in weight of 0.045-0.907 kg, depending on the level of protein in the diet. There is also an impact of adding legume to the diet, but only in animals fed a diet containing KG with CP less than 15%.

Table 4 looks at the effect of body condition score (BCS) on daily change in weight and DMI. BCS did not have any effect on DMI, although there was a trend for increased intake with increasing protein levels, but it did have an effect on ADG. Increased BCS decreased ADG for animals fed a high-protein kikuyu, whereas it increased ADG for animals fed a low-protein kikuyu. Heavier animals required less CP in the diet in order to minimize decreases in body weight with increasing BCS.

**Discussion**

Both Lanna et al. (1996) and Molina et al. (2004) found the CNCPS to be far more reliable in predicting productive capabilities and DMI of cattle raised in tropical conditions. Using Nellore cattle, Lanna et al. (1996) found that the CNCPS accounted for 72% of the variation in live weight gain with a 2% bias and 71% of the variation in milk production with a 10% bias. In the evaluation presented in this paper, there was no significant bias, due to the large variation found the data. The model also accounted for none of the variation in observed daily change in weight.

Characterization of cattle type (breed) and other conditions are critical. Key variables to consider are age of animals, days since calving, lactation number, and body weight and condition score. The aggregate of these estimations will undoubtedly introduce uncertainty into the predictions of the CNCPS. Another aspect that will introduce uncertainty into the predictions is the nutritional characterization of the forages consumed by the animals. The forages consumed by the hypothetical animals in this study were characterized for the various nutrient classes by proximate analysis and the Van Soest detergent techniques.

However, for minerals, only typical mineral values were used, rather than the values for the actual grasses grazed. Likewise, amino acid and vitamin levels were not characterized. More importantly, no information on bioavailability and rates of digestion were available for these grasses. The CNCPS default values were used in these cases, and likely were not entirely accurate. While any one of these issues may not have rendered the model unable to accurately predict daily change in weight, taken in aggregate the model was not able to accurately predict daily change in weight under the conditions of the research conducted here. However, based on the research of Lanna et al. (1996), Juarez Lagunes et al. (1999), and Molina et al. (2004), if the common forages used in Hawaii can be more accurately characterized, including bioavailability and rates of digestion of nutrients, the CNCPS will likely be an adequate tool for predicting animal performance. Further investigations into the use of the CNCPS should take care to evaluate the feed used as thoroughly as possible and to accurately determine the DMI, if at all possible, as well as use purebred and crossbred animals to remove the confounding effect of genetics.

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**Table 1. Animal and Environment Descriptions.**

| Description | Units |
|---|---|
| Animal Type | Lactating Beef Cow |
| Age* | 62 – 105 months |
| Sex | Cow |
| Body Weight* | 439.9 – 594.33 kg |
| Breed Type | Beef |
| Mature Weight | 582.31 kg |
| Condition Score | 5 (1-9 scale) |
| Breeding System | 3-way cross |
| Sire's Breed | Angus |
| Grandsire's Breed | Hereford |
| Granddam's Breed | S. Gertrudis |
| Days Pregnant | 0 days |
| Days since Calving* | 50 days |
| Lactation #* | 4 – 8 |
| Relative Milk Production | 5 |
| Calving Interval | 12 months |
| Expected Calf Birth Weight | 36.28 kg |
| Age at First Calving | 24 months |
| Management Description | |
| Additive | None |
| Added Fat in Diet | No |
| Activity | Intensive Grazing |
| Time Spent Standing | 16 h |
| # of Body Position Changes | 6 per day |
| Flat Distance Walked | 1000.05 m |
| Sloped Distance Walked | 0.0 m |
| Environmental Description | |
| Wind Speed | 24.14 km/hr |
| Previous Temperature* | 16.87 – 19.60°C |
| Current Temperature* | 16.18 – 19.62°C |
| Previous Relative Humidity | 80% |
| Current Relative Humidity | 80% |
| H in Sunlight | 14 |
| Storm Exposure | Yes |
| Hair Depth | 0.508 cm |
| Mud Depth | 0.00 cm |
| Hide | Average |
| Hair Coat | No mud |
| Cattle Panting | None |
| Minimum Night | 12.78°C |

*Vary depending on the animals being evaluated. Range of values given as options.

**Table 2. Nutritional composition of forages used to determine the response of the CNCPS to changes in age of forage (all values except DM are expressed as % DM basis).**

| Forage (n) | DM | NDF | CP | Lignin, % of NDF | Starch, % NFC | Fat (EE) (%DMB) | Ash (%DMB) |
|---|---|---|---|---|---|---|---|
| <12% CP Kikuyu (12) | 36.33 | 66.28 | 9.48 | 8.79 | 25.84 | 2.84 | 6.53 |
| 12-15% CP Kikuyu (15) | 24.92 | 62.63 | 13.65 | 8.13 | 17.12 | 3.19 | 7.37 |
| 15-18% CP Kikuyu (10) | 22.99 | 58.15 | 16.28 | 6.62 | 16.75 | 3.41 | 7.78 |
| >18% CP Kikuyu (9) | 20.59 | 54.31 | 20.57 | 7.39 | 9.91 | 3.51 | 8.25 |
| Mixed Legume (16) | 24.26 | 48.23 | 17.89 | 9.06 | 10.34 | 3.27 | 7.61 |

**Table 3. Daily change in weight and DMI predicted by CNCPS using theoretical animals and typical Hawaii forages; effect of adding legume.**

| Weight (kg) | Daily change in weight (kg/day) | | | | DMI (kg/day) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Kikuyu protein level | | | | Kikuyu protein level | | | | |
| | >18% | 15-18% | 12-15% | <12% | >18% | 15-18% | 12-15% | <12% | |
| 408.2 | 0.091 | 0.045 | -0.181 | -0.317 | 10.135 | 10.058 | 9.824 | 9.648 | |
| 498.9 | 0.181 | 0.136 | -0.136 | -0.272 | 11.544 | 11.455 | 11.156 | 10.977 | Kikuyu only |
| 589.6 | 0.272 | 0.181 | -0.045 | -0.227 | 12.891 | 12.790 | 12.451 | 12.260 | |
| 408.2 | 0.091 | 0.045 | -0.136 | -0.272 | 10.138 | 10.068 | 9.835 | 9.693 | |
| 498.9 | 0.181 | 0.136 | -0.091 | -0.227 | 11.547 | 11.467 | 11.195 | 11.029 | Kikuyu + 10% legume |
| 589.6 | 0.272 | 0.227 | -0.045 | -0.181 | 12.895 | 12.804 | 12.494 | 12.305 | |
| 408.2 | 0.091 | 0.045 | -0.136 | -0.227 | 10.141 | 10.078 | 9.868 | 9.739 | |
| 498.9 | 0.181 | 0.136 | -0.045 | -0.181 | 11.551 | 11.478 | 11.234 | 11.082 | Kikuyu + 20% legume |
| 589.6 | 0.272 | 0.227 | 0.000 | -0.136 | 12.898 | 12.817 | 12.538 | 12.366 | |

**Table 4. Daily change in weight and DMI predicted by CNCPS using theoretical animals and typical Hawaii forages; effect of BCS.**

| Weight (lb) | Daily change in weight (kg/day) | | | | DMI (kg/day) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Kikuyu protein level | | | | Kikuyu protein level | | | | |
| | >18% | 15-18% | 12-15% | <12% | >18% | 15-18% | 12-15% | <12% | |
| 408.2 | 0.091 | 0.045 | -0.181 | -0.317 | 10.135 | 10.058 | 9.824 | 9.648 | |
| 498.9 | 0.181 | 0.136 | -0.091 | -0.363 | 11.544 | 11.455 | 11.156 | 10.977 | BCS 3 |
| 589.6 | 0.317 | 0.136 | -0.091 | -0.317 | 12.891 | 12.790 | 12.451 | 12.260 | |
| 408.2 | 0.091 | 0.045 | -0.181 | -0.317 | 10.138 | 10.068 | 9.835 | 9.693 | |
| 498.9 | 0.181 | 0.136 | -0.136 | -0.272 | 11.547 | 11.467 | 11.195 | 11.029 | BCS 5 |
| 589.6 | 0.272 | 0.181 | -0.045 | -0.227 | 12.895 | 12.804 | 12.494 | 12.305 | |
| 408.2 | 0.045 | 0.000 | -0.181 | -0.272 | 10.141 | 10.078 | 9.868 | 9.739 | |
| 498.9 | 0.136 | 0.091 | -0.136 | -0.227 | 11.551 | 11.478 | 11.234 | 11.082 | BCS 7 |
| 589.6 | 0.227 | 0.136 | -0.091 | -0.227 | 12.898 | 12.817 | 12.538 | 12.366 | |

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# IN VITRO DIGESTIBILITY AND CHEMICAL COMPOSITION OF KIKUYUGRASS AS INFLUENCED BY SOIL SILICON, LIMING AND GENOTYPE

**J. R. Carpenter[1*], B. W. Mathews[2], and L. E. Sollenberger[3]**

[1]Department of Human Nutrition, Food, and Animal Sciences, University of Hawaii at Manoa, Honolulu, Hawaii, USA, [2]College of Agriculture, Forestry, and Natural Resource Management, University of Hawaii at Hilo, Hilo, Hawaii, USA,[3]Department of Agronomy, University of Florida, Gainesville, Florida, USA

**ABSTRACT**: Kikuyugrass (KG) pasture accounts for about 85% of the forage production in Hawaii and other regions in the world. Hawaii studies have shown that KG grown at higher elevations is lower in NDF and higher in IVDOM and CP than KG at the same age of regrowth in lower elevation sites with warmer climates. These factors reduce animal performance in low elevation KG pastures, but influence of elevation on forage nutritive value and animal performance may be confounded in part by differences in soil silicon (Si) between sites. Kikuyugrass is an active Si accumulator and can reduce the organic matter digestibility of forage grasses but no information exists regarding the influence of soil Si and liming on KG Si uptake, chemical composition, and digestibility. Calcium carbonate (crushed coral or CC) applications to a low Si Maile silt loam soil (Acrudoxic Hydrudand) had no effect on the IVDOM concentration of 3 wk-old (4.5-leaf-per-tiller stage of development) Whittet KG regrowth while calcium silicate (wollastonite) applied at the Ca equivalent of the CC rates produced a curvilinear IVDOM response. Whittet KG IVDOM decreased when grass Si concentration exceeded about 22 g kg-1 or when 0.5 M HOAc extractable soil Si exceeded about 230 mg kg-1. The Ca level of Whittet KG linearly increased while Si, Mg, and Mn decreased with increased CC application rate. Three week-old common kikuyugrass regrowth on the low Si Maile soil had a lower Si and a greater IVDOM concentration than that observed for high Si Kohala (Humic Dystrustepts) and Waialua (Pachic Haplustolls) silty clay soils. Common KG had a lower CP and IVDOM concentration and higher fiber concentrations than Whittet KG at the same stage of regrowth. Common KG was also poorly balanced in Ca and P from the animal nutrition perspective. The response of 6-wk old Hosaka KG regrowth to soil type was similar to that observed for 3-wk old common KG but Hosaka had a greater IVDOM concentration. Future research should examine the effects of KG genotype on forage Ca, P, oxalate, Si, and IVDO concentrations under field conditions.

**Key words:** Kikuyugrass composition, In vitro digestibility, Silicon levels

## Introduction

Hawaii studies summarized by Carpenter (1999) and Hanna et al. (2004) have shown that kikuyugrass (KG) grown at higher elevations is lower in fiber (cell wall components) and higher in digestibility and crude protein (CP) than KG at the same age of regrowth in lower elevation sites with warmer climates. These factors contribute to reduced animal performance in low elevation KG pastures. In paired rotational stocking studies under identical management Mathews et al. (1999) reported an average daily gain of 0.4 kg for Hereford, Angus, and Hereford x Angus cattle (*Bos taurus*) at a low elevation site (elevation = 130 m, average daily maximum temperature = 27$^{o}$C, forage digestible DM = 55.0%, CP = 10.0%) compared to 0.6 kg at a high elevation site (elevation = 900 m, average daily maximum temperature = 20$^{o}$C, forage digestible DM = 62.0%, CP = 14.0%). With all other factors held constant an average decrease in dry matter digestibility of 0.6 units for each 1$^{o}$C rise in growth temperature is expected for tropical grasses (Minson, 1990).

The influence of elevation on forage nutritive value and animal performance may be confounded in part by differences in soil silicon between sites. Silicon has often been associated with reduced cell wall organic matter digestibility in grasses (Duble et al., 1971; Van Soest, 1982, 1994; Mayland and Shewmaker, 2001). Soils at the higher elevations sites studied in Hawaii are weathered Andisols (Acrudoxic Hydrudands) that are relatively low in soluble Si while soils at the lower elevations are Inceptisols and Mollisols that have not been extensively desilicated (leached of Si, Chadwick et al. 2003). The mechanisms by which Si reduces forage organic matter digestibility are not well understood but are thought to involve Si incorporation into (complexes) or onto (incrustation) the cell wall structure and the suppression of digestive enzyme activity by soluble Si (Minson, 1990; Van Soest, 1982, 1994; Mayland and Shewmaker, 2001). Kikuyugrass is an active Si accumulator but no information exists regarding the influence of Si on KG digestiblity (Reboredo et al., 2006).

Silicon analysis of archived KG samples (6 wk regrowth) from the Hawaii study described by Mathews et al. (1999) showed a 3-fold increase in forage Si content (3.91 vs 1.35%) for a Kohala silty clay (Humic Dystustepts, low elevation site) than a Maile silt loam (Acrudoxic Hydrudands, high elevation site). Silicon is considered a non-essential beneficial plant nutrient and few studies have been conducted to determine yield responses of tropical pasture grasses to Si (Mathews et al., 2004). With the possible exception of a synergistic effect

on fertilizer applied P, soil silicon levels are not likely to influence uptake of plant essential elements by KG (Reboredo et al., 2006; De Melo, 2007). Yield responses to Si appear to be primarily associated with improved disease, pest, and water stress tolerance from stronger cell walls (Savant et al., 1999; Snyder et al., 2007).

The objective of this study was to determine the effects of liming, soil silicon, and soil type on the chemical composition and *in vitro* digestibility of KG.

## Materials and Methods

The upper 15 cm of a field-moist Maile silt loam (Acrudoxic Hydrudand) soil (Table 1) was passed through a 8-mm screen. In order to simulate field bulk density the soil was placed into 5.0 L (22 cm diam) plastic pots lined with polyethylene bags to provide 2600 g oven-dry equivalent soil per pot. Soil amendments were applied on a surface area basis, 3.8 mg $pot^{-1}$ being equivalent to 1 kg $ha^{-1}$. Calcium applied as calcium carbonate (crushed coral ground to pass a 200 mesh screen, 380 g Ca $kg^{-1}$, BEI Hawaii, LLC, Honolulu, HI) and calcium silicate (wollastonite ground to pass a 200 mesh screen, 342 g $kg^{-1}$ Ca and 239 g $kg^{-1}$ Si, Aldrich Chemical Co., Inc., Milwaukee, WI) was mixed into separate pots at rates of rates of 0, 500, 1000, 2000, and 4000 kg Ca $ha^{-1}$ (730, 1460, 2920, and 5840 mg Ca $kg^{-1}$ soil) with four replicates per treatment. The respective calcium silicate treatments also provide 0, 350, 700, 1400, and 2800 kg Si $ha^{-1}$ (0, 510, 1020, 2040, and 4080 mg Si $kg^{-1}$ soil).

The pots were arranged in a greenhouse as a completely randomized factorial design (2 Ca sources x 5 Ca rates). Greenhouse temperatures ranged from 19 to 32°C and soil moisture was maintained at field capacity throughout the study by addition of deionized water. The soils were allowed to equilibrate for one month prior to fertilization with 40 kg N $ha^{-1}$ as ammonium nitrate ($NH_4NO_3$) and planting 'Whittet' kikuygrass (3.75 g seed $pot^{-1}$), the most widely grown improved cultivar of this species (Ross, 1999). The seedlings were clipped to a 3 cm height 6 wk after planting and allowed to regrow because KG seed germinates somewhat unevenly over a period of 1 to 2 wk and seedling growth is not representative of normal pasture growth. At this time the pots received an additional 60 kg N $ha^{-1}$ as $NH_4NO_3$ and the KG was grown for 3 wk to the 4.5-leaf-per-tiller stage of development (Hanna et al., 2004) prior to harvest.

Two additional experiments were conducted with common and 'Hosaka' kikuyugrass, respectively, under the same greenhouse conditions. These experiments compared the effects of the low extractable Si Maile silt loam and the high extractactable Si Kohala (Humic Dystrustepts) and Waialua (Pachic Haplustolls) silty clay soils (Table 1) on forage chemical composition and *in vitro* digestibility. The soils were screened as described previously and the appropriate amount placed into the 5.0 L pots lined with polyethylene bags to simulate field bulk density (Table 1). In each experiment there were five replicates per soil type arranged in a completely randomized design. Ten KG plants were established in each pot from stolon cuttings that were rooting at the nodes as commercial seed is not available for common and Hosaka KG (Ross, 1999). At planting each pot was fertilized as described earlier and the grass clipped to a 3 cm height 6 wk after planting and allowed to regrow. In contrast, Hosaka KG was grown for 6 wk because the optimal tradeoff between nutritional value and yield of this improved variety is thought to occur at 5- to 6-wk of regrowth (Carpenter, 1999).

In all the experiments forage samples were cut 3 cm above soil level using mechanical shears and all the material collected was immediately oven-dried for 48 h at 55°C. After weighing for DM yield determination the samples were ground to pass a 1-mm stainless steel screen using a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ) and stored for chemical analysis. The two-stage technique of Moore and Mott (1974) was used to determine *in vitro* digestible organic matter (IVDOM) concentration. Total forage cell wall (neutral detergent fiber [NDF]), hemicellulose (calculated as NDF - acid detergent fiber [ADF]), cellulose (calculated as ADF - lignin), and lignin concentrations were determined by near-infrared reflectance spectroscopy (Herrero et al., 1996). Crude protein concentration was determined using the micro-Kjeldahl method (aluminum block digestion) to obtain Kjeldahl N and multiplying the result by 6.25 (Jones and Case, 1990). Concentrations of Ca, Mg, P, K, S, Fe, Cu, Mn, Zn, and B were determined by inductively coupled plasma emission spectroscopy (ICPES) following wet digestion with $HNO_3$-$H_2O_2$. Silicon concentration was determined by ICPES following sample preparation by the NaOH fusion and melt dissolution procedure described by (Allen, 1989).

After harvest the soil in each pot of the calcium carbonate and calcium silicate rate experiment with Maile soil was thoroughly mixed and analyzed moist. All extraction procedures were conducted on an oven-dry (100°C) equivalent basis. Soil samples were analyzed for pH (saturated paste), modified-Truog extractable P (molybdenum-blue colorimetric procedure), and 1 *M* $NH_4OAc$ exchangeable Ca, Mg, and K (ICPES analysis) as described by Hue et al. (2000). Sulfate was extracted with 0.04 *M* $CaH_2PO_4$ (turbidmetric analysis) as outlined by Fox et al. (1987) while Si was extracted with 0.5 *M* HOAc (heteropoly blue colorimetric analysis) as described by (Rodrigues et al., 2003).

Data were analyzed by using Proc GLM of the Statistical Analysis System (SAS Inst., 1999). Main effects and interactions with $P$ values $\leq 0.10$ were considered significant. Trends in response to the calcium carbonate and calcium silicate application rates were determined using orthogonal polynomial contrasts and regression (Gomez and Gomez, 1984). Fisher's $F$-test protected least significant difference (LSD) test was used for mean separation among soil types.

## Results and Discussion

Maile Soil Responses to Calcium Carbonate and Calcium Silicate Rates: There were Ca source, rate, and source x rate effects ($P<0.001$) for pH and exchangeable Ca. Calcium carbonate was more effective than calcium silicate at increasing soil pH and exchangeable Ca at all Ca application rates except for the lowest (500 kg $ha^{-1}$)

rate (Table 2) where the two soil amendments behaved similarly. At the greater Ca application rates calcium carbonate was to 2- to 3-fold more effective than calcium silicate at increasing exchangeable Ca. A similar observation has not been noted in the literature for calcium silicate applications and may be due to the greater crystallinity of hyroxy-Al polymers formed in response to calcium carbonate than calcium silicate applications (Haynes, 1984).

As expected there were Ca source and rate effects ($P < 0.001$) for 0.5 *M* acetic acid extractable Si. Calcium silicate increased extractable Si with increasing application rate (Table 3) while calcium carbonate had no effect on extractable Si ($P = 0.70$, mean = 70 mg $kg^{-1}$, SE = 2). The extractable soil Si concentrations obtained (Table 3) represent well the expected range of 50 to 270 mg $kg^{-1}$ for most KG pasture soils in Hawaii (Mathews, unpublished data). Even lower concentrations (5 to 40 mg $kg^{-1}$) can be observed for extremely weathered Oxisols but KG does not persist naturally on these soils due to their low fertility (Hanna et al., 2004).

Whittet Kikuyugrass Response to Calcium Carbonate and Calcium Silicate Rates: There were Ca source, rate, and source x rate effects ($P < 0.05$) for concentrations of Si, IVDOM, Ca, Mg, and Mn in the 3 wk-old Whittet KG regrowth at the 4.5-leaf-per-tiller stage of development. Grass Si concentration decreased linearly ($P < 0.001$, SE = 0.6) with increased calcium carbonate application rate but this did not influence IVDOM. The mean grass Si concentrations for the 0, 500, 1000, 2000, and 4000 kg Ca $ha^{-1}$ rates of calcium carbonate were 14.5, 13.9, 12.4, 11.9, and 10.4 g $kg^{-1}$. This rate effect can be attributed to reduced Si uptake with increased soil pH (Savant et al., 1999). In contrast, Whittet KG Si concentrations increased with increased calcium silicate rate while the IVDOM response was curvilinear ($P < 0.001$, quadratic effect) (Table 3). The IVDOM concentration increased above the control (0 rate) for the 500 through 2000 kg Ca $ha^{-1}$ rates, and then decreased below the control at the highest rate (4000 kg Ca $ha^{-1}$) (Table 3). The decrease in IVDOM corresponded to when grass Si concentration exceeded about 22 g $kg^{-1}$ (Fig. 1) and extractable soil Si exceeded about 220 mg $kg^{-1}$ (Fig. 2). The relationship between extractable soil Si and Si concentration in Whittet KG is presented in Figure. 3. Calcium silicate application rate had no effect ($P > 0.46$) on grass Ca (mean = 4.3 g $kg^{-1}$, SE = 0.1), Mg (mean = 5.9 g $kg^{-1}$, SE = 0.3) or Mn (mean = 154 mg $kg^{-1}$, SE =12). Both calcium silicate and calcium carbonate slightly decreased KG Zn concentration linearly ($P$ =0.004, SE = 4) from a high of 72±1 mg $kg^{-1}$ at the 0 Ca rate to a low of 60 ± 2 mg $kg^{-1}$ at the 4000 kg Ca $ha^{-1}$ rate. A decrease in Zn concentration is often expected in response to application of liming materials (Sumner and Yamada, 2002).

There were no Ca source or rate effects for Whittet KG DM yield, cellulose, hemicellulose, lignin, total cell wall (NDF), CP, P , K , S, Fe, Cu, or B. Mean values were 15.8 g $pot^{-1}$, 26.9%, 22.0%, 5.1%, 54.0%, 14.8%, 0.32%, 2.38%, 0.33%, 123 ppm, 11 ppm, and 16 ppm, respectively and were all in the adequate ranges for grazing ruminants (Tamimi et al., 1997; Mathews et al., 2004) regardless of the treatment.

Common Kikuyugrass Response to Soil Type: The DM yield of 3 wk-old common KG regrowth was slightly greater ($P < 0.05$) for the Kohala soil (23.0 g $pot^{-1}$) than for the Maile (19.6 g $pot^{-1}$) and Waialua (19.4 g $pot^{-1}$) soils. It is also noteworthy that the yield for common KS on the Maile soil was 1.2 fold greater than that observed for Whittet KG at the same stage of regrowth and on the same soil in the simultaneous study discussed above. In contrast to some observations in Australia (Wilson, 1968), common KG has usually been observed to be more vigorous and persistent than Whittet on commercial grazing lands in.Hawaii and might be attributed in part to the slowness of Whittet to form a dense sward (Hanna et al., 2004).

Common KG grown on the Maile soil low in extractable Si had a lower Si and a greater IVDOM concentration than that observed for the high Si Kohala and Waialua soils (Table 4). The differences in common KG Si between the Maile and Kohala soils were substantially smaller than the 3-fold differences observed for these soils under field conditions (unpublished data from the study described by Mathews et al., 1999). Interestingly, grass IVDOM for the Kohala and Waialua soils was reduced at lower forage Si concentrations (17.9 to18.9 g $kg^{-1}$) than the 22 g $kg^{-1}$ found to decrease Whittet KG digestibility at the same age of regrowth in the simultaneous study discussed above. Common KG Si concentration for the Maile soil (14.0 g $kg^{-1}$) was nearly identical to that observed for Whittet KG on the same soil without addition of soil amendments (14.2 g $kg^{-1}$ for the 0 Ca rate control treatments in the study discussed above) but the IVDOM concentration observed for common was 76 ± 7 g $kg^{-1}$ OM lower (567.2 vs 636.2 to 649.8). There was no effect of soil type on common KG cellulose, hemicellulose, lignin, total cell wall (NDF), or CP (mean values were 31.2, 26.7, 5.7, 63.6 and 12.1%, respectively). These values agree with previous work suggesting lower CP and higher fiber concentrations for common KG at the same stage of growth as Whittet KG (Wilson, 1968; Hanna et al., 2004).

While there were differences among soil types for common KG Ca, Mg, P, and K (Table 5), they were not considered of great significance from the standpoint of plant nutrition as they were adequate for good KG growth (Awad, 1976; Tamimi et al., 1997). However, a major concern from the animal nutrition perspective are low Ca concentrations (1.6 to 2.0 g $kg^{-1}$) coupled with the low Ca to P ratios observed for the Maile (0.7), Kohala (0.6) , and Waialua (0.9) soils. The Ca concentrations are well below the 3.0 g $kg^{-1}$ considered adequate for grazing livestock and the Ca to P ratios are below the recommended range of 1.0 to 2.0 (Hanna et al., 2004; Mathews et al., 2004). Of further apprehension is the fact that Ca bioavailablity and imbalance problems can be aggravated by the high levels of Ca complexing oxalate (not measured) found in KG (Hanna et al., 2004; Mathews et al., 2004). It is worth noting that Whittet KG in the control treatments (0 Ca rate) of the simultaneous study discussed above produced

forage with 2.4 fold more Ca and a Ca to P ratio of 1.4 on the same Maile soil.

There was an effect of soil type on common KG Zn ($P < 0.01$) but not for concentrations of S, Fe, Cu, Mn or B (means were 3.2 %, and 60 ppm, 12 ppm, 228 ppm and 10 ppm, respectively ). Grass Zn concentration was greater for Kohala (52 ppm) than Waialau (41 ppm) which in turn was greater than Maile (30 ppm). The concentrations of Zn, S, Fe, Cu, Mn, and B were in the adequate ranges for KS (Tamimi et al., 1997) and grazing ruminants (Mathews et al., 2004).

Hosaka Kikuyugrass Response to Soil Type: Overall the response of 6-wk old Hosaka KG regrowth to soil type was fairly similar to that observed for 3-wk old common KG. Maile soil produced forage with lower Si and greater IVDOM and CP concentrations than the Kohala and Waialua soils (Table 5). There was no effect of soil type on Hosaka KG cellulose, hemicellulose, lignin, and NDF (means were 32.2, 25.1, 5.56, and 62.9%, respectively). Interestingly, these fiber fraction concentrations are very similar to those observed for 3-wk old common KG (discussed above) while maintaining a greater average IVDOM concentration (57.0% for Hosaka vs. 54.4% for common). While not quantified in the present study this response may be due in part to the leafier morphology of Hosaka KG (Hanna et al., 2004).

There were no yield (61.0 g $pot^{-1}$) or P (0.20%) and K concentration (2.83%) responses. Differences among soil types were observed for forage Ca, Mg, and S concentration (Table 5). These differences were not considered of great significance from the standpoint of plant nutrition as they were adequate for good KG growth (Awad, 1976; Tamimi et al., 1997). However, low Ca was a concern from the standpoint of livestock nutrition as was discussed above for common KG.

There was an effect of soil type on Hosaka KG Zn ($P < 0.05$) but not for concentrations of Fe (47 ppm), Cu (10 ppm), Mn (198 ppm) or B (10 ppm). Kikuyugrass Zn concentration was greater for Waialua (33 ppm) than Maile (17 ppm) while Kohala (24 ppm) did not differ ($P > 0.10$) from either Waialua or Maile. The forage Zn concentration observed for the Maile soil is considered marginal for both KG (< 22 ppm) and grazing ruminants (< 30 ppm) (Mathews et al., 2004).

## Conclusions

Whittet KG Si concentrations at the 4.5-leaf-per-tiller stage of development (3 wk-old regrowth) increased linearly with increased soil Si resulting from calcium silicate application to the Maile soil. The IVDOM response to Si was curvilinear with IVDOM decreasing when grass Si concentration exceeded about 22 g $kg^{-1}$ or when 0.5 *M* HOAc extractable soil Si exceeded about 220 mg $kg^{-1}$. The Ca concentration of Whittet KG linearly increased while Si linearly decreased with increased calcium carbonate application rate but this did not influence IVDOM. Calcium carbonate application also decreased grass Mg and Mn concentration.

Common KG at the 4.5-leaf-per-tiller stage of development and grown on the Maile soil low in extractable Si had a lower Si and a higher IVDOM concentration than that observed for the high Si Kohala and Waialua soils. The differences in common KG Si between the Maile and Kohala soils were of smaller magnitude than the 3-fold differences reported for these soils under field conditions. This observation agrees with the suggestion of Van Soest (1982) that removing the environmental effects of differing locations can reduce the magnitude of soil type effects on grass Si. Common KG Si concentration for the Maile soil was nearly identical to that observed for Whittet KG on the same soil without addition of soil amendments (control treatments) but the IVDOM concentration was lower for common. Common KG also had lower CP and higher fiber concentration than Whittet KG at the same stage of growth. Furthermore, common KG was poorly balanced in Ca and P from the animal nutrition perspective. Overall the response of 6-wk old Hosaka KG to soil type was fairly similar to that observed for 3-wk old common KG but Hosaka had a greater IVDOM concentration. Future research should examine the effects of KG genotype on forage Ca, P, oxalate, Si, and IVDOM concentrations under field conditions.

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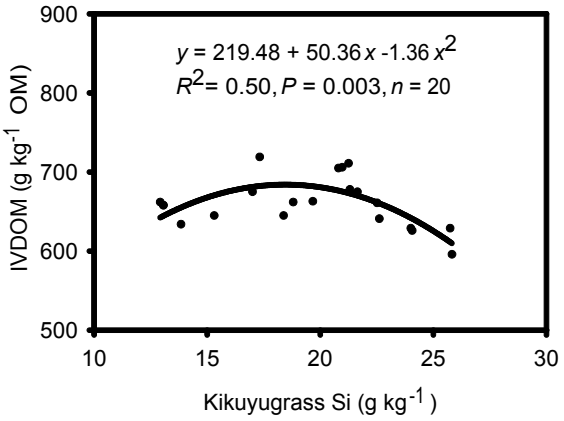


**Figure 1.** Relationship between the concentrations of Si and *in vitro* digestible organic matter (IVDOM) in 3-wk old Whittet kikuyugrass grown on Maile soil amended with calcium silicate at rates of 0, 500, 1000, 2000, and 4000 kg Ca $ha^{-1}$ (0, 350, 700, 1400, and 2800 kg Si $ha^{-1}$).

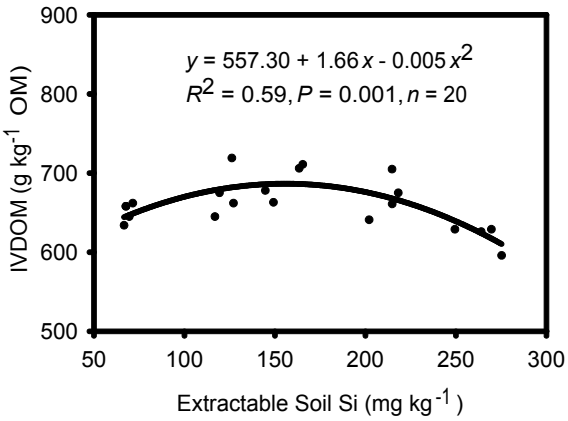


**Figure 2.** Relationship between 0.5 *M* HOAc extractable soil Si and the *in vitro* digestible organic matter (IVDOM) concentration in 3-wk old Whittet kikuyugrass grown on Maile soil amended with calcium silicate at rates of 0, 500, 1000, 2000, and 4000 kg Ca $ha^{-1}$ (0, 350, 700, 1400, and 2800 kg Si $ha^{-1}$).

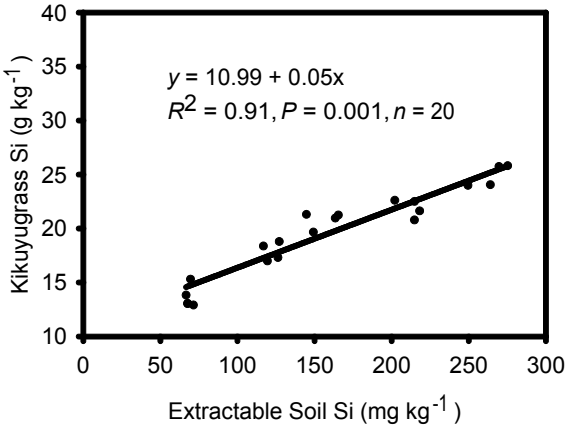


**Figure 3**. Relationship between 0.5 M HOAc extractable soil Si and the Si concentration in 3-wk old Whittet kikuyugrass grown on Maile soil amended with calcium silicate at rates of 0, 500, 1000, 2000, and 4000 kg Ca $ha^{-1}$ (0, 350, 700, 1400, and 2800 kg Si $ha^{-1}$).

**Table 1. Selected chemical characteristics of the pasture soils studied (0- to 15-cm depth).**

| Soil property | Soil series | | |
|---|---|---|---|
| | Maile | Kohala | Waialua |
| pH† | | | |
| Saturated paste | 5.3 | 6.0 | 6.0 |
| Exchangeable cations† | | | |
| Ca, $cmol_c\ kg^{-1}$ | 11.35 | 11.74 | 18.88 |
| Mg, $cmol_c\ kg^{-1}$ | 2.88 | 7.00 | 12.36 |
| K, $cmol_c\ kg^{-1}$ | 0.65 | 3.21 | 1.34 |
| Na, $cmol_c\ kg^{-1}$ | 0.33 | 1.10 | 0.50 |
| Al, $cmol_c\ kg^{-1}$ | 0.20 | 0 | 0 |
| ECEC, $cmol_c\ kg^{-1}$ | 15.41 | 23.05 | 33.08 |
| Extractable P, $SO_4$, and Si‡ | | | |
| P, $mg\ kg^{-1}$ | 38 | 60 | 112 |
| $SO_4$-S, $mg\ kg^{-1}$ | 188 | 82 | 71 |
| Si, $mg\ kg^{-1}$ | 70 | 259 | 263 |
| Organic matter† | | | |
| OC, $g\ kg^{-1}$ | 165.2 | 60.3 | 25.9 |
| Bulk density | | | |
| Density, g cm3§ | 0.52 | 0.92 | 1.18 |

† Soil pH, exchangeable cations, effective cation exchange capacity (ECEC), and organic carbon (OC) determined by the methods outlined by Hue et al. (2000).

‡ Modified-Truog extractable P, 0.04 *M* monocalcium phosphate extractable $SO_4$-S, and 0.5 *M* acetic acid extractable Si determined by the procedures of Hue et al. (2000), Fox et al. (1987), and de lima Rodrigues (2003).

§ Core method.

**Table 2. Influence of calcium carbonate and calcium silicate application rates on Maile soil pH and exchangeable Ca.**

| Calcium rate ($kg\ ha^{-1}$) | Calcium carbonate | | Calcium silicate | |
|---|---|---|---|---|
| | Soil pH | Exchangeable Ca ($mg\ kg^{-1}$) | Soil pH | Exchangeable Ca ($mg\ kg^{-1}$) |
| 0 | 5.24 | 2153 | 5.28 | 2063 |
| 500 | 5.44 | 2348 | 5.35 | 2296 |
| 1000 | 5.57 | 2764 | 5.37 | 2366 |
| 2000 | 5.77 | 3548 | 5.52 | 2600 |
| 4000 | 6.22 | 4969 | 5.62 | 3004 |
| SE† | ----- | 90 | ----- | 45 |
| Linear effect | ***‡ | *** | *** | *** |
| Quadratic effect | NS | NS | NS | NS |

† Standard error of a treatment mean.

‡ *** Significant at the 0.001 probability level or NS (nonsignificant $p > 0.10$).

**Table 3. Influence of calcium silicate application rate on 0.5 M acetic acid extractable Si in the Maile soil and concentrations of Si and in vitro digestible organic matter (IVDOM) in 3-wk-old regrowth of 'Whittet' kikuyugrass.**

| Calcium rate (kg $ha^{-1}$) | Soil Si (mg $kg^{-1}$) | Grass Si (g $kg^{-1}$) | IVDOM (g $kg^{-1}$ OM) |
|---|---|---|---|
| 0 | 69 | 13.8 | 649.8 |
| 500 | 122 | 17.9 | 675.3 |
| 1000 | 156 | 20.8 | 689.5 |
| 2000 | 212 | 21.9 | 670.5 |
| 4000 | 265 | 24.9 | 620.0 |
| SE† | 4 | 0.5 | 11.6 |
| Linear effect | ***‡ | *** | ** |
| Quadratic effect | *** | *** | *** |

† Standard error of a treatment mean.
‡ **, *** Significant at the 0.01 and 0.001 probability levels, respectively.

**Table 4. Influence of soil type on concentrations of Si, in vitro digestible organic matter (IVDOM), Ca, Mg, P, and K in 3-wk-old regrowth of common kikuyugrass (% dry matter basis).**

| Soil | Silicon | IVDOM | Calcium | Magnesium | Phosphorus | Potassium |
|---|---|---|---|---|---|---|
| Maile | 1.40b† | 56.72a‡ | .18b‡ | .30a | .26a | 3.74b |
| Kohala | 1.79a | 52.78b | .16b | .22b | .28a | 4.24a |
| Waialua | 1.89a | 53.54b | .20a | .28a | .21b | 3.89b |
| SE§ | .12 | 1.26 | .01 | .04 | .02 | .09 |

† Means in the same column not followed by the same letter are different at $P < 0.05$ using Fisher's F-test protected LSD test unless otherwise noted.
‡ Means for IVDOM and Ca not followed by the same letter are different at $P < 0.10$ using Fisher's F-test protected LSD test.
§ Standard error of a soil mean.

**Table 5. Influence of soil type on concentrations of Si, in vitro digestible organic matter (IVDOM), Ca, Mg, P, and K in 3-wk-old regrowth of `Hosaka' kikuyugrass (% dry matter basis).**

| Soil | Silicon | IVDOM | Calcium | Magnesium | Phosphorus | Potassium |
|---|---|---|---|---|---|---|
| Maile | 12.5b† | 595.8a | 95.0a | 1.8b‡ | 2.8a | 3.0a‡ |
| Kohala | 15.8a | 559.8b | 73.0b | 1.7b | 2.1b | 2.4b |
| Waialua | 16.0a | 552.2b | 78.2b | 2.1a | 2.4ab | 3.4a |
| SE§ | 0.8 | 7.7 | 4.5 | 0.1 | 0.1 | 0.3 |

† Means in the same column not followed by the same letter are different at $P < 0.05$ using Fisher's F-test protected LSD test unless otherwise noted.
‡ Means for IVDOM and Ca not followed by the same letter are different at $P < 0.10$ using Fisher's F-test protected LSD test.
§ Standard error of a soil mean.

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# NUTRITIONAL VALUE, MILK YIELD AND GAS PRODUCTION OF CORN SILAGE CUT AT DIFFERENT HEIGHT

**D. Domínguez*[1], A. Corral[1], G. Villalobos[1], H. Castillo[1], S. Ramirez[1], J. Ortega[1], A. Muro[2]**
[1]Universidad Autónoma de Chihuahua, Chihuahua, Chih., México
[2]Universidad Autónoma de Zacatecas, Zacatecas, Zac., México

**ABSTRACT:** Corn silage is the most cost-competitive forage supplying energy and fiber of high quality to the dairy industry in México. The corn hybrid PIONEER 33G66, was planted at a density of 125,000 seeds/ha, with a row distance of 0.80 m, and harvested at half milk line. Corn silos of 5 Kg (n=3 per height) were used to evaluate fermentation after 45 d. Silos were open, and analyzed by triplicate for DM, CP, NDF, ADL, and *in vitro* DM and NDF digestibility (IVDMD, IVNDFD, respectively). Milk yield and $NE_l$ were estimated using the MILK 2006 model. Data were analyzed using the PROC GLM of SAS. Production of DM/ha decreased by 9 y 12%, and grain content was increased by 15 and 23%, by rising cutting height to 40 and 60 cm, respectively. The NDF and CP concentration were reduced ($P<0.05$) at 60 cm. ADL, IVDMD and IVNDFD were unaffected by treatments. However, $NE_l$ tended to improve by 11.5 and 14.7 % by cutting at 40 and 60 cm, respectively. Milk yield was not different among treatments. In vitro gas production at 72 h was higher ($P<0.05$) in corn silage cut at 60 vs. 20 cm (85.1 vs.74.0 ml of gas/200 mg.

**Key Wors:** Corn silage, cutting height, nutritional quality.

## Introduction

Dairy rations often contain large amounts of corn silage, reflecting its low cost production and its high supply of energy. However the nutritive value of corn silage is reduced by the poor digestibility of the fraction of the silage (Tolera and Sundstøl, 1999). Nutritive value and digestibility of stover among commercial hybrids is relatively similar (Coors, et al, 1994). The contribution of stover to digestible energy of corn silage is limited indicating the importance of grain content. One approach to improving nutritive value in corn silage is by increasing the height at harvest time, as DM, CP and starch content increase (Restle et al, 2002). Harvest time corn plants at a higher height increased 7.9% milk per ton of corn silage (Neylon and Kung, 2003).

This study evaluated DM production/ha, nutritional quality, milk yield and *in vitro* gas production of corn silage cut at 20, 40 y 60 cm above soil surface.

## Materials and Methods

*Corn silage*. Corn hybrid 33G66 PIONEER (Pioneer Hi-Bred Internacional, Des Moines, IA), was used, which was planted at a 125,000 seeds per hectare at a row distance of 80 cm. Corn was harvested at half milk line. Cut heights were 20, 40 and 60 cm above soil surface. At harvest forage samples were collected at the different cutting heights, which were inoculated with Sil-All® (Alltech) and were ensiled for later analysis.

*Plant morphology.* At harvest five replicates of three plants were randomly collected for each cutting height (20, 40 and 60 cm). Plants were separated into: leaf blade, leaf sheath, tassel, stalk, husk, cob and grain. Plant parts were dried in a forced air oven at 60ºC during five d to determinate dry matter contribution.

*Dry matter and grain yield.* The amount of plants per hectare were determined by counting total number of plants in 4 $m^2$ (n= 10), which was multiplied by 2500 to extrapolate to one hectare. Plants and grain dry weight in each cutting height was multiplied by total number of plants to calculate dry matter and grain yield per hectare.

*Silage chemical analysis.* Silos were opened at 45 d of fermentation to determine chemical composition. The pH was measured at the time of opening the silages using a portable pH meter (HANNA, Instruments). Dry and organic matter were determined according (AOAC, 1990). Crude protein was determined by Kjeldahl procedure (AOAC, 1990). The NDF, ADF and ADL were determined sequentially in ANKOM$^{200}$ fiber analyzer (Ankom Technology, Fairport, NY) using the Ankom® filter bags with a pore size of 30 microns.

*In vitro digestibility.* Digestibility was determined in a DAISY$^{II}$ (ANKOM$^{200}$) digestor. After 48 h incubation period bags were removed from jars and

washed with distilled water at room temperature until the rinse water was clear. Afterwards NDF was determined as above mentioned to calculate NDF digestibility.

*Estimated milk yield.* Net energy for lactation was estimated ($NE_l$), milk yield per hectare and per ton of dry matter using the MILK2006 model (Schwab et al., 2001) for each treatment.

*In vitro gas production.* In order to determine in vitro gas production Hohenheim test (Menke et al., 1979) was used. Two hair wethers fitted with rumen cannulas were used as ruminal fluid donators. In vitro ruminal medium was made by bicarbonate and phosphate buffers, a reducting agent, a nitrogen source, several minerals and resazurin as anaerobiosis indicator. During medium preparation continuous CO2 was applied in order to provide an appropiate anaerobic environment at time of inoculation. A sample weight of 200 mg was used on a glass container (50 ml) adding 30 mL of the artificial saliva:ruminal fluid mixture in 2:1 ratio. At this time initial volume was recorded and glass containers were placed on a continuous shaking incubator for 72 h. Gas yield measurement were performed at 0, 1, 2, 3, 4, 5, 6, 9, 12, 24, 48 and 72 h, accumulated pressure was measured using a FESTO® transductor. Fermentation parameters *a*, *b*, and *c* were estimated using the monophasic model described by Groot et al., (1996):

$$G = \sum_{i=1}^{n} \frac{A_i}{1 + \frac{B_i^{C_i}}{t^{C_i}}}$$

In equation, *G* (mL $g^{-1}$ DM) is amount of gas produced per gram of incubated dry matter in the time *t* after incubation. $A_i$ (mL $g^{-1}$ DM) is asymptotic gas production. $B_i$ (h) is the time after incubation in which half of the asymptote of gas has been formed, and $C_i$ is a constant that determine the profile shape and characteristics. The value of *i* indicates the number of phases in the profile (*i* = 1, n) in the case of several be observed. The C is the constant that determine curvature and in consequence the point of inflexion

*Statistical analysis.* Data collected for plant nutritional value, dry matter, digestibility (DMD) and NDF digestibility, estimated milk yield per ton of DM and per hectare were analyzed by PROC GLM of SAS v. 9.0 (SAS, 2002), were the only factor was cutting height. Fermentation parameters obtained through monophasic model of Groot et al. (1996) were analyzed by PROC GLM of SAS v. 9.0 (SAS, 2002).

## Results

Dry matter production per hectare (Table 1) was decreased in 9 and 12.5% by cutting height at 40 and 60 cm, respectively, compared to normal cutting height of 20 cm. However it was observed an increase of 15 and 23% in grain content in plants cut at 40 and 60 cm, respectively. Likewise stalk ratio decrease 21 and 31% at cutting height of 40 and 60 cm, respectively.

Table 1. Effec of cutting height on morphological composition of the corn plant and DM and grain yield.

| | Cutting height (cm) | | |
|---|---|---|---|
| | **20** | **40** | **60** |
| Stalk (%) | 21.95 | 17.54 | 15.26 |
| Leaf blade (%) | 16.47 | 16.27 | 14.74 |
| Leaf sheath (%) | 8.72 | 7.69 | 7.57 |
| Husk (%) | 13.51 | 14.99 | 15.40 |
| Tassel (%) | 0.91 | 0.79 | 1.20 |
| Cob (%) | 11.58 | 12.76 | 13.75 |
| Grain (%) | 26.30 | 29.65 | 31.77 |
| | **Yield (Ton DM/ha)** | | |
| DM | 31.5 | 28.6 | 27.5 |
| Grain | 8.2 | 8.6 | 8.8 |

NDF and CP content were reduced (P≤0.05) at 60 cm (Table 2). It was observed a numerical increase (P≥0.05)in IVDMD and IVNDFD. Likewise cutting height did not increase milk yield per ton of DM and by hectare (Table 2).

Final volume of gas (Figure 1) produced was higher (P≤0.05) for 60 cm compared to 20 and 40 cm. The parameter *b* was different among cutting heights (P≤0.05), with tendency to decrease at higher cutting heights. The parameter *c* was also different (P≤0.05) between 20 and 60 cm (Table 3).

Table 3. Effect of cutting height on in vitro DM degradability parameters.

| Parameter | Cutting height (cm) | | | SE | *P* |
|---|---|---|---|---|---|
| | **20** | **40** | **60** | | |
| *a* | 139.0 | 122.8 | 106.4 | 2.81 | 0.001 |
| *b* | 59.6 | 34.8 | 15.2 | 3.58 | 0.000 |
| *c* | 0.7 | 0.7 | 0.9 | 0.02 | 0.012 |

## Discussion

Increasing cutting height at harvest is a useful tool for improving nutritional value of corn silage. In this study, increasing cutting height from 20 to 40 cm and 60 cm resulted in decreasing dry matter yield per hectare 9 and 12.5%, respectively. These results are similar to Lewis et al., (2004) and Curran and Posch (2000) who reported a 11% reduction in dry matter yield. Likewise, while increasing cutting height it improves corn silage nutritional value because NDF and ADL decreases. Higher cutting height changes the morphology proportion of the plant, it decreases the stalk percentage and increases that of grain. In this study, stalk proportion was reduced 31% and grain proportion increase 23% when cutting height was changed from 20 to 60 cm. These are similar with 29.1% reduction in stalk proportion reported

by Restle et al. (2002). Domínguez (2004) also reported a decrease in NDF concentration when changing cutting height from 23 to 71 cm. Most of the changes found in this study as well as in others, as a result of elevated cutting height match with the fact that more fibrous and highly lignified parts are left on the ground (Neylon and Kung, 2003).

Milk yield per ton of dry matter resulted in an increase of 14% when cutting height increased from 20 to 40 cm and 16% when increased from 20 to 60 cm. These results are similar to those reported by Neylon y Kung (2003) who when changed cutting height from 10.2 to 51 cm found an increase of 7.9% in milk yield per ton of dry matter.

In the fermentation parameters, *b* was different among cutting heights, this parameter denotes the time in h in which half the total gas is produced (parameter *a*). In the case of parameter *c*, this was increased as cutting height did, it indicates changes ratio (mL gas/h).

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Table 3. Effect of cutting height on Chemical composition of the corn silage, in vitro DM and NDF digestibility and estimated milk yield.

| | Cutting height (cm) | | | SE | *P* |
|---|---|---|---|---|---|
| | 20 | 40 | 60 | | |
| pH | 3.7 | 3.9 | 3.7 | 0.090 | 0.1571 |
| DM (%) | 31.59 | 27.95 | 28.98 | 0.36 | 0.0536 |
| OM (%) | 92.94 | 92.53 | 92.95 | 0.356 | 0.9897 |
| Ash (%) | 7.02 | 7.46 | 7.04 | 0.529 | 0.9786 |
| CP (%) | 7.86 | 7.57 | 6.77 | 0.155 | 0.0023 |
| Fat (%) | 2.5 | 3.3 | 3.85 | 0.620 | 0.1419 |
| NDF (%) | 48.29 | 47.27 | 43.3 | 0.6770 | 0.0026 |
| ADF (%) | 25.46 | 26.89 | 24.16 | 0.836 | 0.389 |
| Lignin (%) | 2.31 | 1.88 | 1.85 | 0.228 | 0.1807 |
| IVDMD (%) | 56.76 | 55.45 | 61.09 | 2.047 | 0.2027 |
| IVNDFD (%) | 46.38 | 49.26 | 50.32 | 3.545 | 0.4264 |
| $NE_L$ | 1.22 | 1.36 | 1.4 | 0.063 | 0.0732 |
| Milk yield (Ton/DM Ton) | 1.07 | 1.22 | 1.25 | 0.859 | 0.1118 |
| Milk yield (Ton/Ha) | 30.3 | 31.6 | 31.8 | 2.302 | 0.6174 |

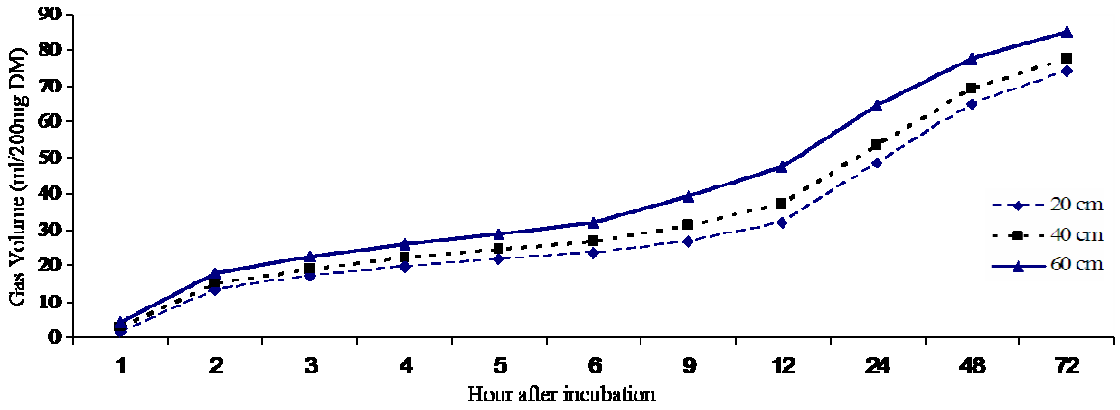


Figure 1. Effect of cutting height on in vitro gas production.

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# THE EFFECTS OF COMPOST, MANURE, AND UREA ON YIELD AND FORAGE QUALITY WHEN TOPDRESSED ON A PERENNIAL FORAGE MIX OF IRRIGATED, COOL SEASON GRASSES

**K.C. Doesken, J. G. Davis, J. E. Brummer, and T. E. Engle**
**Colorado State University, Ft. Collins, CO**
**Kathy.Doesken@colostate.edu (970)491-6984**

**ABSTRACT:** Significant quantities of animal manures are generated by livestock and equine facilities along the Front Range in Colorado. Topdressing raw manure on perennial forage is not recommended due to high potential losses of N into the environment (Waskom et al., 1999). Much of the nitrogen in composted manure is in stable, organic forms. Compost contains lower levels of the ammonium and nitrate forms of N than raw manure. A two year study was conducted to determine the effects of topdressing composted manure, raw manure and urea on the yield and forage quality of a mix of irrigated, cool season perennial grasses. Ccomposted horse manure and raw manure at 22 t $ha^{-1}$ and 45 t $ha^{-1}$ and 22 t $ha^{-1}$ raw manure with wood shavings were topdressed once on a two year old stand of well established grasses. Urea at 207 kg $ha^{-1}$ N was topdressed each spring for two years. Forage yield was measured in two hay cuttings for two years. No yield differences among any of the fertility treatments were apparent during the first year after application. In the second year of the study, in both the first and second hay cuttings, annually applied urea at the agronomic rate resulted in greater yields ($P$=0.0014) than any of the organic treatments. Crude protein (CP), neutral digestible fiber (NDF), and acid digestible fiber (ADF) were measured in the harvested material. Relative feed value was calculated using NDF and ADF values. In both 2006 and 2007 differences occurred in RFVs. In three out of four harvests RFV among both compost and manure applied at 45 t ha-1 and urea applied each spring at 207 kg ha -1 N resulted in RFVs that were not different from each other. One third less N was applied with topdressed compost at the rates studied here than when the agronomic rate of N was applied as urea. The application of compost poses less risk of loss of N to the environment than annually applied urea or raw manure. However, further study with higher rates of compost over several growing seasons is needed to identify optimum rates of topdressed compost on irrigated perennial cool season grass mixes, after considering both yield and forage quality.

Keywords: compost, cool season perennial grasses, forage quality, manure, relative feed value, yield

## Introduction

Manure from large dairies and feedlots is plentiful along the Front Range of the Rocky Mountains in CO. Significant quantities of manure are produced in concentrated areas, necessitating movement off-farm for proper utilization. Equine facilities, both large and small, also abound here. Due to urbanization along the Front Range and an increase in the number of small equine facilities, horse manure utilization can be problematic due to insufficient agricultural land and lack of knowledge about how to use it. While the use of synthetic fertilizers is well understood and convenient several problems are associated with its use. One metric ton of urea cost $90 in 1970, $203 in 1990, and $500 in 2007 (http://www.ers.usda.gov/Data/FertilizerUse/). Rising fertilizer prices and an abundance of locally produced manure may result in an increased interest in managing soil fertility with manure and composted manure. However, nitrogen losses from fertilizer and manure have been recognized as potential environmental contaminants. Nitrogen volatilization from synthetic nitrogen fertilizers can be high in alkaline soils, especially when fertilizer cannot be incorporated or is applied during warm weather. These losses are both an economic problem and a growing environmental concern. This source of nitrogen in the atmosphere is now linked to nitrogen deposition in Rocky Mountain National Park and subsequent ecosystem impacts. Most of the N in composted manure is in stable organic forms, resulting in less risk of losses of N to the environment when topdressed.

Increasing the use of manure and composted manure to maintain soil fertility on agricultural crops will be a practical course of action as prices of commercially produced nitrogen fertilizers increase, the manure supply is plentiful and environmental problems with its use continue to emerge. Composting manure reduces its volume, produces a finer textured material which spreads well and contains very little ammonium or nitrate N. Most of the nitrogen in compost is in organic forms which are mineralized in the soil when moisture is adequate, soil microorganisms are present and soil is warm.

Published information about agronomic rates of compost application on perennial grasses is limited, which is a deterrent to the use of compost. The objective of this research is to measure yield and forage quality resulting from topdressing compost on irrigated perennial grass forage. Results obtained from this study can be applied to organic forage production. An increase in published information will encourage the appropriate use of manure produced in this region by livestock and equine facilities.

## Materials and Methods

This study began in spring 2004 when meadow brome (*Bromus bilbersteinii*), smooth brome (*Bromus inermis*), and orchardgrass (*Dactylis glomerata*) in a ratio of approximately 1:1:1 were drill seeded on plots located at Colorado State University's Agricultural Research, Development and Education Center (ARDEC), located near Fort Collins, CO.

**Compost Preparation**
The compost used in this study was made on-site during the fall and winter of 2005-2006. It was made with equine manure from the Equine Teaching and Research Center at CSU, alfalfa, wheat straw and fallen leaves.

**Crop Establishment**
Prior to planting, 180 kg $ha^{-1}$ nitrogen N was applied from urea (46-0-0) and incorporated. No additional phosphorus was applied. This pre-plant fertilization rate was based on soil test results from soil sampled the fall before planting and recommendations developed at Colorado State University for grasses and alfalfa (Davis et. al, 1996). The new planting was irrigated during the 2004 growing season and cut once to suppress annual weeds. Hay was also cut during the growing season in 2005 but no data was taken.

**Experimental Design**
The experimental design was a randomized complete block design with six treatments replicated four times. Each rectangular plot measured 6 m by 12 m.

Table 1. Soil Fertility Treatments.

| Soil Amendments | | | | |
|---|---|---|---|---|
| **Soil Treatment** | **Application rate** | **% N** | **N (kg $ha^{-1}$)** | **Carbon ($ha^{-1}$)** |
| Compost 1 (C1) | 22 t $ha^{-1}$ † | 0.67 | ~70 | ~762 kg |
| Compost 2 (C2) | 45 t $ha^{-1}$ † | 0.67 | ~139 | ~1525kg |
| Manure 1 (M1) | 22 t $ha^{-1}$ † | 0.95 | ~213 | ~4.5 t‡ |
| Manure 2 (M2) | 45 t $ha^{-1}$ † | 0.95 | ~426 | ~9.0 t‡ |
| Manure 1 + Wood Shavings (M1 + WS) | 22 t $ha^{-1}$ + WS 40% by volume | 0.95 | ~213 | ~ 6.7 t‡ (M1=4.5, WS=2.2) |
| Urea spring 06 § (U) | 451 kg $ha^{-1}$ | 46.0 | 207 | 0 |
| Urea spring 07 § (U) | 451 kg $ha^{-1}$ | 46.0 | 207 | 0 |

†Applied "as is;" all % N on dry basis.
‡Assume 9.5 kg "as is" manure, 50% moisture, manure C:N 20:1.
§Urea applied annually at agronomic rate, based on crop grown, yield expectations, fall soil test, and CSU fact sheet 0.537, Fertilizing Alfalfa and Grasses. All other treatments applied once in spring of 2006.

**Irrigation**
Irrigation was sprinkler applied using a Zimmatic Lateral Move System (http://www.lindsaymanufacturing.com/zim_agsysystem_latmov.asp) weekly throughout both growing seasons.

**Climatic Factors Affecting Crop Growth at ARDEC**
There is no official weather station at ARDEC. Unofficial precipitation measurements were obtained through the Community Collaborative Rain, Hail and Snow Network (http://www.cocorahs.org/). Precipitation was less than 20.3 cm during the 2005-2006 water year between October 1 and September 30. During the 2006-2007 water year 33.4 cm of precipitation were measured at ARDEC. Precipitation in 2007 was close to the long-term average, while precipitation for 2006 was only slightly over half of the long-term average.

**Fertility Treatments**
Six fertility treatments were applied (Table 1) in spring 2006. Composted horse manure and raw manure were both applied once in spring 2006 at 22 and 45 t $ha^{-1}$ (C1, C2, M1, and M2, respectively). Horse manure at 22 t $ha^{-1}$ (M1) was also applied at the same time with the addition of 40% by volume of softwood shavings (M1 + WS) which are locally used for equine stall bedding. Shavings high in carbon often are present in horse manure that is land-applied. The control treatment, urea (46-0-0), was spring applied in 2006 at the agronomic rate, 207 kg $ha^{-1}$ (Davis et. al, 1996), based on soil test results from fall 2005, yield estimates and crop. Urea was again applied in the spring of 2007 at the same rate based on soil tests from fall 2006, yield estimates and crop.

Organic treatment rates were not based on agronomic recommendations for compost and manure due to lack of information. Mineralization rates, which are derived from field studies, are necessary to predict what proportion of N in the organic amendment will become available to plants in a growing season, which is necessary information for determining the agronomic rate. This information is not available for organic amendments topdressed on perennial forages in the Great Plains. Measuring N mineralization is another phase of this field study, not reported here. Application rates were selected that are commonly used in this region.

**Crop Management**
Forage was cut and baled for hay twice during each of the two growing seasons. The first cuttings in both years were made between jointing and anthesis stages, which fell both years in early June. In 2006 the second cutting was made in August. In 2007 it was made in September.

**Yield Measurement**
Prior to cutting samples for yield, 1 m alleys were cut between the plots using a Carter flail harvester (http://www.cartermfgco.com/product2.htm). To obtain yield data, the center 1 m strip, minus the alley width at

each end of the swath, was cut from each plot, resulting in a sample area of 1 m by 11.3 m. The harvester equipment blew cut forage through a chute into a large trash container mounted on a weigh pad. Weights were recorded manually from an electronic readout in front of the tractor operator. Grab samples were taken from the forage as it was harvested, bagged and weights were recorded. Samples were oven dried for several days until dry, then weighed again. Wet and dry weights were used to calculate moisture content and dry matter content.

**Forage Quality**

The dried grab samples from each harvest collected for moisture content and yield, saved, and later ground through a 1-mm sieve using a Retsch SM 2000 Heavy-Duty Cutting Mill. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured with an Ankom fiber digester (Ankom, 1998). Relative feed value (RFV) was calculated from NDF, ADF, digestible dry matter (DDM), and dry matter intake (DMI) where DDM = 88.9-(0.779*%ADF), DDI = 120/%NDF, and RFV = (DDM*DMI)/129 (Stokes and Prostko, 1998). Total N was analyzed with a LECO Total CHN 1000 Elemental Analyzer (St. Joseph, MI). Crude Protein was calculated from % N measured with the LECO (CP=%N x 6.25).

Table 2. Forage Yields

| soil treatment | Forage Yields (t ha$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 2006 | | | 2007 | | |
| | Jun | Aug | Total | Jun | Sept | Total |
| C1 | 2.7 | 2.7 | 5.4 | 0.7b | 4.5b | 5.2b |
| C2 | 1.6 | 2.0 | 3.6 | 0.7b | 4.9b | 5.6b |
| M1 | 1.3 | 1.1 | 2.5 | 0.7b | 4.3b | 4.9b |
| M2 | 1.6 | 1.3 | 2.9 | 0.7b | 4.0b | 4.7b |
| M1 +WS | 3.1 | 1.8 | 4.9 | 0.7b | 4.0b | 4.7b |
| Urea | 4.3 | 2.0 | 6.3 | 0.9a | 6.0a | 7.0a |
| Avg. yield | 2.5 | 1.8 | 4.3 | 0.7 | 4.7 | 5.4 |
| *P*-value | NS | NS | NS | 0.0014 | 0.0100 | .0050 |
| Statistics: SAS 9.1, proc glm, mean separation test LSD. | | | | | | |

**Statistical Analysis**

Statistical analyses were performed on the data using SAS 9.1 using the proc glm model and the least significant difference mean (LSD) separation test. *P* values are reported in each data table where statistically significant differences occur.

## Results and Discussion

**Forage Yields**

The soil amendments applied in this study spanned a wide range of N (Table 1), ranging from about 70 kg ha$^{-1}$ N from compost applied at 22 t ha$^{-1}$ (C1) to 426 kg ha$^{-1}$ N from raw manure applied at 45 t ha$^{-1}$ (M2). The 207 kg ha$^{-1}$ N from urea (U) applied spring 2006 and 2007 totaled 414 kg ha$^{-1}$ N over the two year study, which is similar to the 426 kg ha$^{-1}$ N from manure applied at 45 t ha$^{-1}$ (M2) applied once in spring 2006.

In addition to the range in levels of actual N applied, carbon application also covered a wide range (Table 1). In addition to N, compost, raw manure, and wood shavings all contain carbon. The compost used in this study had a C:N of 11:1. Raw horse manure contained more carbon, with a C:N of about 20:1. The wood shavings added to 22 t ha$^{-1}$ manure (M1 + WS) added about 2.2 t ha$^{-1}$ carbon in addition to what was already in the manure, which was about 4.5 t ha$^{-1}$. This treatment was added to study the effect of surface applied stable waste, which can contain large amounts of bedding. Softwood shavings, produced by local mills from logging and slash removal in the nearby mountains, are commonly used for equine bedding in this region. Adding carbon to soil is known to temporarily immobilize nitrogen, resulting in decreased plant growth. While the low rate of manure (M1) resulted in significantly lower yield than the annually applied urea (U), the yield from the treatment M1 + WS was the same as yields from plots amended with manure applied at the same rate but without the added wood shavings (M1). This indicates that the expected immobilization of N due to the addition of carbon either did not happen, or has not happened yet. Visual inspection of these plots indicated that the wood shavings may have acted like surface mulch that prevented some water loss.

In 2006 there were no differences in yield (Table 2) among any of the fertility treatments, in spite of the range in N and C applied (Table 1). While this study was irrigated, 2006 precipitation was about half of the long-term average. Below average precipitation plus limited irrigation water may have contributed to the lack of treatment effects in 2006.

In 2007 yields from each of the two harvests in plots fertilized each spring for two years with urea (U) were greater (*P*=0.0014, *P*=0.005, respectively) than yields in any of the plots amended with organic treatments once at the beginning of the study. There were no differences among the yields of any of the organic treatments (C1, C2, M1, M2, M1+WS) in either harvest in 2007.

**Forage Quality**

In addition to yield, forage quality is important in evaluating fertility treatments. Relative feed value, calculated from NDF and ADF, is widely used to compare forage quality. In general, a larger value for RFV is associated with better forage quality.

Table 3. Forage Quality: Relative Feed Value

| Soil Treatment | 2006 | |
|---|---|---|
| | June | August |
| | RFV | RVF |
| C1 | 102.6bc | 101.0ab |
| C2 | 107.2a | 102.3a |
| M1 | 101.4c | 97.5ab |
| M2 | 105.7ab | 97.0bc |
| M1 + WS | 103.5abc | 92.1c |
| Urea | 103.6abc | 99.6ab |
| *P*-value | 0.08 | 0.0017 |

| Soil Treatment | 2007 | |
|---|---|---|
| | June | September |
| | RFV | RFV |
| C1 | 59.1bcd | 58.6ab |
| C2 | 58.3d | 58.4ab |
| M1 | 59.4abc | 57.8bc |
| M2 | 58.4cd | 58.6ab |
| M1 + WS | 60.0ab | 57.4c |
| Urea | 60.3a | 59.2a |
| *P*-value | 0.0004 | 0.0019 |
| SAS 9.1, proc glm, least significant difference (LSD) mean separation<br>RFV: relative feed value | | |

In both years of this study, differences occurred in RFVs among treatments at both harvests (Table 3). In three out of the four hay harvests, RFV among both compost and manure applied at 45 t $ha^{-1}$ and urea applied each spring at 207 kg $ha^{-1}$ N resulted in RFVs that were not different from each other. In the June 2007 hay harvest manure at 22 t $ha^{-1}$ plus wood shavings resulted in a RFV that was not different from urea, while compost at 45 t $ha^{-1}$ resulted in a lower RFV. In both years CP levels in forage showed no differences due to treatment.

Results from this study indicate that topdressed compost can result in forage quality comparable to applications of annually applied urea at the agronomic rate. However, the amount of N applied in 45 t $ha^{-1}$ of compost applied once was one third as much N as was applied in the two annual applications of urea at the agronomic rate. The compost application rates in this study pose fewer risks of loss of N to the environment than annually applied urea. Since yields were reduced in all the organic treatments when compared with annually applied urea, further study with higher rates of compost over several growing seasons is needed to identify optimum rates of topdressed compost on irrigated perennial cool season grass mixes, after considering both yield and forage quality.

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# PLANTING SYSTEM AND MATURITY STAGE ON PRODUCTION AND NUTRITIONAL VALUE OF OAT HAY

**D. Domínguez[*1], S. Ramírez[1], J. J. Salmerón[2], G. Villalobos[1], A. Corral[1], and J. A. Ortega[1]**
Universidad Autónoma de Chihuahua, Chihuahua, Chih., México[1]. INIFAP, Cuauhtémoc, Chih., México[2]

**ABSTRACT:** Oat (*Avena sativa L.*), is the most important forage source for the beef and milk production system at the western of the State of Chihuahua. In order to evaluate the effect of traditional sowing in plane surfaces (SPS), and furrow diking sowing (FDS) on production and nutritional value of oat hay harvested at boot (BS), dough (DS) and hard grain (HS) stages, oat varieties Bachiniva, Menonita, Teporaca, Karma, Cevamex, Cuauhtemoc and Babicora were cultivated under non irrigated conditions in five sites of western Chihuahua. Production of dry matter per hectare (DM/ha), and content of CP, NDF, ADF, and ADL were determined; dry matter digestibility (DMD), and concentration of $NE_l$ were estimated. The grain:foliage ratio and their NDF content were determined to explain fiber dilution effect. Data was analyzed by a split plot factorial design using the PROC MIXED of SAS, and mean comparison was performed using the LSD test. The FDS did not improved DM/ha. A positive linear increase ($P$<0.05) in DM/ha was observed across maturity stages of BS, DS and HS (1247 ± 322, 3120 ± 322 y 4475 ± 322 Kg, respectively). CP content had a quadratic tendency across plant maturity ($P$<0.05; 17.4 ± 1, 10.9 ± 1 y 10.6 ± 1%, respectively). Concentration of NDF (57.1 ± 1.3, 52.0 ± 1.3 y 47.4 ± 1.3%, respectively), and ADF had a negative linear tendency ($P$<0.05) by plant maturity effect. ADL content was not affected by maturity stage ($P$>0.05). Whole plant NDF dilution was associated with a grain weight increase, and its decreased NDF concentration, due to plant maturity. The DMD and $NE_l$ were linearly increased by maturity ($P$<0.05). The FDS did not affect DM production and nutritional value. Oat hay harvested at HS maximizes forage production and nutritional value, recommended genotypes being Teporaca, Cevamex and Bachiniva.

**Key words:** planting system, maturity stage, oat varieties, dry matter production, nutritional quality.

## Introduction

Oat hay is cultivated extensively under non irrigated conditions at the western of the State of Chihuahua. This state accounts for more than 75% of Mexico land cultivated with oat, representing the first place production. Oat is the basic forage source in the menonita milk system (Salmerón et al., 2003).

National Research Institute of forestry, agronomy and animal production (INIFAP) has developed 21 oat varieties. Production and agronomic characteristics has been studied, but knowledge of their nutritive value is limited. Commonly, in Chihuahua oat is planted in plane surfaces and harvested at hard grain stage, in order to maximize dry matter production. In this way, dry matter production can be decreased by low water utilization, as well as nutrient and oat seeds washed during high precipitation conditions (Brhane et al., 2006). Furrow diking sowing has showed to increase water filtration, and dry matter production of corn (McFarland et al., 1991) and sorghum (Brhane et al., 2006) compared to sowing in plane surfaces. By the way, oat hay produced has low content of CP (10.5%) but high NDF concentration (61.4%; Salmeron et al., 2003) compared to harvesting at boot and dough stages (21 and 12%, and 44 and 54%, respectively; FAO, 2004).

The objective of this study was to evaluate the furrow diking sowing on yield and nutritive value of seven varieties of oat harvested at boot, dough and hard grain stages.

## Materials and Methods

The study was conducted in five sites of western of Chihuahua, where Bachiniva, Menonita, Teporaca, Karma, Cevamex, Cuauhtémoc and Babícora oat varieties were planted from august 2nd to July 25th of 2005 at 100 Kg $ha^{-1}$ densit in a 1/2 of hectare, and fertilized with doses of 30-40-00 Kg of N-P-K.

Each plot oat was sowed in plane surfaces (SPS), and furrow diking sowing (FDS). Each parcel was also divided in three sublots accounting for the three maturity stages. Oat samples were harvested at boot (BS; 47 ± 2 d), dough (DS; 78 ± 4 d) and hard grain (HS; 92 ±3 d) after planting date.

Oat samples were dried in forced air oven at 60 C during 48 h to determine DM content. (AOAC, 1995). Dry matter production $ha^{-1}$ was determined in 1 m2 replicated six times and adjusted to one hectare. Oat samples were ground to 1mm in a Wiley mill (Arthur H. Tomas, Philadelphia, PA) to determine absolute dry matter, OM and CP (AOAC, 1995).

NDF, ADF and ADL were sequentially determined in the ANKOM $^{200}$ (Ankom Technology, Fairport, NY), using Ankom® filter bags F57 (Van Soest et al., 1991 and Goering y Van Soest (1970).

Grain, leaves, and stalk of Bachiniva oat

variety harvested at three maturity stages (n=10) were manually separated to determine grain:foliage ratio and were analyzed for CP, NDF and ADL content..

Dry matter digestibility was estimated by the equation: DMD (%) = 88.9 – (0.779 x ADF% (% DM)) (Moore y Undersander, 2002), and $NE_l$ with the equation: $EN_l$ (Mcal $lb^{-1}$) = 0.7936 – (0.00344 x ADF, % DM) (Undersander *et al*., 1993), which was adjusted to Mcal $Kg^{-1}$ DM.

Data was analyzed by PROC MIXED of SAS (SAS, 1999) adjusting a split plot factorial design (7x3x2), and mean comparison was performed using the LSD test. Significance was declared at $P \leq 0.05$.

## Results

The FDS did not improved ($P>0.05$) DM $ha^{-1}$ vs. SPS at BS (1237 vs. 1257 kg $ha^{-1}$), DS (3030 vs. 3210 kg $ha^{-1}$) and GS (4181 vs. 4770 kg $ha^{-1}$). Dry matter production was affected by maturity stage ($r^2$=0.99), showing a positive ($P<0.05$) lineal increase (Table 1). Similar DM $ha^{-1}$ ($P>0.05$) was observed for oat varieties at the different maturity stages, with the exception of Karma oat variety (Table 1). Sowing system did not affect ($P>0.10$) nutritional value of oat varieties at BS, DS and HS.

Crude protein content of oat varieties showed a quadratic tendency ($P<0.05$; $r^2$ = 0.58) through the maturity stages. Grain:foliage ratio for Bachiniva oat variety was 15:85, 53.1:46.9 and 51.3:48.7 for BS, DS, and HS, with CP contents of 26.7 y 17.6, 14.5 y 8.9, y 15.1 y 4.1%, respectively. Therefore, each fraction accounted for 21.1 y 78.9, 64.6 y 35.4, y 79.4 y 20.6%, respectively of CP content at BS (19.0%), DS (11.9%) and HS (9.7%). There was observed an interaction effect for maturity x variety ($P<0.05$) for CP concentration. Teporaca, Karma, Cevamex and Bachiniva had the highest CP content through the maturity stages (Table 1).

Concentration of NDF and ADF showed a negative lineal tendency ($P<0.05$) decreasing, on average, between 5.0 and 3.4 percentage units throughout the different maturity stages (table 2). We also observed an interaction between maturity and variety ($P<0.05$) on NDF, which implied that there is not a single variety with a constant fiber content along maturity stages. In general, those varieties with a constant ADF and NDF were Teporaca, Karma, Cevamex and Bachiniva.

The NDF and ADF content for grain and foliage was 52.5, 56.9, 25.1 y 63.5, 19.2, 68.8 and 22.8 y 33.9, 11.4 y 38.9, 8.4 and 41.9, respectively. This showed that the NDF accounted for the 14.0, 86.0, 30.9 and 69.1, 22.6 y 77.4% and the ADF accounted for the 10.6 y 89.4, 25.0 y 75.0, 17.4 y 82.6% of the total sample.

In general, the ADL content did not show a significant trend since the average concentration was not affected by maturity stage ($P>0.05$) and were 2.56, 2.70 y 2.52% during BS, GS and HS, respectively (table 2). Only the Menonita genotype showed a quadratic effect ($P<0.05$; $r^2$ = 0.23), whereas the Karma and Cevamex genotypes showed a quadratic trend ($P \leq 0.10$; $r^2$ = 0.14 y 0.17, respectively). On the grain:foliage ratio, ADL concentration for each fraction throughout the different stages (BS, GS and HS) was 2.3:2.6, 1.7:3.7 and 1.2:4.2, respectively. Here, each component accounted for the 13.5 and 86.5%, 34.0 and 66.0%, 23.8 and 76.2% of the total lignin contained on the sample, which was 2.5, 2.7 and 2.7%. On the other hand, the ADL only showed effect by variety ($P<0.05$). Teporaca, Karma and Cevamex were the varieties that showed a constant ADL concentration during the three different maturity stages (table 2).

The DMD increased linearly ($P<0.05$; $r^2$ = 0.99) with 2.7 percentage units from one stage to stage. The highest DMD ($P<0.05$) was during HS with 68.6% (table 5). DMD only showed a significant effect by HS ($P<0.05$) and variety ($P<0.05$) with the highest DMD during HS (68.6; Table 2). Teporaca, Karma, Cevamex and Bachiniva oat varieties had the highest DMD.

The $NE_l$ was lineal increased ($P<0.05$; $r^2$ = 0.99) by 0.03 units through maturity stages. The $NE_l$ content at BS, DS and HS was 1.50, 1.53 y 1.55 Mcal/Kg de MS, respectively (Table 2).

## Discussion

Furrow diking sowing did not improved dry matter production may be due to the high precipitation observed during the growth season, as well as experimental plots in the sites of study did not have a strong steeper, which are some of the mainly factors to observe a positive response of this planting system (McFarland et al., 1991). However, this technique has improved dry matter productions of corn (McFarland et al., 1991) and sorghum (Brhane et al., 2006).

The average of crude protein content of oat varieties at BS and DS, are in agreement with those reported by Baron et al. (2000) and FAO (2004), and with Salmerón et al. (2003) for HS.

The increase in the grain:foliage ratio across muturity stages, decreased NDF, and ADF content of oat, but did not affected ADL concentration. This behavior has been reported in small grain cereals (Khorasani et al., 1993, and Domínguez et al., 2007). The DMD and $NE_l$ were increased by maturity stage, due to the fiber concentration of oat was decreased.

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Table 1. Effect of maturity stage on dry matter production, crude protein, neutral detergent fiber and acid detergent fiber concentration of seven oat varieties.

| | DM ha$^{-1}$ (Kg) | | | PC (% DM) | | | FDN (% DM) | | | FDA (% DM) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stage of Maturity | | | | | | | | | | | |
| Variety | BS[1] | DS[2] | HS[3] | BS[1] | DS[2] | HS[3] | BS[1] | DS[2] | HS[3] | BS[1] | DS[2] | HS[3] |
| Bachiniva | 1144$^{ab}$ | 3220$^{a}$ | 4448$^{abc}$ | 17.6$^{b}$ | 11.3$^{a}$ | 10.9$^{ab}$ | 59.0$^{a}$ | 51.5$^{bc}$ | 44.9$^{d}$ | 33.4$^{a}$ | 28.6$^{bc}$ | 24.8$^{cd}$ |
| Menonita | 1325$^{ab}$ | 3353$^{a}$ | 4490$^{abc}$ | 17.4$^{b}$ | 10.6$^{ab}$ | 10.5$^{ab}$ | 58.4$^{ba}$ | 54.1$^{a}$ | 50.2$^{a}$ | 33.8$^{a}$ | 29.9$^{ab}$ | 27.4$^{a}$ |
| Teporaca | 1243$^{ab}$ | 3128$^{a}$ | 4740$^{ac}$ | 18.2$^{a}$ | 11.6$^{a}$ | 10.3$^{ab}$ | 55.3$^{c}$ | 51.0$^{bc}$ | 46.3$^{cd}$ | 31.6$^{c}$ | 28.1$^{bc}$ | 25.2$^{cd}$ |
| Karma | 957$^{b}$ | 2674$^{b}$ | 4148$^{b}$ | 18.5$^{a}$ | 11.4$^{a}$ | 10.4$^{ab}$ | 56.6$^{bac}$ | 50.3$^{c}$ | 45.2$^{d}$ | 32.7$^{bac}$ | 27.4$^{c}$ | 24.4$^{d}$ |
| Cevamex | 1225$^{ab}$ | 3013$^{ab}$ | 4753$^{c}$ | 18.4$^{a}$ | 11.8$^{a}$ | 9.9$^{b}$ | 55.1$^{c}$ | 53.1$^{ba}$ | 48.6$^{bac}$ | 31.7$^{c}$ | 29.2$^{ab}$ | 26.5$^{ba}$ |
| Cuauhtémoc | 1565$^{a}$ | 3148$^{a}$ | 4426$^{abc}$ | 15.4$^{c}$ | 10.5$^{ab}$ | 11.3$^{a}$ | 56.5$^{cb}$ | 52.5$^{bac}$ | 47.8$^{bc}$ | 32.9$^{bac}$ | 29.3$^{ab}$ | 26.3$^{bca}$ |
| Babicora | 1269$^{ab}$ | 3302$^{a}$ | 4320$^{ab}$ | 16.6$^{bc}$ | 9.7$^{b}$ | 10.9$^{ab}$ | 58.7$^{ba}$ | 51.7$^{bc}$ | 48.6$^{bac}$ | 33.7$^{a}$ | 29.2$^{ab}$ | 27.2$^{a}$ |

[1, 2, 3] Correspond to boot stage, dough stage and hard grain stage, respectively
Means within columns with different superscripts are different ($P< 0.05$)

Table 2. Effect of maturity stage on acid detergent lignin content, dry matter digestibility and net energy of lactation of seven oat varieties.

| | ADL (% DM) | | | DMD (% DM) | | | $NE_l$ (Mcal/kg DM) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Stage of Maturity | | | | | | | | |
| Variety | BS[1] | DS[2] | HS[3] | BS[1] | DS[2] | HS[3] | BS[1] | DS[2] | HS[3] |
| Bachiniva | $2.64^{a}$ | $2.68^{b}$ | $2.51^{d}$ | $63.0^{b}$ | $66.6^{ab}$ | $69.6^{a}$ | $1.50^{b}$ | $1.53^{abc}$ | $1.56^{ac}$ |
| Menonita | $2.62^{a}$ | $2.99^{a}$ | $2.80^{a}$ | $62.6^{b}$ | $65.6^{b}$ | $67.5^{b}$ | $1.49^{b}$ | $1.52^{c}$ | $1.54^{b}$ |
| Teporaca | $2.57^{ab}$ | $2.67^{b}$ | $2.29^{ed}$ | $64.2^{a}$ | $67.0^{a}$ | $69.3^{a}$ | $1.51^{a}$ | $1.54^{ab}$ | $1.56^{ac}$ |
| Karma | $2.48^{ab}$ | $2.60^{b}$ | $2.33^{cde}$ | $63.4^{ab}$ | $67.5^{a}$ | $70.0^{a}$ | $1.50^{ab}$ | $1.54^{a}$ | $1.56^{c}$ |
| Cevamex | $2.35^{b}$ | $2.66^{b}$ | $2.57^{bd}$ | $64.2^{a}$ | $66.2^{b}$ | $68.2^{b}$ | $1.51^{a}$ | $1.53^{bc}$ | $1.55^{b}$ |
| Cuauhtémoc | $2.61^{a}$ | $2.61^{b}$ | $2.53^{bcd}$ | $63.2^{ab}$ | $66.1^{b}$ | $68.4^{b}$ | $1.50^{ab}$ | $1.53^{bc}$ | $1.55^{ab}$ |
| Babícora | $2.62^{a}$ | $2.70^{b}$ | $2.59^{bda}$ | $62.6^{b}$ | $66.2^{b}$ | $67.7^{b}$ | $1.49^{b}$ | $1.53^{bc}$ | $1.54^{b}$ |

[1, 2, 3] Correspond to boot stage, dough stage and hard grain stage, respectively

Means within columns with different superscripts are different ($P< 0.05$)

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# EVALUATION OF WINTER CEREALS FOR PASTURE IN MONTANA

**A. N. Hafla[1], S. D. Cash[1], L.M.M. Surber[1], J. A. Paterson[1], A. W. Lenssen[2], A. L. Todd[1], M. S. Huffman[1]**
[1]Department of Animal and Range Sciences; Montana State University, Bozeman, MT 59717.
[2]Northern Plains Agricultural Systems Research Unit, USDA-ARS, Sidney, MT 59270.

**ABSTRACT:** In the southern Great Plains, it is common to graze winter wheat pastures prior to grain harvest to take advantage of economic returns from the grain crop and value added to livestock. In Montana, a similar management practice could provide complementary pasture for livestock in the late spring to relieve pressure on cool season native rangelands. A two year study was conducted to evaluate the forage yield and quality of winter cereals, when grazed at three growth stages, vegetative (V), boot stage (B), and at heading (H), prior to hay and grain harvest. Western white faced ewes were used to graze plots at each date. Ungrazed forage yield and quality was measured at each date and on each date following grazing. 'Willow Creek' winter wheat (*Triticum aestivum* L.) and 'Trical 102' triticale (X*Triticosecale* Wttm.) were planted in the fall of 2005, using best management practices for grazing experiments in 2006. For the 2007 trial, only winter wheat was evaluated. In 2006, available forage yield of wheat and triticale did not differ ($P = 0.37$) at V, however triticale had higher yields ($P \leq 0.05$) at B, H, and at haying. Following grazing by ewes, hay yields were reduced in wheat by 30, 54, and 75% and in triticale by 21, 66, and 86% when grazed at V, B, and H, respectively. Forage utilization by ewes of wheat and triticale were similar at V and H, but wheat was lower ($P \leq 0.05$) than triticale at B. Forage quality and nitrate concentrations were highest at V, and declined similarly in wheat and triticale through B. Ungrazed wheat at H had higher ($P \leq 0.05$) 48 h in situ dry matter disappearance (ISDMD), CP, and DM and lower NDF and ADF than triticale. In 2007, forage yield of ungrazed wheat at haying was 12.92 t/ha. Hay yield losses for wheat were 34, 85, and 65% for grazed forage at V, B and H, respectively. Forage utilization by ewes was 66, 62, and 55% respectively. Forage quality was highest at V, and declined through haying. These results indicate that spring grazing of winter cereals in an integrated crop livestock system should occur during the vegetative state. Cereal pasture at this stage had excellent forage quality and grazing had limited impacts on subsequent hay yields.

Key Words: Ewes, Forage quality, Grazing, Winter wheat

## Introduction

Annual cereals harvested as hay have become a valuable source of livestock feed and gained popularity as an alternative feed source to traditional hays due to their forage quality and yield (Todd et al., 2007). Cereal forages provide a relatively high protein source for livestock and produce a high total dry matter yield.

Winter wheat is often used in the southern Great Plains as pasture for stocker cattle, to maximize returns by providing producers with income from grain and cattle enterprises (Christiansen et al., 1989; Holman et al., 2005; Winter and Thompson 1990). Winter cereals grown in Montana have several advantages when compared to spring seeded cereals. Planting in the fall allows forage harvest to occur earlier than spring cereal forage, and can help reduce spring workloads for producers who have livestock and crop enterprises. Additionally, winter cereals generally have greater forage production potential due to their better water use patterns (Cash et al., 2007). Very little literature is available regarding impacts of livestock grazing on subsequent forage yield of winter cereals. A better understanding of spring grazing of winter cereals in Montana is necessary to identify management practices to maximize livestock utilization while minimizing subsequent forage yield reduction at hay harvest. The objective of this study was to assess the spring grazing potential of winter cereals under dryland conditions in Montana by evaluating forage yield, forage quality, and grain yield when grazed by ewes at three growth stages.

## Materials and Methods

*Research Sites and Animals.* In a two year grazing study, Western White faced ewes were used to evaluate grazing effects on forage yield, quality, and grain yield, on plots of winter cereal cultivars. 'Willow Creek' winter wheat and 'Trical 102' triticale were evaluated. These cultivars are tall, early maturing, and have been developed for forage use in this region. Crops were planted in the fall of the years prior to each study using best management practices for grazing experiments at the Fort Ellis Research and Teaching Farm near Bozeman, MT. Temporary grazing cells of 24 $m^2$ were constructed in a uniform area within each field and each cell was randomly assigned a treatment (grazing date) and a replication (2006 r = 3, 2007 r = 4). Both wheat and triticale were evaluated in 2006, and only wheat was evaluated in 2007. Grazing dates varied by year according to stage of maturity and were imposed at 14-d intervals to include the vegetative stage (**V**) (late May), boot stage (**B**) (early June), and heading (**H**) (mid June). Four to six ewes (depending on forage availability) were randomly assigned to respective cells at each grazing date and allowed to graze the forage to a height of approximately 10 cm.

*Measurements.* Forage biomass was monitored throughout the season from V until grain harvest. Total available forage yield was measured by 0.5 $m^2$ clip samples taken from ungrazed cells at each grazing date and at

haying. Clip samples were taken from the inside of cells immediately following grazing to estimate forage utilization at each date. Grazing cell locations were maintained through the season and repeated clip samples were taken from grazed cells at 14-d intervals to evaluate forage regrowth at each grazing date following grazing and at haying. All forage yield estimates were calculated on a DM basis following drying 96 h in a forced air drying oven at 40º C. Grain yield was determined for both ungrazed cells and regrowth from each grazing date by a 0.5 $m^2$ clipping taken at grain maturity. The clip sampled at grain maturity was threshed to determine grain yield. Ungrazed forage samples at each grazing date and at haying were analyzed for forage quality. Regrowth forage was sampled at 14-d intervals following grazing and at haying for forage quality analysis. Forage samples were ground through a 5-mm screen in a Wiley mill and analyzed for 48 h in situ dry matter disappearance (**ISDMD**) (Van Soest et al., 1991). The unused remainder of each sample was ground through a 1-mm screen and analyzed for DM, N, nitrate concentration (**$NO_3$-N**) (AOAC, 2000), NDF and ADF (Van Soest et al., 1991).

*Statistical Analysis.* The experimental design was a completely random design with grazing cells considered the experimental units. Ungrazed and regrowth forage yield, quality parameters, and grain yield for 2006 were analyzed by ANOVA at each date. Means for each variable were compared by t-test at $P = 0.05$. Data for biomass ungrazed and regrowth forage yield, quality parameters, and grain yield in 2007 were reported as mean and standard error for each date. Grazing dates were considered fixed effect.

## Results and Discussion

*Forage Yield and Utilization.* In 2006, forage yield measured from ungrazed cells of wheat and triticale did not differ ($P = 0.37$) at V; however triticale had significantly higher yields ($P \leq 0.05$) at B, H, and at haying (Table 1.). Forage utilization by ewes of wheat and triticale were similar at V and H, but wheat utilization was lower ($P \leq 0.05$) than triticale at B (Table 1.). Forage yield regrowth when measured at haying was higher ($P \leq 0.05$) for triticale when grazed at V. Following grazing by ewes, hay yields were reduced in wheat by 30, 54, and 75% and in triticale by 21, 66, and 86% when grazed at V, B, and H, respectively. In 2007, forage yield of ungrazed wheat at haying was 12.9 t/ha (Table 2.). Forage utilization of wheat by ewes was 66, 62, and 55% when grazed at V, B and H respectively and hay yield losses were 34, 85, and 65% for grazed forage. During both years, grazing at V caused minimal loss of subsequent forage yield. Low moisture levels were likely the cause of limited regrowth occurring in cells grazed at or after B. While winter wheat used for pasture in the southern Great Plains is most often grazed in the fall, results of many studies still suggest grazing occur at the vegetative stage to minimize grain yield losses at harvest (Dunphy et al., 1983; Epplin et al., 1999)

*Forage Quality and Nitrate Concentration.* Ungrazed forage quality and nitrate concentrations were highest at V in 2006, and declined similarly in wheat and triticale through B (Table 3.). At B, wheat had superior ($P \leq 0.05$) forage quality compared to triticale, with higher 48 h ISDMD and CP, and lower NDF and ADF concentrations; however $NO_3$-N was higher in wheat at this stage (Table 3). When measured at haying, winter wheat had higher 48 h ISDMD and CP, but was similar to triticale in all other quality parameters and $NO_3$-N. When grazed at V and B, wheat regrowth maintained higher ($P \leq 0.05$) CP until haying (Table 3.). Additionally, wheat grazed at V and B maintained higher ($P \leq 0.05$) 48 h ISDMD than triticale when measured at haying (Table 3). Ungrazed forage quality and $NO_3$-N of Willow Creek winter wheat in 2007 was highest at V; and declined through haying (Table 4). In 2006 and 2007, grazing delayed forage maturity and increased forage quality parameters and $NO_3$-N concentrations when compared to winter cereals of the same date that had not been previously grazed.

*Grain Yield.* In 2006, grain yields for ungrazed triticale were higher ($P \leq 0.05$) than winter wheat grain yields (data not reported). Both cultivars experienced similar grain yield losses when grazed at V ($P = 0.71$), B ($P = 0.12$) and H ($P = 0.44$) (data not reported). Average grain yield losses were 17, 38, and 39% when grazed at V, B, and H, respectively. In 2007, grain yield losses of wheat were 34, 44, and 100% at V, B, and H respectively.

*Conclusions.* The results of this grazing study suggest that spring grazing of winter cereals should occur at the vegetative stage. Cereal pasture at this stage had excellent forage quality and forage yield losses measured at haying were minimal. Our results suggest that early grazing delays maturity and increases forage quality of regrowth. Using winter cereals in an integrated crop-livestock system in Montana and the northern Great Plains could provide livestock producers with more flexible management options. Fall seeding would potentially lighten the spring workload for many producers who have both cattle and crop enterprises. Harvesting winter cereals for hay that have been grazed in the spring would give producers the opportunity to take advantage of delayed forage maturity which causes increased forage quality. Additionally, allowing lactating livestock to graze winter cereal pasture during spring would reduce pressure on native cool season ranges, reserving forage for later in the season.

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Table 1. Forage biomass, utilization, and regrowth yield of winter cereals in 2006.

| | Julian date | | | |
|---|---|---|---|---|
| Item | 139 d | 153 d | 167 d | 186 d (Haying) |
| Ungrazed forage yield, t/ha | | | | |
| Triticale | 0.2[a] | 1.1[b] | 3.3[c] | 8.5 |
| Winter wheat | 0.1[a] | 0.3[b] | 1.5[c] | 4.0 |
| *P*-value | 0.37 | 0.01 | 0.01 | 0.01 |
| Forage utilization by ewes[1], t/ha | | | | |
| Triticale | 0.2[a] | 0.4[b] | 2.2[c] | - |
| Winter wheat | 0.1[a] | 0.2[b] | 0.6[c] | - |
| *P*-value | 0.25 | 0.00 | 0.08 | - |
| Regrowth forage after grazing, t/ha | | | | |
| Triticale grazed at Vegetative | - | 0.6[a] | 2.3[b] | 6.6 |
| Winter wheat grazed at Vegetative | - | 0.1[a] | 0.7[a] | 2.8 |
| *P*-value | - | 0.02 | 0.04 | 0.05 |
| Triticale grazed at Boot | - | - | 0.4[a] | 2.9 |
| Winter wheat grazed at Boot | - | - | 0.2[a] | 1.9 |
| *P*-value | - | - | 0.16 | 0.00 |
| Triticale grazed at Heading | - | - | - | 1.2[a] |
| Winter wheat grazed at Heading | - | - | - | 1.0[a] |
| *P*-value | - | - | - | 0.39 |

[1] Forage remaining after grazing on that date.
[a] V = vegetative, [b] B = boot, and [c] H = heading, designates growth stage.

Table 2. Forage biomass, utilization, and regrowth yield of Willow Creek winter wheat in 2007.

| | Julian date | | | |
|---|---|---|---|---|
| Item | 151 d | 165 d | 179 d | 194 d (Haying) |
| Ungrazed forage, t/ha (SE) | 3.2 (0.18)[a] | 4.1 (0.36)[b] | 9.8 (1.36)[c] | 12.9 (2.04) |
| Utilization by ewes[1], t/ha (SE) | 1.2 (0.15)[a] | 1.8 (0.10)[b] | 5.5 (1.96)[c] | - |
| Regrowth forage after grazing, t/ha (SE) | | | | |
| Grazed at 151 d | - | 1.5 (0.19)[a] | 4.4 (0.29)[c] | 8.5 (0.99)[c] |
| Grazed at 165 d | - | - | 1.6 (0.13)[a] | 1.9 (0.46)[b] |
| Grazed at 179 d | - | - | - | 4.5 (0.87)[a] |

[1] Forage remaining after grazing on that date.
[a] V = vegetative, [b] B = boot, and [c] H = heading, designates growth stage.

Table 3. Forage quality parameters of winter cereals in 2006.

| Item | Julian date | ISDMD[1,2], % | $NO_3$-N, ppm[1] | CP, %[1] | NDF, %[1] | ADF, %[1] |
|---|---|---|---|---|---|---|
| | | Forage quality parameters | | | | |
| Ungrazed forage | | | | | | |
| Triticale (Vegetative) | 139 d | 89.8 | 24.1 | 23.1 | 37.8 | 20.4 |
| Winter wheat (Vegetative) | 139 d | 89.6 | - | 27.8 | 40.1 | 22.0 |
| *P*-value | | 0.89 | - | - | 0.07 | 0.01 |
| Triticale (Boot) | 153 d | 81.8 | 29.1 | 19.0 | 47.4 | 24.7 |
| Winter wheat (Boot) | 153 d | 86.8 | 74.6 | 29.4 | 44.4 | 22.3 |
| *P*-value | | 0.02 | 0.01 | 0.01 | 0.02 | 0.02 |
| Triticale (Heading) | 167 d | 66.6 | 6.9 | 11.5 | 60.9 | 33.2 |
| Winter wheat (Heading) | 167 d | 78.1 | 46.1 | 19.9 | 53.4 | 26.6 |
| *P*-value | | 0.00 | 0.07 | 0.00 | 0.01 | 0.01 |
| Triticale (Haying) | 186 d | 52.1 | 7.2 | 10.2 | 58.5 | 36.4 |
| Winter wheat (Haying) | 186 d | 60.8 | 25.2 | 14.3 | 59.7 | 38.9 |
| *P*-value | | 0.02 | 0.13 | 0.03 | 0.68 | 0.45 |
| Regrowth forage grazed at Vegetative | | | | | | |
| Triticale | 153 d | 86.7 | 88.6 | 26.6 | 46.8 | 24.3 |
| Winter wheat | 153 d | 89.5 | 109.7 | 30.5 | 43.3 | 22.0 |
| *P*-value | | 0.09 | 0.08 | 0.00 | 0.05 | 0.09 |
| Triticale | 167 d | 67.8 | 24.1 | 14.9 | 56.2 | 30.8 |
| Winter wheat | 167 d | 80.8 | 61.0 | 24.0 | 49.8 | 27.3 |
| *P*-value | | 0.01 | 0.00 | 0.01 | 0.04 | 0.13 |
| Triticale | 186 d | 51.2 | 6.5 | 10.3 | 63.5 | 35.6 |
| Winter wheat | 186 d | 63.5 | 34.8 | 15.7 | 59.9 | 30.1 |
| *P*-value | | 0.00 | 0.00 | 0.01 | 0.04 | 0.26 |
| Regrowth forage grazed at Boot | | | | | | |
| Triticale | 167 d | 81.6 | 87.6 | 27.1 | 62.5 | 42.1 |
| Winter wheat | 167 d | 85.7 | 112.4 | 29.1 | 60.8 | 41.7 |
| *P*-value | | 0.07 | 0.05 | 0.01 | 0.15 | 0.76 |
| Triticale | 186 d | 62.3 | 25.5 | 13.7 | 62.2 | 33.3 |
| Winter wheat | 186 d | 68.7 | 53.1 | 18.3 | 57.3 | 28.7 |
| *P*-value | | 0.03 | 0.06 | 0.05 | 0.01 | 0.00 |
| Regrowth forage grazed at Heading | | | | | | |
| Triticale | 186 d | 70.3 | 27.7 | 17.6 | 55.0 | 30.5 |
| Winter wheat | 186 d | 75.0 | 57.9 | 21.4 | 54.3 | 29.5 |
| *P*-value | | 0.39 | 0.11 | 0.31 | 0.83 | 0.64 |

[1] All values reported on a DM basis
[2] 48 h in situ dry matter disappearance.

Table 4. Forage quality parameters of Willow Creek winter wheat in 2007.

| Item | 48 hr ISDMD, %[1,2] | $NO_3$-N, ppm[1] | CP, %[1] | NDF, %[1] | ADF, %[1] |
|---|---|---|---|---|---|
| | Forage quality parameters | | | | |
| Ungrazed forage, mean(SE) | | | | | |
| Julian date 151 d (Vegetative) | 79.7 (0.82) | 60.0 (4.26) | 25.5 (0.71) | 45.1 (0.25) | 22.6 (0.35) |
| Julian date 165 d (Boot) | 68.6 (0.88) | 60.0 (3.31) | 16.6 (0.50) | 55.9 (1.30) | 28.6 (0.51) |
| Julian date179 d (Heading) | 51.8 (0.15) | 71.3 (3.33) | 11.2 (0.67) | 64.8 (0.50) | 36.0 (0.51) |
| Julian date 194 (Haying) | 57.4 (1.86) | 51.3 (8.63) | 9.3 (0.44) | 56.9 (2.24) | 31.3 (1.74) |
| Regrowth forage grazed at V[a], mean(SE) | | | | | |
| Julian date 165 d | 69.5 (2.50) | 68.9 (7.61) | 20.0 (1.11) | 53.0 (1.12) | 27.6 (0.51) |
| Julian date 179 d | 61.6 (1.35) | 86.7 (14.20) | 15.1 (1.43) | 62.0 (1.31) | 32.6 (0.75) |
| Julian date 194 d (Haying) | 57.4 (0.83) | 45.6 (7.97) | 10.4 (0.38) | 58.3 (1.36) | 31.6 (1.26) |
| Regrowth forage grazed at B[b], mean(SE) | | | | | |
| Julian date179 d | 60.9 (1.77) | 53.1 (6.74) | 11.9 (0.54) | 59.1 (0.91) | 30.8 (0.73) |
| Julian date 194 (Haying) | 65.2 (1.86) | 68.9 (6.54) | 18.3 (1.53) | 55.1 (1.10) | 28.3 (1.22) |
| Regrowth forage grazed at H[c](SE) | | | | | |
| Julian date 194 (Haying) | 49.4 (1.81) | 62.5 (5.76) | 8.2 (0.41) | 65.9 (0.75) | 41.7 (0.62) |

[1] All values reported on a DM basis
[2] 48 h in situ dry matter disappearance.
[a] V = Vegetative, [b] B = Boot, [c] H = Heading, designates growth stage.

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# NUTRITIONAL IMPLICATIONS OF FATTY ACID COMPOSITION OF MARROW OF FOUR BONES FROM GRASS-FED CATTLE

**D. C. Rule[1], C. M. Murrieta[1], K. Fulton[2], and L. Cordain[2]**
[1]Department of Animal Science, University of Wyoming, Laramie, WY 82071
[2]Department of Health and Exercise Science, Colorado State University, Fort Collins, CO, 80523

**ABSTRACT:** Bone marrow could be a source of beneficial fatty acids. Previous studies on bone marrow from cattle and several wild ruminant species did not report on fatty acids of current importance. We hypothesize that marrow fatty acids of grass-fed cattle will contain high proportions of n-3 fatty acids, CLA, and vaccenic acid, and will vary according to bone type and location. Marrow was obtained from proximal, medial, and distal femur, humerus, radius, and tibia of four crossbred steers harvested at 28 mo of age that had grazed a mixture of cool season grasses and legumes. Marrow total lipids were extracted, transesterified with methanolic KOH, and fatty acid methyl esters analyzed by GLC. Data were analyzed as a split plot using the MIXED procedure of SAS. No interactions ($P > 0.42$) between bone and location within bone were observed. Main effects of location within bone were observed only for weight percentage of 18:1*t*11, which was lowest ($P = 0.03$) for medial compared with either distal or proximal locations. For femur vs. humerus, as well as for radius vs. tibia, no differences ($P > 0.05$) in fatty acid profiles were observed. However, monounsaturated fatty acids (14:1, 16:1, 17:1, 18:1*c*9, and 18:1*c*11) were lower ($P = 0.01$ to 0.05) and saturated fatty acids (16:0, 17:0, and 18:0) were greater ($P = 0.01$ to 0.05) for femur and humerus vs. radius and tibia suggesting that marrow lipids of bones located within the core of the animal had higher proportions of saturated fatty acids and lower proportions of monounsaturated fatty acids than bones at the extremities. Overall proportions of 18:3 n-3 and 18:1t11 were 0.60% and 2.50%, respectively. Rumenic acid (18:2*c*9, *t*11) was greater ($P = 0.02$) for radius and tibia (1.32%) than for femur and humerus (0.86%). We conclude that bone marrow fatty acids are more unsaturated in the extremities than in the core and that n-3 and CLA fatty acids are within the magnitude of meat lipids of grass-fed beef.

Key Words: Bone Marrow; Fatty Acids; Grass-Fed Cattle

## Introduction

Fat was once a limited commodity; consequently marrow was eagerly sought by hunter gatherers (Madrigal and Blumenschine, 2000) because it represented a high fat food. Currently, the types of fatty acids have a more important role in determining coronary heart disease (CHD) risk than the total amount of fat in the diet (Laaksonen et al., 2005). Without knowledge of bone marrow fatty acid composition conclusions regarding its capacity to influence CHD risk are not possible. Although fatty acid studies of bone marrow date to 1908 (Nerking, 1908), the full component of fatty acid isomers in marrow was not fully resolved due to measurement limitations inherent in the technology of the era. Nevertheless, marrow had a healthful fatty acid profile as indicated by high concentrations of 18:1*c*9 (oleic acid; 43 – 78 % of total fatty acids) and 18:0 (stearic acid; 14 – 21 %). More current studies have examined marrow fatty acids in caribou (Meng et al., 1969; Soppela and Nieminen, 2001), dall sheep (West and Shaw, 1975) and desert big horn sheep (Turner, 1979). Consistent with earlier studies of bovine marrow (Mello et al., 1976; Miller et al., 1982), marrow in the wild ungulates contained high concentrations of 18:0 and MUFA; however, values for CLA and *trans*-fatty acid isomers were not reported. Accordingly, a need exists to make comprehensive measurements of the full fatty acid spectrum in bovine marrow to determine its potential as a source of healthful dietary animal fats. Moreover, grass-fed beef retailers include bone marrow as one of their byproduct foods. Our hypothesis suggests that marrow fatty acids of grass-fed cattle will contain high proportions of n-3 fatty acids, CLA (rumenic acid), and vaccenic acid (18:1*t*11), and will vary according to bone type and location. Our objective was to determine the fatty acid composition of marrow of humerus, femur, tibia, and radius, as well as in the proximal, medial, and distal location within each bone, of four grass-fed steers.

## Materials and Methods

Bones were sampled from four crossbred steers harvested at 28 months of age. Steers had grazed a mixture of cool season grasses and legumes. The mixture contained smooth brome, meadow brome, orchard grass, festuolium, manska pubescent wheatgrass, Garrison creeping foxtail, and alfalfa. Bones were cut with a band saw during fabrication into thirds to represent the proximal, medial, and distal sections of the humerus, femur, tibia, and radius. Approximately 50 mg of marrow from each from each side was weighed into a 16 x 125 mm tube containing 1.0 mg of glyceryl-tridecanoate as internal standard. Samples were subjected to direct transesterifcation using 0.2 *M* methanolic-KOH as catalyst (Murrieta et al., 2003); water was used instead of saturated sodium chloride during extraction of fatty acid methyl esters (**FAME**) with 2 mL

of hexane. Fatty acid methyl esters were separated with a GLC (Agilent Technologies, Santa Clara, CA) equipped with a 100 m capillary column (SP-2560, Supelco, Inc., Bellefonte, PA) and flame ionization detector. Identification of individual FAME was accomplished using commercially available standards (Matreya, Inc., Pleasant Gap, PA), which were quantified using ChemStation Software (Agilent Technologies, Santa Clara, CA). Data were analyzed by using the MIXED procedure of SAS for a split-plot designed experiment as outlined by Kaps and Lamberson (2004). The model included bone, location within bone, carcass side, and the bone by location interaction; carcass side and side by bone interaction were used as random effects; and LS means were reported.

## Results and Discussion

Main effects of location within bone indicated lower ($P$ = 0.03) weight percentage of 18:1*t*11 in medial bone compared with either distal or proximal bone locations (2.37, 2.51, 2.62, respectively). No other effects ($P$ = 0.08 to 0.99) of location within bone were observed for other FAME. Fatty acid weight percentages of marrow of the four bones are shown in Table 1. Results indicate that *cis*-MUFA and SFA were affected by bone location. However, for radius vs. tibia fatty acids, as well as for femur vs. humerus fatty acids, profiles were quite similar. For the *cis*-MUFA (14:1, 16:1, 17:1, and 18:1) weight percentages of both radius and tibia marrow were greater ($P$ = 0.01 to 0.05) than for femur and humerus marrow. The opposite effect was noted for marrow SFA (16:0, 17:0, and 18:0) wherein weight percentages were greater ($P$ = 0.01 to 0.05) for femur and humerus than for radius and tibia. Weight percentages of vaccenic acid were greater ($P$ = 0.02) for femur and humerus compared with radius and tibia. On the other hand, rumenic acid was lower ($P$ = 0.02) for femur and humerus compared with radius and tibia.

Average weight percentage of marrow vaccenic acid was 2.50% for the current study, which would provide more of this fatty acid than typical meat sources, unless the cattle or sheep were supplemented with dietary fat. For example, 18:1 *trans* fatty acids were less than 1% in lipids of longissimus muscle of forage fed beef cattle (Rule et al., 2002), but in muscle of lambs fed supplemental vegetable oil, weight percentage of vaccenic acid was over 3% (Bolte et al., 2002). Average weight percentage of 18:3 n-3 was 0.60%, which is typical for most beef muscle lipids. However, grass-fed beef has been reported to be much greater than this (Baublits et al., 2006; Mann, 2005). Weight percentage of CLA was greater ($P$ = 0.03) in extremity bone marrow than of marrow of bones within the core of the animal. This response to bone region was similar to that observed for the MUFA and SFA, suggesting that delta-9 desaturase activity could be lower in the marrow of core region bones.

Additional evaluation of data in Table 1 suggests that marrow of bones located further out toward the extremities had a different fatty acid profile than marrow of bones located within the core of the animal. Data were re-analyzed such that marrow of radius and tibia were considered the extremities while marrow of the femur and humerus were considered the core. Total *cis*-MUFA (14:1, 15:1, 16:1, 17:1, and 18:1) weight percentage for marrow of the extremities was greater ($P$ = 0.03) than marrow of the core bones (53.7 vs. 43.4), whereas total SFA (14:0, 15:0, 16:0, 17:0, and 18:0) of the extremities was lower ($P$ = 0.02) than for the core bones (38.6 vs. 48.6). This observation suggests that desaturation of SFA was greater in the bones of the extremeties. A limited number of studies have examined the fatty acid concentration bone marrow in caribou (Meng et al., 1969; Soppela and Nieminen, 2001), dall sheep (West and Shaw, 1975) and desert big horn sheep (Turner, 1979). The marrow in these wild ungulates contained high concentrations of 18:0 and MUFA (particularly 18:1*c*9); however, values for CLA and *trans*-fatty acid isomers were not reported. Additionally data from these wild North American animals showed that the relative degree of saturation decreases distally in both the front and rear legs, perhaps as a result of increasing proximal to distal body temperatures (Petrakis, 1966).

We conclude that bone marrow is a rich source of fatty acids in grass-fed beef, some of which deem healthful and of current biomedical importance. Conjugated linoleic acid, vaccenic acid, and 18:3 n-3 occur at proportions expected in meat lipids of grass-fed and feedlot finished beef; however, bone type influenced the level of CLA. There appears to be greater desaturation of SFA in the marrow of bone of the extremities; thus, CLA development from desaturation of vaccenic acid would be less in femur and humerus than in radius and tibia.

## Implications

Bone cross-sections are currently sold by grass-fed beef, and likely other meat retailers. The marrow is used as an ingredient in a number of recipes, which call for a fat source to enhance texture and flavor. Results of this study clearly show that there is no difference in n-3 fatty acids between the long, heavy core bones and the lighter bones of the extremities. However, weight percentages of conjugated linoleic acid were greater in the extremity bones than in the heavier core bones. Moreover, saturated fatty acids were higher and monounsaturated fatty acids were lower in the heavier core bones, which would impact the nutritional quality of this fat source.

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Table 1. Effects of bone type on marrow fatty acid weight percentages

| Fatty acid[1] | Femur | Humerus | Radius | Tibia | SEM[2] | *P*-value[3] |
|---|---|---|---|---|---|---|
| 14:0 | 2.65 | 2.72 | 2.40 | 2.63 | 0.17 | 0.62 |
| 14:1 | 0.30[a] | 0.28[a] | 0.59[b] | 0.55[b] | 0.03 | 0.01 |
| 15:0 | 0.83 | 0.87 | 0.72 | 0.75 | 0.03 | 0.11 |
| 15:1 | 0.27 | 0.29 | 0.23 | 0.24 | 0.02 | 0.29 |
| 16:0 | 26.40[b] | 26.73[b] | 23.92[a] | 24.49[a] | 0.45 | 0.05 |
| 16:1 | 2.55[a] | 2.28[a] | 4.32[b] | 4.08[b] | 0.26 | 0.02 |
| 17:0 | 1.23[b] | 1.24[b] | 0.91[a] | 0.99[a] | 0.05 | 0.04 |
| 17:1 | 0.42[a] | 0.36[a] | 0.77[b] | 0.70[b] | 0.03 | 0.01 |
| 18:0 | 16.38[b] | 18.03[b] | 9.26[a] | 11.08[a] | 0.66 | 0.01 |
| 18:1*t9* | 0.05 | 0.06 | 0.05 | 0.04 | 0.004 | 0.32 |
| 18:1*t10* | 0.11 | 0.11 | 0.11 | 0.11 | 0.007 | 0.80 |
| 18:1*t11* | 2.83[b] | 2.76[b] | 2.16[a] | 2.26[a] | 0.08 | 0.02 |
| 18:1*c9* | 38.04[a] | 37.04[a] | 46.14[b] | 43.44[b] | 1.01 | 0.02 |
| 18:1*c11* | 2.62[b] | 2.39[a] | 3.20[b] | 3.18[b] | 0.13 | 0.05 |
| 18:2*c9,12* | 1.30 | 1.33 | 1.29 | 1.31 | 0.05 | 0.96 |
| 18:3*c9,12,15* | 0.64 | 0.59 | 0.57 | 0.61 | 0.02 | 0.41 |
| 18:2*c9,t11* | 0.91[a] | 0.81[a] | 1.33[b] | 1.31[b] | 0.07 | 0.02 |
| Unk <16:0 | 0.73 | 0.81 | 0.58 | 0.62 | 0.05 | 0.10 |
| Unk 16:0-18:0 | 1.35 | 1.22 | 1.36 | 1.47 | 0.10 | 0.48 |
| Unk >18:0 | 0.35 | 0.10 | 0.06 | 0.13 | 0.10 | 0.32 |
| Total, mg/g | 879.21 | 949.64 | 928.71 | 893.33 | 30.96 | 0.47 |

[1]Fatty acids denoted as number of carbon atoms:number of double-bonds.
[2]n-4.
[3]Means in a row with different superscripts are different.

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# PERENNIAL FORAGE KOCHIA FOR IMPROVED PRODUCTIVITY OF GRASS DOMINATED WINTER GRAZING PASTURES

**L.K. Greenhalgh[1], K.C. Olson[2], D.R. ZoBell[1], B.L. Waldron[3], A.R. Moulton[1], B.W. Davenport[4]**

[1]Utah State University Cooperative Extension, UMC 4815, Logan, Utah 84322
[2]South Dakota State University, West River Ag Center, 1905 N. Plaza Blvd, Rapid City, South Dakota 57702-9302
[3]USDA-ARS Forage and Range Research Lab, Logan, Utah 84322
[4]USDA-NRCS, 185 N. Main, Tooele, Utah 84074

**ABSTRACT: G**razing forage kochia (*Kochia prostrata*) during fall/winter has been shown to improve livestock health and reduce winter feeding costs. The objectives of this study were to compare the differences of traditional winter pastures versus pastures with forage kochia. Fifty mature, pregnant, Black Angus crossbred cows were body condition scored (BCS) and randomly divided into two groups of 25 head. One group was placed in a forage kochia/crested wheatgrass (*Agropyron desertorum)* pasture, the other (control) group in a crested wheatgrass/cheatgrass (*Bromus tectorum*) pasture. Both groups were placed in the pastures on 10/02/07 and removed on 01/03/08. Upon removal, cows were combined in one group and condition scored. Forage availability measured by the double sampling method showed the control and study pastures to contain 496 kg/ha and 3370 kg/ha of forage respectively. Crude protein was 2.5 and 9.8 percent for crested wheatgrass and forage kochia respectively. Estimated carrying capacities were 0.66 AUM/ha for the control and 4.46 AUM/ha for the forage kochia pasture. Initial (control = 5.33, kochia = 5.09) and final (control = 5.64, kochia = 5.63) BCS were similar ($P > 0.10$) among treatment groups. Although not statistically significant ($P = 0.12$), there was a trend for greater increase in BCS for the kochia pasture (+ 0.54) compared to the control pasture (+ 0.31). Overall, this study found that both pastures had adequate forage to maintain body condition; however, carrying capacity was almost seven times greater in the forage kochia pasture than the crested wheatgrass pasture. This study further indicates that winter grazing can be enhanced by including forage kochia as one of the plant components.

**Key Words:** beef cattle, body condition score, perennial forage kochia

## Introduction

DelCurto and Olson (2000) and Hathaway (2003) reported that winter feed costs is one of the major challenges for beef producers in the Western United States. One alternative is finding ways to maximize low-quality forage utilization by cattle, but minimize the use of extensive supplements. Arthun et al. (1992) reported that one way to do this is to include forbs and shrubs in low-quality forage-based diets to reduce protein supplementation, and Otsyina et al. (1984) reported that shrubs are particularly important in winter grazing systems. Improving winter grazing is important economically because it can reduce costs associated with feeding stored hay (Waldron et al., 2006; Gade and Provenza, 1986; Waldron, 2004).

Forage kochia (*Kochia prostrata*) has been shown to be good forage for livestock, especially during the fall and winter grazing seasons (Waldron et al., 2006; Otsyina et al., 1984; Stevens et al., 1985). During winter, dormant grasses are high in energy (fiber) but low in protein (Waldron et al., 2006; Cook, 1972). Simultaneously, shrubs such as forage kochia are low in energy and high in protein (Waldron et al., 2006; McKell et al., 1990). Therefore combining grasses and shrubs can optimize protein and energy levels by meeting microbial crude protein requirements of 7% (Van Soest, 1994) during nutritionally stressful times (Arthun et al., 1992). Maximizing the amount of energy utilization will also increase reproductive efficiency (Dunn et al., 1969; Selk et al., 1988).

Reported benefits of forage kochia prompted researchers and local entities to conduct a study in Tooele County of traditional winter pastures versus pastures with forage kochia. The objectives were to assess the differences between forage production, forage quality, carrying capacity and body condition score (BCS).

The site in Tooele Valley was first grazed in the mid 1800's and became overcrowded and overgrazed. In 1929, the first dust storm was reported and conditions continued to deteriorate to the point that dust storms made life unbearable and hazardous in the area. In 1934, the worst of these storms finally brought about action (Helm and Quate, circa 1980). The area was fenced and excluded from grazing. The land was eventually given to the Grantsville Soil Conservation District and Tooele Army Depot and seeded with crested wheatgrass. Restoration efforts and more careful grazing management eliminated dust storms.

This area of the Tooele Valley is still important winter grazing land. It is sensitive to overgrazing and subject to wildfire that could seriously threaten the surrounding communities. For these reasons it is imperative to maintain proper grazing which reduces fuel loads and thus the likelihood of devastating fires. Forage kochia planted in strips can serve as effective fire-breaks (Harrison et al., 2002, Newhall et. al., 2004), and help

prevent blowout areas (Newhall et. al., 2004, Stevens et. al., 1984; Rasmussen et al., 1992).

## Materials and Methods

*Site Information.* The Tooele Valley ecological site (lat 40º 34' 16.91" N, long 112° 24' 25.74" W) classification is Semi-Desert Alkali Loam (Black Greasewood). Elevation is 1311 to 1615 meters. The average annual precipitation is 254 to 305 mm, mean air temperature 7 to 11° C and the average frost free period is 110 to 140 days. (USDA Soil Survey of Tooele Area). Based on this description, average total dry weight forage production should be 732 kg/ha.

*Kochia Establishment.* Thirty-two hectares in Tooele Valley were prepared by disking in November 2004 and seeded with forage kochia in January 2005 at a rate of 2.25 kg/ha of pure live seed. In the spring of 2005 the forage kochia was observed to have germinated but because of an especially wet spring was overshadowed by crested wheatgrass and was hard to detect.

*Forage Evaluation.* Forage production was estimated using the double sampling method described by (Herrick et al., 2005). Ten 0.89 $m^2$ subplots were randomly selected for each pasture. Samples were taken on 10/27/08, six days before cattle began grazing. In three subplots, each species was clipped and bagged separately. Air dry weight in grams was recorded at least one week later. Species weights in all subplots were estimated ocularly. A correction factor was applied to each estimated weight based on the total clipped weight divided by the total estimated weight. Multiplying the estimate of the grams from each 0.89 $m^2$ plot by 11.2 produces an estimate for kg/ha.

Samples of forage kochia and crested wheatgrass were collected and analyzed for crude protein.

*Cattle Performance.* On 11/02/07, fifty mature, pregnant, Black Angus crossbred cows were visually body condition scored and randomly divided into two groups of 25 head. One group was placed in a forage kochia/crested wheatgrass (*Agropyron desertorum)* pasture, the other (control) group was placed in a crested wheatgrass/cheatgrass (*Bromus tectorum*) pasture. On 03 January 2008 both groups were gathered, combined and given a final condition score. The same person did initial and final scoring.

*Statistical Analysis.* Body condition score and forage availability data were analyzed using SAS.

## Results and Discussion

*Kochia Establishment.* It was hoped that the forage kochia pasture would be ready to graze in the late fall of 2006. However, forage kochia establishment did not proceed as expected and it was determined to wait until the fall of 2007 to graze. In the summer of 2007, it was apparent that there would be enough forage kochia to proceed with the study in the fall.

*Forage Evaluation.* Forage availability showed the control and study pastures to contain 496 kg/ha and 3370 kg/ha of forage respectively (Table 1). This correlates with Waldron et al., (2006) results that showed forage kochia greatly increases the yield potential of western rangelands.

Table 1. Forage Availability (kg/ha)

| Species | Control | Kochia |
|---|---|---|
| *Agropyron desertorum* | 461 | 186 |
| *Bromus tectorum* | 24 | 79 |
| *Kochia prostrata* | | 3065 |
| *Poa bulbosa* | | 29 |
| *Poa secunda* | 11 | 11 |
| Total | 496[a] | 3370[b] |

Values with different letters are significantly different ($P < 0.001$).

Average crude protein value was greater for forage kochia than for crested wheatgrass (Table 2). This also is in agreement with previous studies which show crude protein to be consistently higher in forage kochia than in dormant grasses (Waldron et al., 2006, McKell et al., 1990).

Table 2. Average Crude Protein (%)

| Crested Wheatgrass | Forage Kochia |
|---|---|
| 2.5 | 9.8 |

Carrying capacities were 0.66 AUM/ha for the control and 4.46 AUM/ha for the forage kochia pasture (Table 3). This nearly seven fold increase in carrying capacity is significant from a management and economical standpoint.

Table 3. Difference in AUM/hectare

| Site | Control | Kochia |
|---|---|---|
| Tooele Valley | 0.66 | 4.46 |

*Cattle Performance.* Body condition scores were similar for the two groups of cattle both initially and finally (Table 4). While not statistically significant, there was a trend for greater increase in BCS for the kochia pasture compared to the control pasture (Table 4).

Table 4. Differences in body condition score

| Treatment | Initial | Final | Change |
|---|---|---|---|
| Control | 5.33 | 5.64 | 0.31 |
| Kochia | 5.09 | 5.63 | 0.54 |
| P – Value | 0.29 | 0.97 | 0.12 |

## Implications

The findings of this study are in agreement with previous research on forage kochia. Both quality and availability of forage increased for the forage kochia pasture. In this particular study both pastures had adequate forage to maintain body condition; but, carrying capacity was almost seven times greater in the forage kochia pasture than the crested wheatgrass pasture.

One of the interesting aspects of forage kochia is its ability to thrive in dry, harsh climates. The large majority of forage kochia forage production took place in the spring and summer of 2007 – a year with considerably lower than average precipitation and higher than average temperatures. Overall, we can conclude that winter grazing is enhanced when forage kochia is one of the plant components.

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# ESTIMATING LIVESTOCK FORAGE DEMAND: DEFINING THE ANIMAL UNIT (AU)

**T.L. Meyer[1], D.C. Adams[1], T.J. Klopfenstein[2], J.D. Volesky[1], L.A. Stalker[1], and R.N. Funston[1]**
[1]West Central Research and Extension Center, North Platte, NE, and [2]University of Nebraska-Lincoln

**ABSTRACT:** The definition of an animal unit varies between publications with many assuming an animal's daily intake is 2.6% of BW. The objective of this experiment was to evaluate the effect of a beef animal's physiological state on forage intake. The experiment was repeated over two yr with six replications of three treatments: cow-calf pair (**CC**; BW = 649 kg), dry cow (**DC**; BW = 508 kg), and yearling steer (**S**; BW = 310 kg). The cow and calf were treated as one unit, with calves being approximately 42 d of age and weighing 73 kg at the start of each yr. Animals were housed in individual pens and fed high quality (11% CP) meadow hay ad libitum daily. Daily diet samples were composited by week and analyzed for OM, IVDMD, NDF, and UIP. Refusals were collected, composited by week per pen, and analyzed for OM, IVDMD, and NDF. Refusals were also composited for each yr by pen and evaluated for UIP. All data were analyzed using the MIXED procedure of SAS. Dry matter intake was different ($P < 0.01$) among treatments. Daily DM intake was 16.4, 11.7, and 6.6 kg for CC, DC, and S, respectively; 2.5, 2.3, and 2.1% as %BW for CC, DC, and S, respectively; and 15.6, 13.5, and 10.8 $\%BW^{.75}$ for CC, DC, and S, respectively. In addition, OM intake, IVDMD intake and NDF intake ($P < 0.01$) were different among treatments. Our results indicate intake differences among cattle of different physiological state or class should be considered when calculating forage demand for stocking rate or feeding purposes.

**Keywords:** animal unit, forage intake

## Introduction

Grazing is a vital component of beef cattle production. The term, animal unit (**AU**), or more commonly, animal unit month (**AUM**), is commonly utilized in grazing management strategies. Various definitions for the terms AU, animal unit day (**AUD**), AUM, and animal unit yr (**AUY**) exist; but they all have one common theme – define forage intake on the basis of a standard animal. Across a variety of publications, general consensus is the standard animal consumes about 2.6% of their BW on a DM basis.

Scarnecchia and Kothmann (1982) defined the animal unit as a unit of animal demand equivalent to approximately 11.8 kg DM/day. An animal with a demand rate more or less than 11.8 kg DM/day will have an animal unit equivalent which is a proportionate fraction or multiple of one animal unit. An AUM would then be defined as 354 kg DM and an AUY equal to 4,245 kg.

Scarnecchia (1985) suggested the animal unit concept should be calculated based only on animal related factors, including weight, lactation, gestation, and other animal factors which affect animal demand. Defining the standard animal used in the animal unit is where variation occurs.

In popular and extension publications, deviations of the AU definition occur. Waller et al., (1986) and Ohlenbusch and Watson (1994) defined an animal unit as a 454 kg cow of above average milking ability with a calf less than 3-4 mo postpartum. This AUM forage value was given as 308 kg of forage dry matter (10.3 kg = 1 AUD; 340-354 kg air dry). Redfearn and Bidwell described an animal unit simply as a 454 kg cow with calf, with no age of calf given. Reynolds et al., (2001) defines an animal unit as 0.10 ×45.4 kg of animal weight; then uses the Waller et al. (1986) definition for one AU, but defines a 454 kg cow, non-lactating, as only 0.9 AU. Reynolds et al. (2001) also assigns 0.50 AU for yearling cattle, ages 7 to 12 mo; and 0.75 for yearling cattle, ages 12 to 17 mo. The Society for Range Management (1989) and Iowa State University (1998) consider an animal unit one mature cow of about 454 kg, either dry or with calf up to 6 mo, or their equivalent, based on a standardized amount of forage consumed, 11.8 kg of forage a day (DM basis). Gerrish and Roberts (1999) define an animal unit as a 499 kg cow without calf, 1.4 yearling cattle or 5 non-lactating ewes, with an AUM considered to be roughly 454 kg of forage dry matter.

Allison (1985) listed body size, physiological status, body condition, supplementation, forage preference, forage availability, and grazing systems as factors affecting intake. The factor accounted for in many of the animal unit definitions is body size, with physiological status being the most erratic factor in defining an animal unit. Therefore, the objective of this experiment was to evaluate the effect of a beef animal's physiological state on forage intake.

## Materials and Methods

*Facilities.* This project was replicated over two yr, with yr 1 (**Y1**) located at the University of Nebraska Gudmundsen Sandhills Laboratory (**GSL**), near Whitman, NE (elevation 3,520 ft, lat 42°05' N, long 101°26' W) and yr 2 (**Y2**) at the University of Nebraska West Central Research and Extension Center (elevation 2,284 ft, lat 41°08' N, long 100°77' W), North Platte, NE.

*Animals, Design, and Treatments.* All animal procedures were approved by the University of Nebraska Institutional Animal Care and Use Committee. The experiment was repeated over two yr with six replications of three treatments each yr: cow-calf pair (**CC**; BW = 649 kg; $BW^{.75}$ = 105 kg), dry cow (**DC**; BW = 508 kg; $BW^{.75}$ = 88 kg), and yearling steer (**S**; BW = 310 kg; $BW^{.75}$ = 61 kg). The cow and calf

were treated as one unit, with calves averaging 42 d and weighing 73 kg at the start of the experiment each yr.

*Diet.* Animals were offered hay harvested from sub-irrigated meadows at GSL. Tables 1 and 2 provide the analysis of hay supplied. Hay was weighed and offered daily in amounts to allow *ad libitum* intake. Dry matter was determined from samples collected daily and composited within week. Refusals from each pen were weighed weekly and dry matter determined in Y1 and daily in Y2.

*Data Collection.* At the beginning, middle, and end of each trial, all animals were weighed for three consecutive days. Diet and refusal samples were dried in a forced air oven for 48 hr at 60°C. Daily diet and refusal samples were composited by week. All samples were then ground to pass through a 2-mm screen, with a subsample ground to pass through a 1-mm screen.

*Sample Analysis.* Laboratory analyses consisted of DM, OM, IVDMD, NDF and UIP. Ruminally fistulated cows fed a basal diet of meadow hay provided inoculant for IVDMD, as well as in situ incubation. *In vitro* dry matter disappearance was determined using the Tilley and Terry (1963) method modified by the addition of 1 g/L of urea to McDougall's buffer (Weiss, 1994). The IVDMD was then adjusted to better reflect *in vivo* digestibility as described by Geisert et al. (2006).

*In situ* incubations were replicated using two bags per sample per ruminally fistulated cow, providing 4 bags per sample. Dacron bags (5×10 cm; Ankom Inc., Fairport, NY) with an average pore size of 50 µM were filled with 1.25 g of dried composited refusal sample ground to 2 mm. Incubation times included 0 hours and 27 hours. Following incubation, bags were hand washed (39°C) for five cycles consisting of agitation and rinsing. Bags were then refluxed in a neutral detergent solution using a fiber analyzer (Ankom Inc., Fairport, NY) to remove microbial contamination (Mass et al., 1999), and dried for 48 hours at 60°C. Bags were weighed and then air-equilibrated, re-weighed, and residues were analyzed for N by combustion (AOAC, 1996; Leco FP-528, St. Joseph, MI).

*Statistical analysis.* Average daily intake during each wk of the experiment was analyzed as a repeated measure using the MIXED procedure of SAS with a first order autoregressive (**AR1**) covariance structure. An AR1 covariance structure was chosen because it minimized Akaike's information criterion. The model included the effects of treatment as a fixed effect and yr, wk, and trt by wk interaction as random effects. Individual animal or cow/calf pair was used as the experimental unit.

## Results and Discussion

Differences ($P < 0.001$) occurred among treatments for all variables analyzed as shown in Table 3.

Actual daily DMI was over 28% higher for CC when compared to DC and almost 60% higher when compared to S. When DMI is compared as %BW, CC still had an 8% greater intake than CC and 16% greater intake than S. According to data presented in the NRC (1996), maintenance requirements of lactating cows are approximately 20% higher than those of nonlactating cows. Patterson (2007) reported dry cows removed 28% less forage than pairs during the same time frame (August-November) as cow-calf pairs.

Data presented in NRC (1987) indicated voluntary intake in beef cows is similar to growing cattle when adjusted for the effect of milk production. One would then assume a dry cow and yearling steer would consume a similar percentage of BW, which was not the case in this experiment (2.3% vs. 2.1% for DC and S, respectively; $P < 0.001$).

Actual daily OMI was over 28% higher for CC when compared to DC and almost 60% higher when compared to S. Hollingsworth-Jenkins et al. (1995) measured intake of calves about the same age as the present study, and found they consume from 1.1 to 1.5 % of their BW on an OM basis. Lactating cows in the same study consumed 2.0 to 2.6% of their BW on an OM basis. These intakes are greater than the present study. Hollingsworth-Jenkins et al. (1995) also concluded nursing calves grazing native Sandhills summer range selects diets higher in rumen degradable protein than their dam. Results from research conducted by Ansotegui et al. (1991) indicated calves nursing low-milk-producing cows consume more forage than those calves nursing high-producing cows, but the increased forage consumption by the calf did not imply more forage was necessary for the low-producing cow and her calf.

Vanzant et al. (1991) reported OM intake was 17% greater for lactating heifers versus non-lactating controls approximately 26 d after parturition. There was no difference in ruminal fill and capacity between the two groups. When grazing native tallgrass prairie during the winter months, pregnant heifers approximately 2 mo before calving had greater intakes than control heifers. Lactation prompted an increase in intake and grazing time.

Differences in voluntary dry matter intake account for more than 50% of the variation in digestible nutrient consumption by ruminants (Allen, 1996). Consumption of less-digestible, low-energy (often high fiber) diets is controlled by physical factors such as ruminal fill and digesta passage, whereas consumption of highly digestible, high-energy (often low-fiber, high-concentrate) diets is controlled by the animal's energy demands and by metabolic factors (NRC, 1987).

In a review by Allison (1985) evidence is cited voluntary intake is limited by reticulo-rumen capacity and by rate of disappearance of digesta from this organ in predominantly forage diets. When ruminants are offered roughages such as hay and dried grass, evidence exists cattle and sheep eat to a constant rumen fill. Stanley et al. (1993) used late gestation and early lactation crossbred cows to monitor peripaturient changes in DMI, ruminal capacity and digestion and fermentation characteristics. They concluded increased passage rate was one way that increased nutrient demand is accommodated in the presence of decreasing ruminal capacity. Patterson et al. (2001) reported forage intake of bred heifers grazing Sandhills winter range from 2.1% decreasing to 1.3% as parturition approached.

Loy et al. (2004) demonstrated intake of primiparous heifers declined prior to calving and increased rapidly after parturition. Patterson et al. (2001) hypothesized advancing growth of the fetus and fluids reduce rumen volume prior to calving. Decreased rumen volume coupled with heifers'

higher nutritional requirements than mature cows puts them at risk for a negative energy balance during late gestation.

Comparing results of this experiment to predicted intake values given in popular and extension publications, differences are evident. A comparison of selected values is shown in Table 4. It would appear our intake values for a lactating cow are similar to the values offered by ISU (1998), SRM (1989), and Scarnecchia and Kothmann (1982). Many of the other values would over predict forage intake of a dry cow or yearling steer from this experiment. The definition Waller et al. (1986) provided accounts for a lactating cow but resembles the present study's dry cow intake, overestimating for a yearling steer based on our results. If values recorded for Hollingsworth-Jenkins et al. (1995) were on a DM basis they may appear similar to Gerrish and Roberts, (1999) intake values.

## Implications

Many estimates of livestock forage demand and AU definitions factor in the animal's weight. When determining pasture stocking rates for example, average mature cow weight can vary substantially from herd to herd and it is important to use the appropriate weight value. Our results indicate intake differences among cattle of different physiological state or class should be considered when calculating forage demand. This would further increase accuracy of forage demand estimates for stocking rate or feeding purposes.

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Table 1.Characteristics of hay fed to treatment animals during yr 1

| | Hay offered | Hay refused | Actual Diet |
|---|---|---|---|
| % Dry matter | 84.1 | 76.4 | -- |
| % Organic matter | 90.5 | 85.5 | 91.3 |
| % Neutral detergent fiber | 64.3 | 70.0 | 63.8 |
| % Crude protein | 11.6 | 10.5 | -- |
| % In vitro dry matter digestibility | 52.6 | 48.4 | 53.2 |
| Undegradable intake protein, % of CP | 40.8 | 46.4 | -- |

Table 2. Characteristics of hay fed to treatment animals during yr 2

| | Hay offered | Hay refused | Actual Diet |
|---|---|---|---|
| % Dry matter | 79.7 | 85.8 | -- |
| % Organic matter | 89.9 | 89.8 | 89.9 |
| % Neutral detergent fiber | 67.2 | 76.5 | 66.2 |
| % Crude protein | 10.7 | 10.2 | -- |
| % In vitro dry matter digestibility | 51.8 | 46.5 | 52.9 |
| Undegradable intake protein, % of CP | 44.9 | 53.2 | -- |

Table 3. Average BW, DM, OM, IVDMD, and NDF intake of cow-calf pairs, dry cows and steers

| | Cow-calf pair | Dry Cow | Steer | SE | P value |
|---|---|---|---|---|---|
| BW, kg | 649.0 | 508.0 | 310.0 | 19.55 | < 0.0001 |
| MBW, kg | 105.4 | 87.5 | 60.6 | 2.5 | < 0.0001 |
| DMI, kg | 16.4 | 11.7 | 6.6 | 0.38 | < 0.0001 |
| DMI, % of BW | 2.5 | 2.3 | 2.1 | 0.0006 | < 0.0001 |
| DMI, % of MBW | 15.6 | 13.5 | 10.8 | 0.003 | < 0.0001 |
| OMI, kg | 14.9 | 10.6 | 6.0 | 0.35 | < 0.0001 |
| OMI, % of BW | 2.3 | 2.1 | 1.9 | 0.0005 | < 0.0001 |
| OMI, % of MBW | 14.1 | 12.2 | 9.8 | 0.003 | < 0.0001 |
| IVDMD, kg | 8.7 | 6.2 | 3.5 | 0.22 | < 0.0001 |
| IVDMD, % of BW | 1.3 | 1.2 | 1.1 | 0.0004 | 0.0013 |
| IVDMD, % of MBW | 8.3 | 7.1 | 5.8 | 0.001 | < 0.0001 |
| NDF, kg | 10.6 | 7.6 | 4.3 | 0.24 | < 0.0001 |
| NDF, % of BW | 1.7 | 1.5 | 1.4 | 0.0004 | < 0.0001 |
| NDF, % of MBW | 10.1 | 8.7 | 7.0 | 0.002 | < 0.0001 |

Table 4. Comparison of suggested and actual intakes from selected publications

| Study | Animal description | Suggested Daily DMI(kg/ 454 kg) | Actual Daily DMI (kg/ 454 kg) | Daily DMI, %BW | Daily OMI, % BW |
|---|---|---|---|---|---|
| Present study | Lactating cow with calf < 3 months of age | -- | 11.4 | 2.5 | 2.3 |
| Present study | Dry cow | -- | 10.4 | 2.3 | 2.1 |
| Present study | Yearling steer | -- | 9.5 | 2.1 | 1.9 |
| Hollingsworth-Jenkins et al., 1995 | 103-231 kg nursing calf, approx. age 3-6 mo | -- | -- | -- | 1.1-1.5 |
| Hollingsworth-Jenkins et al., 1995 | Mid lactation cow, 454-540 kg | -- | -- | -- | 2.4-2.6 |
| Scarnecchia and Kothmann, 1982 | Animal not defined | 11.8 | -- | 2.6 | -- |
| Waller et al., 1986 | 454 kg cow, above average milking ability, with a calf < 3-4 mo postpartum | 10.3 | -- | 2.3 | -- |
| SRM, 1989; ISU, 1998 | Mature cow of about 454 kg, either dry or with calf up to 6 mo of age | 11.8 | -- | 2.6 | -- |
| Gerrish and Roberts, 1999 | 499 kg cow without calf | 15.1 | -- | 3.0 | -- |

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# EVALUATION OF ANNUALS FORAGES AS ALTERNATIVES TO NATIVE RANGE AS FALL-WINTER FORAGE IN SOUTH-CENTRAL NORTH DAKOTA

B. W. Neville[1], D. L. Whitted[1], P. E. Nyren[2], G. P. Lardy[1], and K. K. Sedivec[1]

[1]Department of Animal and Range Sciences, North Dakota State University, Fargo, ND
[2] Central Grasslands Research Extension Center, Streeter, ND

**ABSTRACT:** The objective of this study was to determine the effects of grazed annual forage type on beef cow performance during fall and early winter grazing in North Dakota. One-hundred fifty-nine mature pregnant beef cows stratified by initial BW (533.6 ± 42.3 kg) and initial BCS (5.29 ± 0.41) were assigned to graze one of four treatment forages: 1) foxtail millet; 2) turnips; 3) a forage mix (**café**) consisting of turnip, forage radish, cowpea, soybean, sunflower, and foxtail millet; or 4) native range (which was the control). Annual forages were seeded on 13-July. Stocking rates were determined based on measured forage production and estimated utilization. Cows were allowed to graze from 16-October to 27-November. Cow BW increased 0.9 ± 0.16 $kg \cdot hd^{-1} \cdot d^{-1}$; however, these increases did not differ ($P$ = 0.29) between treatments. There were increases in BCS for cows grazing café and foxtail millet when comparing initial and final BCS (5.27 vs. 5.63; $P$ = 0.004 and 5.30 vs. 5.57; $P$ = 0.05 for café and foxtail millet, respectively); however, there were no differences between treatments for final BCS ($P$ = 0.31) or change in BCS ($P$ = 0.10). Calculated costs for grazing were $0.75, 0.83, 1.80, and 1.27 $hd \cdot d^{-1}$; respectively for foxtail millet, turnips, café, and native range. Much of the increased cost for grazing the café mix of annuals was due to inclusion of soybeans and cowpeas which increased seeding costs by $28.88/ha. Use of more cost effective legume species will likely increase the cost effectiveness of these forage mixtures. Given that both the foxtail millet and turnips produced more forage than café; producers could benefit from increased stocking rates when utilizing these forage crops in their livestock production systems. Annual forage mixes, like the café treatment, show promising results when considering beef cow performance; however work is needed to decrease planting costs.

**Key words:** Annual forages, Beef Cows, Winter Grazing

## Introduction

Livestock producers continually try to extend the grazing season with the knowledge that extending grazing reduces feed costs (D'Souza et al., 1990; Adams et al., 1994). Allowing cattle to graze stockpiled perennial forages decreased the amount of hay needed to maintain body condition (Hitz and Russell, 1998). Grazing annual forages is another such way to not only graze livestock longer into the fall or early winter, but also provides potentially higher quality forages. Brassicas, such as turnips, are one example of an annual forage that can be effectively grazed by sheep (Koch et al., 2002) or cows. A warm-season annual, foxtail millet has previously been evaluated as a standing, bale-fed, or swath-grazed forage (Munson et al., 1999) for wintering beef cows. More recently, forage mixtures often including warm-season annual grasses, legumes, and Brassicas have received more interest; not only because of benefits to cattle performance, but also possibly improving soil health. These benefits include reducing soil compaction and nitrogen scavenging and addition to the soil (Sustainable Agriculture Network, 2007). We know of no published literature comparing cow performance when mixtures of these species are grazed (turnips, foxtail millet, and annual grasses); therefore, our objective was to determine the effects annual forage type on beef cow performance under grazing conditions during the fall and early winter in North Dakota.

## Materials and Methods

One-hundred fifty-nine mature, pregnant Angus-Simmental cross beef cows were stratified by initial BW (533.6 ± 42.3 kg) and initial BCS (5.29 ± 0.41) and assigned randomly to graze one of four treatment forages from 16-October to 27-Novermber, 2007. At the beginning and end of the trial two day body weights and BCS (Wagner et al., 1988) were collected. Treatments were: 1) foxtail millet (*Setaria italica*); 2) turnips (*Brassica rapa* var. *rapa.*); 3) a forage mix (**café**) consisting of turnip (*Brassica rapa* var. *rapa*), forage radish (*Raphanus sativus*), cowpea (*Vigna unguiculta*), soybean (*Glycine max*), sunflower (*Helianthus annuus*), and foxtail millet (*Setaria italica*); or 4) standing dormant native range (which was the control). The most prevalent species on native range were previously described by Hirschfeld et al. (1996) and were blue grama (*Bouteloua gracilis*), needle and thread (*Heterostipa comata*), sunsedge (*Carex heliophila*), western snowberry (*Symphoricarpos occidentalis*), and Kentucky bluegrass (*Poa pratensis*).

This study was conducted at the Central Grasslands Research and Extension Center (CGREC) located in south central North Dakota, approximately 14 km NW of Streeter, ND. The study was located on T 138 North R 70 West, with grazing treatment pastures located on section 14 and native range pastures located on section 25. This region of North Dakota is near the eastern edge

of the Missouri Coteau, an area of young morainic hills formed from recent glaciation (Lura, 1985; Hirschfeld, 1996). Climate of the study area is characterized by seasonal variations in both temperature and precipitation. The south central region of North Dakota experiences approximately 120 frost-free days with a range of average high and low monthly temperature from –13.7ºC in January to 20.0ºC in July (Jensen, 1972; NDAWN, 2008). Mean annual precipitation of 44.1 cm is seasonal with over 70% occurring from May through September (30.9 cm; NDAWN, 2008).

***Forage establishment***

Seeding of annual forages occurred on July 13th. Seeding rates for foxtail millet and turnips were 22.4 and 3.9 kg/ha, respectively. The cafeteria treatment (**café**) pasture was seeded with the seed mixture containing 22.4, 16.8, 4.5, 1.1, 1.1, and 0.6 kg/ha for soybean, cowpea, foxtail millet, sunflower, radish, and turnip respectively. At time of seeding fertilizer 56 kg/ha (2/3 urea, 1/3 N 11: P 52) was applied. Rainfall events totaled 7.59, 10.03, 5.13, and 3.81cm/month for July, August, September, and October respectively (NDAWN, 2008); these totals were on average 1.75cm/month below the previous five year average for this area.

***Forage sampling***

Forage sampling for production data began 7-September and was conducted a total of twice prior to grazing. At each pre-grazing sampling date 10 0.25 $m^2$ plots were clipped per paddock, or 30 plots per treatment. Turnip plots were sorted for turnip tops and bulbs, as well as other forbs and grasses. Café plots were sorted by species contained within the mixture, as well as other grasses and forbs. Native range was split into grasses and forbs, while the foxtail millet was not separated due to the limited amount other species present. Forage samples were collected at the initiation of and then weekly throughout grazing period.

***Grazing***

All animal care and handling procedures were approved by the NDSU Institutional Animal Care and Use Committee prior to the initiation of the study. For the annual forage treatments, each treatment pasture was divided into three, 4 ha paddocks using electric fence, providing three (4 ha) replications for each annual grazing treatment. Electric cross fencing was used to limit access in an attempt to increase forage utilization. The first area grazed was immediately adjacent to water source, and cross fences were moved to allow access to water and previously grazed areas. Native range treatment groups (3 replicates) were allowed to graze entire pasture (16.6 ha) to simulate a typical fall-winter management scenario. Cattle grazing turnips were offered oat straw on a free-choice basis to prevent digestive upset commonly associated with Brassicas. Water was provided in stock tanks, which were filled daily. Stock tanks were heated with propane tank heaters to allow cattle constant access to water.

Stocking rates were determined based on forage production and estimated utilization. We estimated cattle would consume 70% of all forage in the café, 70% of the foxtail millet, 25% of the grasses and 15% of the forbs in the native range, and 90% of turnip foliage and 30% of turnip bulbs in the turnip paddocks.

***Laboratory Analysis***

Forage samples were dried using a forced-air oven (55° C; The Grieve Corporation, Round Lake, IL) for 48 h. Dried samples were ground using a Wiley Mill (Aurther H. Thomas Co., Philadelphia, PA) to pass a 2 mm screen. Forage samples were analyzed for DM, Ash, and CP (AOAC, 1990). Concentrations of NDF (Robertson and Van Soest, 1991, as modified by Ankom Technology, Fairport, NY) and ADF (Goering and Van Soest, 1970, as modified by Ankom Technology, Fairport, NY) were determined using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Samples were also analyzed for in vitro dry matter disappearance (IVDMD), and in vitro organic matter disappearance (IVOMD) using a modified procedure of (Tilley and Terry, 1963).

***Economic Comparison***

Rental and custom rates were used to calculate cost of forage establishment. The costs used were $34.65/ha and $99/ha for land rental of non-irrigated pasture and non-irrigated cropland, respectively (NASS, 2007a). Custom rates for tillage, spraying, and planting were determined from NASS (2007b).

***Statistical Analysis***

Cow performance data was analyzed as a completely random design using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The experimental unit was paddock, and treatment was forage type. Statistical analysis was conducted for differences in initial BW, initial BCS, final BW, final BCS, ADG, and BCS change. Initial BW was tested as a covariant and was not significant, thus it was removed from the model.

## Results and Discussion

***Forage Production and Composition***

Forage production at the time stocking rates were calculated was 2588, 5629, 2965, and 5866 kg/ha for café, foxtail millet, native range, and turnips respectively. However, certain species such as turnips continued to grow into the grazing season as evidenced by increased production numbers (data not presented). Percent composition of the café treatment pastures is presented in Table 1. At the time stocking rates were calculated, 4-October, average production was 25.5, 1208.0, 73.0, 56.4, 249.7, 259.3, and 716.0 kg/ha (DM basis) for cowpeas, foxtail millet, radish, soybean, sunflower, turnip tops, and turnip bulbs respectively. Desiccation and, to a lesser extent, grazing by wildlife decreased the amount of cowpeas, soybeans, and sunflowers present in the café pastures as the grazing season progressed. In fact, cowpeas had all but disappeared from the café treatment at the time grazing began; while soybean disappeared by

31-October. Other constituents such as radish and naturally occurring forbs also nearly disappeared by the end of the grazing season. The fact that cowpeas and soybeans disappeared prior to the beginning of or during the grazing season further illustrates that these species may not be well suited for this type of grazing or at least under the conditions present in this study. Further alterations in seeding rates, soil preparation, or fertilization could change these outcomes.

Forage quality at the beginning of the grazing season is presented in Table 2. Crude protein values for all forages were above the 7.0% CP requirement for mature gestating beef cows listed by NRC (1996). At the time of publication, laboratory analysis for forage samples collected during the grazing season had not been completed.

***Cattle Performance***

Initial BW was affected ($P = 0.005$) by treatment. This is likely due to cattle being stratified from a weight taken 5 d prior to the initiation of this study. Weights reported in this study did not include this weight but, rather the average of a two day weight taken one day prior to and the day of initiation of this study. Cow BW increased 0.9 ± 0.16 $kg \cdot hd^{-1} \cdot d^{-1}$; however, these ADG did not differ ($P = 0.29$) between treatments. While there were no treatment differences in ADG; this data indicates that any of these annual forages would be an acceptable alternative to grazing native range during the early winter. There were increases in BCS for café and foxtail millet grazed cows when comparing initial and final BCS (5.27 vs. 5.63; $P = 0.004$ and 5.30 vs. 5.57; $P = 0.05$); however, a difference between treatments for either final BCS ($P = 0.31$) or change in BCS ($P = 0.10$) could not be established. Interestingly, the foxtail millet and café grazed cattle had numerically lower ADG, but numerically greater change in BCS. The differences are likely due to nutritional differences between forages that occurred as the grazing season progressed. Differences between café and foxtail millet could be due, in part, to variability in forage DM between treatments, as well as the intake of oat straw for turnip grazing cows leading to increased gut fill.

***Economic Comparison***

Calculated costs for grazing forages were \$0.75, 0.83, 1.80, and 1.27 $hd \cdot d^{-1}$; respectively for foxtail millet, turnips, café, and native range. Much of the increased cost for grazing the café mix of annuals was incurred due to the inclusion of soybeans and cowpeas. These two forages increased seeding costs by \$28.88/ha, while providing little to the total forage production of the café paddocks. It is not known how much these legumes contributed to the production of the other annual species due to their ability to fix nitrogen. Further use of more cost effective legume species, such as red clover, may increase the cost effectiveness of these forage mixtures. Additionally, the application of this type of grazing in a double-cropping system, where a forage crop is removed prior to planting of the winter pasture forage could prove more economically beneficial.

**Implications**

Given that both the foxtail millet and turnips produced more forage than café and that there were no statistical differences observed in cow performance; producers could benefit from increased stocking rates when utilizing these annual forage crops in their livestock production systems. Annual forage mixes, like the café treatment, show promising results when considering beef cow performance; however work is needed to decrease the cost of planting these mixtures to make application more economical.

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Table 1. Species composition (% of total DM) of Café treatment pastures over time at Central Grasslands Research Extension Center, Streeter ND in 2007

| | Date | | | | |
|---|---|---|---|---|---|
| | 7-September[1] | 4-October[1] | 16-October[2] | 31-October[2] | 11-November[2] |
| Cowpea (%) | 1.8 | 1.5 | 0.3 | 0.0 | 0.1 |
| Foxtail Millet (%) | 45.0 | 52.5 | 45.8 | 57.0 | 30.0 |
| Other Forbs (%) | 13.5 | 3.8 | 2.0 | 1.0 | 0.0 |
| Radish (%) | 5.0 | 3.6 | 5.9 | 1.8 | 0.3 |
| Soybean (%) | 7.0 | 3.7 | 3.8 | 0.0 | 0.5 |
| Sunflower (%) | 13.7 | 8.3 | 6.4 | 10.8 | 5.6 |
| Turnip Tops (%) | 14.0 | 9.1 | 17.8 | 10.6 | 8.3 |
| Turnip Bulbs (%) | - | 17.5 | 18.1 | 18.8 | 55.3 |

[1] Samples collected prior to grazing (n=10/paddock).
[2] Samples collected during grazing study (n=3/paddock).

Table 2. Forage quality of annual forages and native range at the initiation of grazing (16-October) at Central Grasslands Research Extension Center, Streeter ND in 2007

| | Treatment[1] | | | |
|---|---|---|---|---|
| | Café[2] | Foxtail Millet | Native Range | Turnips |
| Crude Protein (%) | 10.13 ± 0.34 | 12.02 ± 0.36 | 8.15 ± 1.44 | 13.61 ± 1.96 |
| NDF (%) | 41.92 ± 3.16 | 61.74 ± 1.93 | 65.26 ± 2.85 | 21.58 ± 0.95 |
| ADF (%) | 23.83 ± 1.62 | 33.02 ± 1.10 | 36.16 ± 0.76 | 16.98 ± 1.23 |
| IVDMD (%) | 74.18 ± 1.18 | 64.72 ± 1.86 | 50.59 ± 0.44 | 87.00 ± 1.05 |
| IVOMD (%) | 58.53 ± 5.13 | 63.65 ± 1.59 | 50.80 ± 0.90 | 87.70 ± 0.50 |
| Ca (%) | 1.46 ± 0.45 | 0.45 ± 0.05 | 0.54 ± 0.10 | 1.47 ± 0.33 |
| P (%) | 0.38 ± 0.01 | 0.25 ± 0.02 | 0.15 ± 0.01 | 0.38 ± 0.03 |

[1] Weighted means (mean ± standard deviation).
[2] When insufficient sample was present to analyze for the value of interest an average of the remaining values was used.

Table 3. Performance of beef cows grazing annual forages and native range at Central Grasslands Research Extension Center, Streeter ND in 2007

| | Café | Foxtail Millet | Native Range | Turnips | SE | Treatment *P*-value |
|---|---|---|---|---|---|---|
| Initial BW, kg | 534.6[ab] | 537.4[a] | 530.9[b] | 530.9[b] | 1.01 | 0.005 |
| Initial BCS | 5.27 | 5.30 | 5.38 | 5.22 | 0.04 | 0.15 |
| Final BW, kg | 572.0 | 569.0 | 570.6 | 574.1 | 4.34 | 0.85 |
| Final BCS | 5.63 | 5.57 | 5.47 | 5.48 | 0.06 | 0.31 |
| ADG, kg | 0.88 | 0.75 | 0.94 | 1.03 | 0.10 | 0.29 |
| ΔBCS | 0.36 | 0.26 | 0.10 | 0.26 | 0.06 | 0.10 |

[abc] Means are different at $P < 0.05$.

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# IN VITRO DIGESTIBILITY OF BROMEGRASS HAY AS AFFECTED BY ADDITION OF CRUDE GLYCERIN

**V. Nayigihugu, P. L. Price, and B. W. Hess**
Department of Animal Science, University of Wyoming, Laramie, WY, 82071

**ABSTRACT:** An in vitro experiment was conducted to determine the effect of adding different levels of crude glycerin on fermentation characteristics and digestibility of bromegrass hay. Triplicate in vitro filter bags containing 0.5 g bromegrass hay (7.19% CP, 39.83% ADF, 69.15% NDF; DM basis) or crude glycerin (25 w/w% glycerol) replacing bromegrass at 7.5, 15, or 30% of the substrate were incubated for 2, 4, 8, 12, 24, and 48 h. At each time point, a 10-mL of ruminal fluid was acidified with 7.2 N $H_2SO_4$ and frozen for later VFA and $NH_3$ analyses. After 48 h incubation, bags were rinse in tepid distilled water and dried in a 55$^o$ C forced air oven for 48 h to determine apparent DMD. Bags were then rinsed in NDF solution to determine true DMD and rumen degradable protein (RDP). Inclusion of crude glycerin in the substrate linearly decreased ($P \leq 0.05$) apparent DMD at 0, 4, 8, 24, and 48 h after incubation, but the rate of apparent DMD did not differ ($P = 0.23$) among treatments. True DMD did not differ ($P = 0.65$) among treatments at any of the incubation time points, although the rate of true DMD decreased ($P = 0.05$) as crude glycerin levels increased. Addition of crude glycerin did not affect ($P \geq 0.20$) extent or kinetics of NDF disappearance. After 8 and 12 h of incubation, RDP decreased ($P = 0.04$) linearly primary due to reduced RDP for the 30% crude glycerin treatment. Although fraction A was similar ($P = 0.16$) among treatments, fraction B was greater and fraction C was less with the inclusion of 30% crude glycerin. Crude glycerin addition did not affect total ($P = 0.42$) or molar proportions ($P \geq 0.44$) of individual VFA or $NH_3$ concentrations ($P = 0.98$) in the in vitro fluid. Replacing up to 30% of dietary forage with crude glycerin (25 w/w% glycerol) may have minimal impacts on ruminal digestibility and fermentation in ruminants.

**Keywords:** crude glycerin, bromegrass hay, in vitro digestibility

## Introduction

Glycerol, a liquid substance of sweet taste and high energy concentration (Fisher et al., 1971; 1973; Sauer et al., 1973), is a co-product of the biodiesel industry. Crude glycerin may have benefits for ruminant animals, because glycerol can be converted to glucose by the liver (Krebs et al., 1966) and kidneys (Krebs and Lund, 1966) providing energy for cellular metabolism. However, previous studies on ruminal metabolism of glycerol have indicated that glycerol is extensively fermented in the rumen (Kijora et al., 1998). Khalili et al. (1997) reported that glycerol addition of 36 g/ kg of barley silage decreased the molar proportion of acetate and increased molar proportions of propionate and butyrate in the rumen of dairy cows. Schröder and Südekum (1999) concluded that crude glycerin can substitute up to 10% of readily fermentable DM in mixed diets fed to ruminants without compromising digestibility. Limited information is available on level of crude glycerin inclusion in forage-based diets. Therefore, the objective of this experiment was to evaluate the effect of adding various levels of crude glycerin on fermentation characteristics and digestibility of bromegrass hay.

## Materials and Methods

An in vitro experiment was conducted to determine the effect of various levels of crude glycerin on fermentation characteristics and digestibility of bromegrass hay. Treatments included bromegrass hay without crude glycerin or crude glycerin (25 w/w% glycerol) replacing 7.5, 15, and 30% of the hay substrate. Treatment mixtures were thoroughly blended by hand to produce homogenous samples. Bromegrass mixtures were then analyzed for DM and ash (AOAC, 1990), N (LECO model FP-528 Nitrogen Determinator, LECO, St. Joseph, MI), ADF and NDF (Ankom 200, ANKOM Technology, Fairport, NY). Residues from ADF and NDF were then analyzed for total N, as previously described, to estimate acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN), respectively. The difference between NDIN and ADIN was computed to estimate $B_3$ fraction. In vitro filter bags (Ankom F57; 25 μm pore size; ANKOM Technology, Faiport, NY) were pre-rinsed in acetone for 5 min to remove surfactants that may inhibit microbial digestion and triplicate bags containing 0.5 g of each treatment mixture were incubated in 1 of 4 jars (ANKOM Technology, Fairport, NY) randomly assigned to 1 of 3 Daisy$^{II}$ incubators (ANKOM Technology, Fairport, NY) resulting in 3 replications per treatment. A set of filter bags for each treatment were submerged in a non-inoculated media, which constituted the 0 h time point. The remaining in vitro filter bags were placed into corresponding jars that contained pre-warmed McDougall's buffer that was inoculated with ruminal fluid (2:1 ratio) collected from 2 cows consuming bromegrass hay. After 2, 4, 8, 12, 24, and 48 h of incubation, triplicate filter bags and 1 blank bag were serially removed from each jar and immediately frozen.

At each sampling point, 10 mL of in vitro fluid was collected and acidified with 7.2 *N* $H_2SO_4$ and frozen for later VFA and $NH_3$ analysis. In vitro fluid was analyzed for VFA concentration (Goetsch and Galyean, 1983) using a Hewlett-Packard 5850 gas liquid chromatography (Hewlett Packard, Avondale, PA) equipped with a 15-m x 0.53-mm

(i.d.) column (Nukol, Supelco, Bellafonte, PA). The initial oven temperature was 110°C and final temperature was 150°C with the rate of 8° C/min. Hydrogen was used as a carrier gas with a column flow rate of 20 mL/min. Injector and flame ionization temperature were 250°C. In vitro $NH_3$ concentrations were determined by the phenol-hypochlorite procedure (Broderick and Kang, 1980).

Filter bags were thawed, rinsed in tepid water until rinse water was clear, and dried in a 55°C force-air oven for 48 h to determine apparent DMD. Filter bags were then rinsed in NDF solution to correct for microbial contamination for determination of true DMD. Residues were then analyzed for total N to estimate RDP (Mass et al., 1999).

Rate of NDF disappearance was calculated using non-linear regression (Martens and Loften, 1980). The non-linear model of Orskov and McDonald (1970) was used to calculate protein fractions A, B, and C. All resulting data were then subjected to ANOVA using the GLM procedures of SAS (SAS Institute, Cary, NC) for a randomized complete block design. The block effect was incubator and the treatment effect was glycerol level. Fermentation pattern data were analyzed as a split-plot design. Treatment effects were tested using jar within treatment as the error term (error a). The time effect and treatment × time interaction were tested using the residual error term (error b). Single degree of freedom orthogonal contrast procedures were used to evaluate treatment differences (Steel and Torrie, 1980).

## Results and Discussions

Chemical composition of bromegrass-crude glycerin substrates is presented in Table 1. Südekum (2007) reported similar total tract digestibilities when 15% of different purity glycerol was added to a 40:60 forage to concentrate ration. In the current experiment, apparent and true DMD increased across incubation time (Table 2) which is indicative of microbial digestion. Inclusion of crude glycerin in the substrate linearly decreased ($P \leq 0.05$) apparent DMD after 0, 4, 8, 24, and 48 h and a quadratic decrease ($P = 0.02$) was observed after 8 h of incubation. Crude glycerin did not affect ($P = 0.23$) rate of apparent DMD. Although there was a numerical advantage of adding 30% crude glycerin to bromegrass hay, in vitro true DM disappearance did not differ ($P = 0.65$) among treatments at any incubation time point. Rate of true DM disappearance decreased ($P = 0.05$) as crude glycerin level increased. Addition of crude glycerin did not affect ($P \geq 0.27$) extent or kinetics of NDF disappearance (data not shown). Schröder and Südekum (1999) fed sheep 48, 78, 131, or 185 g/d of glycerol (DM basis) in a low starch, concentrate diet and found either no effect or positive effects on digestibility of OM, starch, and cell-wall components. They further reported, however, that feeding the same levels of glycerol in high-starch concentrate diets resulted in a decrease in cell-wall digestibility but did not affect digestion of OM or starch.

At the 8 h time point, RDP increased linearly ($P = 0.04$) from 0 to 30% crude glycerin treatment (Table 3). Addition of crude glycerin increased ($P \leq 0.01$) RDP at the 12 and 24 h incubation points. A quadratic ($P = 0.01$) response was observed at 12 and 24 h of incubation points. Addition of crude glycerin did not affect ($P = 0.16$) fraction A. Fraction B increased ($P = 0.003$) linearly from 0 to 30% crude glycerin. Fraction C exhibited a quadratic ($P = 0.03$) response because it increased with the addition of 7.5% crude glycerin and then decreased when crude glycerin replaced 30% of the substrate. Treatment × time interactions were not observed for $NH_3$ ($P = 0.70$) or total VFA ($P = 0.16$), (data not shown). In contrast to results reported by Khalili et al. (1997) and Schröder and Südekum (1999), crude glycerin addition did not affect $NH_3$ ($P = 0.98$), total VFA ($P = 0.42$) or molar percentages of individual VFA ($P \geq 0.44$), (data not shown).

We concluded that replacing up to 30% of dietary forage with crude glycerin (25 w/w% glycerol) may have minimal impacts on ruminal digestibility of forage-based diets.

## Implications

Crude glycerin may be included in diets of ruminants without affecting ruminal fermentation. However, additional studies are necessary to determine the optimum level of crude glycerin inclusion in forage-fed diets of ruminants.

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Table 1. Chemical composition of bromegrass hay with various levels of crude glycerin

| | Crude glycerin concentrations, % | | | |
|---|---|---|---|---|
| Item | 0 | 7.5 | 15 | 30 |
| DM, % | 94.71 | 93.89 | 91.65 | 87.79 |
| Ash, % of DM | 7.67 | 7.72 | 7.84 | 8.10 |
| NDF, % of DM | 69.15 | 65.19 | 63.53 | 55.51 |
| ADF, % of DM | 39.83 | 37.10 | 35.39 | 32.20 |
| CP, % of DM | 7.19 | 7.13 | 6.50 | 5.61 |
| ADIN, % of CP | 8.77 | 8.71 | 7.64 | 10.37 |
| NDIN, % of CP | 41.71 | 38.84 | 42.32 | 40.50 |
| B3 fraction | 32.94 | 30.13 | 34.68 | 30.13 |

Table 2. Apparent and true DM digestibility of bromegrass hay with various levels of crude glycerin

| | Concentration of glycerol, % | | | | | | Orthogonal contrasts | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 7.5 | 15 | 30 | SEM[1] | *P* | Linear | Quadratic | Cubic |
| Incubation time, h | | | | | | | | | |
| Apparent | | | | | | | | | |
| 0 | 17.0 | 17.0 | 14.8 | 14.8 | 0.6 | 0.05 | 0.02 | 0.94 | 0.15 |
| 2 | 19.6 | 18.5 | 16.7 | 15.5 | 0.6 | 0.008 | 0.48 | 0.79 | 0.52 |
| 4 | 20.5 | 20.1 | 17.9 | 15.9 | 0.5 | 0.002 | 0.0004 | 0.17 | 0.38 |
| 8 | 24.4 | 24.7 | 22.5 | 19.8 | 0.5 | 0.001 | 0.0003 | 0.02 | 0.38 |
| 12 | 31.3 | 28.4 | 27.8 | 23.3 | 1.5 | 0.05 | 0.01 | 0.61 | 0.40 |
| 24 | 39.6 | 37.4 | 37.0 | 34.2 | 0.8 | 0.01 | 0.003 | 0.70 | 0.27 |
| 48 | 49.9 | 50.8 | 48.1 | 45.7 | 0.9 | 0.03 | 0.008 | 0.11 | 0.37 |
| Rate, %/h | 4.3 | 2.1 | 3.3 | 2.8 | 0.01 | 0.23 | 0.33 | 0.24 | 0.15 |
| True | | | | | | | | | |
| 0 | 35.9 | 37.9 | 36.1 | 41.0 | 4.1 | 0.81 | 0.50 | 0.74 | 0.59 |
| 2 | 37.5 | 39.9 | 37.8 | 42.3 | 3.7 | 0.77 | 0.48 | 0.79 | 0.52 |
| 4 | 38.5 | 40.6 | 39.4 | 43.8 | 3.5 | 0.74 | 0.38 | 0.76 | 0.60 |
| 8 | 42.0 | 44.7 | 42.9 | 46.4 | 3.1 | 0.76 | 0.44 | 0.89 | 0.51 |
| 12 | 46.5 | 47.9 | 46.7 | 50.1 | 2.8 | 0.80 | 0.49 | 0.73 | 0.60 |
| 24 | 55.1 | 55.3 | 54.0 | 58.2 | 2.7 | 0.71 | 0.52 | 0.47 | 0.57 |
| 48 | 63.5 | 63.6 | 62.6 | 66.8 | 2.4 | 0.65 | 0.44 | 0.42 | 0.58 |
| Rate, %/h | 4.3 | 3.2 | 3.2 | 2.7 | 0.01 | 0.18 | 0.05 | 0.53 | 0.53 |

[1] n = 12



Table 3. Rumen degradable protein and protein fractions of bromegrass hay with various levels of crude glycerin

| | Concentration of glycerol, % | | | | | | Orthogonal contrasts | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 7.5 | 15 | 30 | SEM[1] | *P* | Linear | Quadratic | Cubic |
| Incubation time, h | | | | | | | | | |
| 0 | 53.9 | 61.6 | 59.0 | 58.5 | 3.4 | 0.50 | 0.48 | 0.27 | 0.45 |
| 2 | 62.4 | 65.3 | 68.2 | 65.9 | 2.6 | 0.51 | 0.29 | 0.35 | 0.67 |
| 4 | 62.5 | 66.9 | 70.8 | 70.1 | 2.9 | 0.26 | 0.08 | 0.41 | 0.75 |
| 8 | 68.9 | 72.3 | 72.9 | 74.5 | 1.5 | 0.16 | 0.04 | 0.59 | 0.58 |
| 12 | 72.0 | 75.0 | 76.3 | 75.2 | 0.6 | 0.01 | 0.006 | 0.01 | 0.77 |
| 24 | 76.9 | 78.3 | 80.1 | 83.4 | 0.7 | 0.003 | 0.001 | 0.24 | 0.79 |
| 48 | 84.0 | 84.8 | 84.7 | 86.8 | 1.6 | 0.67 | 0.30 | 0.72 | 0.69 |
| Fraction A | 61.1 | 64.0 | 54.6 | 60.1 | 0.03 | 0.16 | 0.32 | 0.64 | 0.05 |
| Fraction B | 24.0 | 20.2 | 27.9 | 30.9 | 0.02 | 0.01 | 0.003 | 0.05 | 0.04 |
| Fraction C | 14.9 | 15.8 | 17.5 | 9.1 | 0.02 | 0.05 | 0.09 | 0.03 | 0.22 |

[1] n = 12

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# EFFECT OF DRY DISTILLERS GRAINS PLUS SOLUBLES SUPPLEMENTATION LEVEL ON CHARACTERISTICS OF FORAGE INTAKE AND CHARACTERISTICS OF DIGESTION OF BEEF HEIFERS GRAZING WHEAT PASTURE

**A. Islas, M. Horvath, L. E. Camacho, and S. A. Soto-Navarro**

**New Mexico State Univ., Las Cruces 88003**

**ABSTRACT:** Sixteen ruminally cannulated mixed-breed heifers (378 ± 28.4 kg) grazing wheat pasture were used in a complete randomized design to evaluate effects of supplemental corn dry distillers grains plus soluble level (DDGS; 0, 0.2, 0.4, and 0.6% of BW) on intake, digestibility and rumen fermentation characteristics. The experiment was conducted during the second and third week of April. Heifers grazed in a single wheat pasture with supplements offered individually once daily at 0700. Forage and total OM, CP, and NDF intake were not affected ($P > 0.10$) by DDGS level. Digestibility of OM and CP were not affected ($P > 0.10$) by DDGS level. Digestibility of NDF increased (linear, $P < 0.01$) with increasing DDGS level. Rates of DM in situ disappearance for DDGS and wheat forage were not affected ($P > 0.10$) by DDGS level. In situ CP rate of disappearance of DDGS was not affected ($P > 0.10$) by DDGS level. However, in situ CP rate of disappearance of wheat forage decreased (quadratic, P = 0.06) with increasing DDGS level (5.37, 3.79, 2.26, and 3.90 ± 0.72 %/h for 0, 0.2, 0.4 and 0.6% of BW, respectively. The UIP from DDGS decreased (linear, $P = 0.08$) with increasing DDGS level (55.1, 48.6, and 47.8 ± 2.56% for 0.2, 0.4, and 0.6% of BW, respectively), but UIP from forage was not affected ($P = 0.35$) by increasing DDGS level. Ruminal concentration of acetate, propionate, butyrate, acetate to propionate ratio, ruminal pH, and total ruminal VFA production were not affected ($P > 0.10$) by increasing DDGS level. DDGS can be used in wheat pasture supplements without negatively affecting forage intake, digestibility, or ruminal fermentation.

Key Words: DDGS supplementation, digestion, wheat pasture

## INTRODUCCION

In the United States southern Great Plains, many wheat producers' income is derived from both grain crop and cattle production. Wheat pasture is generally available, from 120 to 150 d, usually in late fall, winter and early spring when other forage sources are low in quantity and quality. Weight gain of cattle grazing wheat forage adds value to stocker and mature cattle by increasing cattle price appreciation (Horn, et al., 2005). A common technique often used to increase ADG of cattle grazing wheat forage is supplementation of energy. Horn, et al. (1995) reported that supplementing cattle grazing wheat forage with high starch or high digestible fiber supplements can increase stoking rate and ADG as much as one third and 0.15 kg, respectively, compared with non-supplemented animals. Wheat pasture is an important resource of high-quality forage that contains over 20% CP and over 70% DM digestibility (Mader, and Horn, 1986; Branine and Galyean, 1990). However, wheat forage protein is highly degradable in the rumen, limiting energy availability for MCP and reducing availability MP (Klopfenstein, 1996; Creigton et al., 2003).

The ethanol industry is expanding rapidly and by-products such as corn dried distillers grains with solubles (**DDGS**) are becoming widely available (Renewable Fuels Association, 2005). Corn dried distillers grains with solubles are high in fiber (37 to 48% NDF, OM basis, Spiehs, 2002), undegradable intake protein (15 to 20%, DM basis), and fat content (8 to 12 %; MacDonald 2007). The supplementation of DDGS to cattle grazing wheat pasture might provide a more balanced nutrient supply. However, there is no information available on the effect of DDGS supplementation on characteristics of digestion and ruminal fermentation of cattle grazing wheat pasture. Therefore, the objectives of this experiment were to evaluate effects of level of DDGS supplementation on forage intake, digestibility, and rumen fermentation characteristics of beef heifers grazing wheat forage.

## MATERIALS AND METHODS

All procedures and experimental protocols were approved by the New Mexico State University Institutional Animal Care and Use Committee. Sixteen crossbred heifers (375 ± 28.4 kg) fitted with ruminal cannulas were used in a completely randomized design. Heifers were randomly assigned to 1 of 4 corn DDGS levels. The DDGS levels were: 1) control or not supplemented with DDGS (**0% BW**), 2) DDGS at 0.02% BW (**0.02% BW**), 3) DDGS at 0.04% BW (**0.04% BW**), and 4) DDGS at 0.06% BW (**0.06% BW**). The experiment consisted of a 15-d experimental period; the first 10 d were used for adaptation to wheat pasture grazing and supplement, and the last 5 d for sample collection. The experiment was conducted during early April. Heifers grazed a single wheat pasture (Pound Plus B; Kelly Green Seeds, Inc, Farwell, TX; wheat, triticale and oat mixture*; triticum aestivum*, *Triticosecale rimpaui* and *Avena sativa*, respectively), with

DDGS supplement offered individually once daily at 0700. Heifers were allowed access to their supplements for 30 min, after which uneaten supplement was placed into the rumen through the ruminal cannula.

***Collections***. Chromic oxide was used to estimate fecal output. Gelatin capsules containing chromic oxide (8 g) were dosed ruminally twice a daily (0700 and 1900) on d 7 through the 15-d of the experimental period. Feces from rectal grab samples were collected and prepared for DM, OM, CP, and chromium analysis.

Two mature cows, fed a grass hay diet, were ruminally evacuated in a holding pen at 1100 1 d before the experimental period. Digesta was place in plastic bags lining 133-L plastic containers. After evacuation cows were allowed to graze in the wheat pasture for 60 min. Cows were then gathered, and masticate samples were collected and dried in a forced- air oven (50°C) to a constant weight, and a 10% sub sample was kept to estimate forage in situ digestibility. The dry forage sub sample was ground in a Wiley Mill (2-mm screen), and composited on an equal dry weight basis within treatment.

In situ digestibility was determined for both ground composited forage and supplement. Five-gram samples were sealed, with an impulse sealer, into dacron bags (10 × 20 cm, 50 ± 15 µm pore size; Ankom, Fairport, NY). On d 12 to 15, composited forage in situ bags were ruminally incubated within nylon lingerie washing bags (30.5 × 25.4 cm) for 72, 48, 36, 24, 14, 9, 5, 2, and 0 h in all steers. 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 using the delicate cycle. The machine was filled with 45 L of cold water, bags were agitated for 1 min, and the machine was drained, and spun for 2 min. This cycle was repeated 5 times for all bags. Bags were dried in a forced-air oven at 50ºC, weighed, and stored at room temperature for analysis of DM, NDF (forage and supplement), CP and purines.

On d 11, CoEDTA (200 mL; Uden et al., 1980) was dosed intra-ruminally at 0700 for a marker of fluid passage rate. Ruminal fluid samples were collected at 0 (before dosing), 3, 6, 9, 12, 18, 24, 36, and 48 h after dosing. Ruminal fluid pH was determined immediately after collection and samples were then acidified with 7.2 $NH_2SO_4$ at a rate of 1 mL/100 mL rumen fluid and frozen (-10°C) in whirl pack bags for later analysis of Co, ammonia, and VFA. Also on d 11, Dy-labeled wheatgrass (850 g; Sindt et al., 1993) and Yb- labeled DDGS (650g) were intra-ruminally dosed at 0700 for marked of particle passage rate. Ruminal content samples were collected at 0 (before dosing), 3, 6, 9, 12, 18, 24, 36 and 48 h after dosing. Wheat forage was labeled with Dy as described by Sindt et al. (1993). Briefly, wheat forage was allowed to soak in a tub with 10 g of Dy and 3.3 L of distilled water/kg of wheat forage for 12 h at 25ºC. Excess marker solution was strained through 4 layers of cheese cloth. Tap water was added to the feed and pH was adjusted to 4.5 with HCL. Then feed plus water were allowed to soak for additional 6 h and were rinsed with tap water 4 times. During rinsing, excess tap water was strained through 4 layers of cheese cloth, and label wheat forage was dried in a force-air oven at 55ºC for 48h. The same procedure was followed to label DDGS but using Yb.

At 1900 on d 15 of the experimental period a 2 kg sub-sample of ruminal contents was obtained and mixed with 1 L of saline solution (0.9% NaCl; wt/vol) for isolation of bacterial cells (Zinn and Owens, 1986). Ruminal content samples were frozen (-10ºC) for later proximate analysis.

***Laboratory Analysis***. Fecal, supplement, and masticate samples were dried in a forced-air oven (50°C) for 48 h. Samples were then allowed to equilibrate at room temperature and ground in a Wiley Mill (2-mm screen). Fecal, masticate, and supplement samples were analyzed for DM, OM, and CP (Methods 930.15, 942.05, and 990.02, respectively; AOAC, 1997). Also, NDF analysis was performed according to Robertson and Van Soest (1991) using Ankom 200 fiber analyzer (Ankom Co., Fairport, NY).

Ruminal fluid samples were centrifuged at 27,000 × g for 10 min and analyzed for $NH_3$-N (Broderick and Kang, 1980), VFA (Goetsch and Galyean, 1983), and cobalt was determined using an air-plus-acetylene flame using atomic absorption spectroscopy as described by Uden et al. (1980). Ytterbium and Dysprosium were extracted as outlined by Hart and Poland (1984), and markers concentrations were determined by atomic absorption spectroscopy using a nitrous oxide-plus-acetylene flame.

***Calculations***. Forage intake was calculated using fecal output and forage in situ indigestibility after incubation for 48 h. Forage fecal output (DM) was converted to an OM basis using the OM content of feces. Forage fecal output on an OM basis was determined by subtracting the indigestible fraction of the supplement from feces of supplemented heifers using in situ indigestibility after the incubation of the supplement for 48 h. To determine forage OM intake, forage fecal output of OM was divided by forage in situ OM indigestibility. Liquid dilution rate was calculated by regressing the natural *log* of Co concentration on sampling time. Forage and supplement particle dilution rates were also calculated by regressing the natural *log* of Dy and Yb concentration on sampling time.

In situ data were evaluated using the Ørskov and McDonald (1979) model, ($d = a + b\ (1 - e^{-kd})$), where $a$ is the soluble fraction, $b$ is the slowly degradable fraction, $d$ is the extent of digestion, and $kd$ is the rate of degradation. Protein remaining in supplement in situ bags was adjusted for microbial protein contribution. Microbial protein was calculated using the N to purine ratio of ruminally isolated bacteria and purine content of supplement in situ remaining material.

***Statistical Analysis***. Data were analyzed as a complete randomized design with the GLM procedures of SAS (SAS Inst., Inc., Cary, NC). The model included the fixed effect of DDGS level. Orthogonal contrasts were used to test for linear, quadratic, and cubic effects of increasing supplemental DDGS level.

## RESULTS AND DICUSSION

The effects of DDGS supplementations levels on OM intake and digestibility are shown in Table 1. Forage and total OM, CP, and NDF intake were not affected ($P > 0.10$) by DDGS levels. Digestibility of OM and CP were

not affected ($P > 0.10$) by DDGS level. Wheat pasture is a high-quality forage source that contains over 20% CP and over 70% DM digestibility (Mader and Horn, 1986; Branine and Galyean, 1990). However, it is low in structural carbohydrate content (Horn et al., 1995). Therefore, improvements in digestion characteristics were not expected. Our results agree with those of MacDonalds et al. (2007) that supplemented dry distillers grains to heifers grazing high-quality smooth bromegrass (IVDMD = 65.7, CP = 20.8%, UIP = 2.17%, DM basis) and found no effects on forage intake. However, these authors found that ADG increased with dry distiller grains. Improvements were attributed to the UIP content of dry distillers grains which is greater than in DDGS. Loy et al. (2007) supplemented DDGS to heifers consuming medium-quality grass hay (8.2% CP) and found a decrease in hay DMI and increased total DMI. Supplementation of corn distillers solubles to cattle consuming low quality hay have shown no effects on forage intake and DM digestibility (Gilbery et al., 2006).

Digestibility of NDF increased (linear, $P < 0.01$) with increasing DDGS level. These results agree with those reported by Burroughs et al. (1950), who found that addition of dried distiller with solubles to an artificial rumen increased fiber digestion. This increase is most likely due to the increase in highly digestible NDF from DDGS observed with increasing DDGS level.

The effects of DDGS supplementation levels on ruminal kinetics are presented on Table 2. Rates of DM in situ disappearance for DDGS and wheat forage were not affected ($P > 0.10$) by DDGS level. These rates agree with the OM digestibility observed in the present study. In situ CP rate of disappearance of DDGS was not affected ($P > 0.10$) by DDGS level. However, in situ CP rate of disappearance of wheat forage decreased (quadratic, $P = 0.06$) with increasing DDGS level (5.37, 3.79, 2.26, and 3.90 ± 0.72 %/h for 0, 0.2, 0.4 and 0.6% of BW, respectively. The UIP from DDGS decreased (linear, $P = 0.08$) with increasing DDGS level (55.1, 48.6, and 47.8 ± 2.56% for 0.2, 0.4, and 0.6% of BW, respectively), but UIP from forage was not affected ($P = 0.35$) by increasing DDGS level. An increase in UIP from wheat forage was expected due to the decrease in rate of in situ CP disappearance observed in this study. Because total UIP reaching the small intestine for absorption is the sum of that from wheat forage and DDGS, total UIP increases with increasing DDG level.

Effects of DDGS supplementations levels on characteristics of ruminal fermentation are presented on Table 3. Ruminal concentration of acetate, propionate, butyrate, acetate to propionate ratio, ruminal pH, and total ruminal VFA production were not affected ($P > 0.10$) by increasing DDGS level. This lack of effects on characteristics of ruminal fermentation agrees with the lack of effects on intake and digestibility discussed above.

## IMPLICATIONS

Supplementation of DDGS to cattle grazing wheat pasture has no adverse effects on intake, digestibility and characteristics of ruminal fermentation. Based on these finding, DDGS can be used as supplements to improve the nutrient supply to cattle grazing wheat pasture.

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**Table 1.** Effects of corn distiller dry grains and solubles supplementation levels on OM, CP, and NDF and intake and digestibility of beef heifers grazing wheat pasture.

| | DDGS, % of BW[1] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Contrast[3] | | | | | | | | | |
| Items | 0 | 0.02 | 0.03 | 0.04 | SE[2] | P | L | Q | C |
| OM Intake, kg/d | | | | | | | | | |
| Forage | 8.70 | 9.27 | 7.13 | 7.771 | 0.72 | 0.21 | 0.15 | 0.96 | 0.12 |
| DDGS | 0.00 | 0.49 | 0.99 | 1.502 | 0.04 | 0.01 | 0.01 | 0.93 | 0.96 |
| Total | 8.70 | 9.77 | 8.12 | 9.27 | 0.75 | 0.47 | 0.98 | 0.96 | 0.13 |
| Total OM intake, kg/d BW | 23.98 | 25.46 | 21.69 | 24.24 | 1.67 | 0.47 | 0.69 | 0.75 | 0.15 |
| CP Intake, kg/d | | | | | | | | | |
| Forage | 1.50 | 1.60 | 1.23 | 1.34 | 0.12 | 0.21 | 0.15 | 0.96 | 0.12 |
| DDGS | 0.00 | 0.18 | 0.36 | 0.55 | 0.01 | 0.01 | 0.01 | 0.91 | 0.96 |
| Total | 1.50 | 1.78 | 1.59 | 1.89 | 0.13 | 0.22 | 0.13 | 0.95 | 0.13 |
| NDF intake, kg/d | | | | | | | | | |
| Forage | 5.44 | 5.80 | 4.46 | 4.85 | 0.45 | 0.21 | 0.15 | 0.96 | 0.12 |
| DDGS | 0.00 | 0.47 | 1.00 | 1.59 | 0.47 | 0.01 | 0.01 | 0.24 | 0.97 |
| Total | 5.44 | 6.26 | 5.45 | 6.44 | 0.48 | 0.35 | 0.33 | 0.87 | 0.14 |
| Digestibility, % of intake | | | | | | | | | |
| OM | 77.40 | 76.20 | 76.50 | 76.55 | 0.57 | 0.57 | 0.43 | 0.33 | 0.54 |
| CP | 73.87 | 74.63 | 74.38 | 74.50 | 0.01 | 0.95 | 0.71 | 0.75 | 0.76 |
| NDF | 74.35 | 74.38 | 77.70 | 79.78 | 0.01 | 0.06 | 0.01 | 0.21 | 0.21 |

[1]DDGS supplementation levels = The experimental treatments were DDGS (**0% BW**), DDGS at 0.02% BW (**0.02% BW**), DDGS at 0.04% BW (**0.04% BW**), and DDGS at 0.06% BW (**0.06% BW**).

[2]Standard error of treatment means; n = 4 heifers per treatment.

[3]Probabilities for the linear (L), quadratic (Q), and cubic (C) effects of level of DDGS.

**Table 2.** Effect of corn distiller dry grains and solubles supplementation levels on CP ruminal kinetics, and DM ruminal disappearance particulate flow rate in beef heifers grazing wheat pasture.

| | DDGS, % of BW[1] | | | | | | Contrast[3] | | |
|---|---|---|---|---|---|---|---|---|---|
| Items | 0 | 0.02 | 0.03 | 0.04 | SE[2] | P | L | Q | C |
| Forage UIP, % CP | 3.78 | 3.81 | 4.31 | 4.01 | 0.021 | 0.35 | 0.27 | 0.48 | 0.21 |
| DDGS UIP, %CP | - | 55.08 | 48.61 | 47.81 | 0.026 | 0.15 | 0.07 | 0.39 | - |
| CP Kinetic parameter | | | | | | | | | |
| DDGS | | | | | | | | | |
| Degradation rate, %/h | - | 4.15 | 3.67 | 3.15 | 0.01 | 0.72 | 0.43 | 0.99 | - |
| Forage | | | | | | | | | |
| Degradation rate, %/h | 5.36 | 3.78 | 2.25 | 3.89 | 0.01 | 0.097 | 0.11 | 0.06 | 0.36 |
| Ruminal DM disappearance, %h | | | | | | | | | |
| DDGS | - | 5.73 | 5.86 | 4.15 | 0.54 | 0.12 | 0.37 | 0.54 | - |
| Forage | 5.91 | 5.60 | 5.43 | 6.74 | 0.64 | 0.01 | 0.48 | 0.33 | 0.70 |

[1]DDGS supplementation levels = The experimental treatments were DDGS (**0% BW**), DDGS at 0.02% BW (**0.02% BW**), DDGS at 0.04% BW (**0.04% BW**), and DDGS at 0.06% BW (**0.06% BW**).
[2]Standard error of treatment means; n = 4 heifers per treatment.
[3]Probabilities for the linear (L), quadratic (Q), and cubic (C) effects of level of DDGS.

**Table 3.** Effects of distillers dry grains and solubles supplementation levels on ruminal pH, ammonia, and VFAs concentrations in beef heifers grazing wheat forage.

| | DDGS, % of BW[1] | | | | | | Contrast[3] | | |
|---|---|---|---|---|---|---|---|---|---|
| Items | 0 | 0.2 | 0.3 | 0.4 | SE[2] | P | L | Q | C |
| N | 4 | 4 | 4 | 4 | | | | | |
| pH | 6.05 | 6.09 | 6.23 | 6.21 | .12 | .64 | .26 | .79 | .62 |
| Ammonia N, mg/dl | 4.55 | 4.16 | 6.45 | 5.00 | .76 | .21 | .31 | .50 | .08 |
| VFA | | | | | | | | | |
| Total, mM | 153.89 | 127.79 | 117.80 | 112.39 | 19.52 | .47 | .15 | .61 | .90 |
| mol/100mol | | | | | | | | | |
| Acetate | 59.76 | 57.43 | 58.66 | 57.11 | 1.29 | .48 | .27 | .77 | .29 |
| Propionate | 19.63 | 20.17 | 19.40 | 21.789 | 0.91 | .30 | .19 | .33 | .29 |
| Butyrate | 18.78 | 18.27 | 18.25 | 17.59 | .89 | .61 | .56 | .25 | .83 |
| Isobutyrate | 0.63 | 0.58 | 0.74 | 0.60 | .06 | .28 | .81 | .47 | .08 |
| Acetate: propionate ratio | 3.10 | 2.87 | 3.13 | 2.76 | .20 | .54 | .42 | .73 | .25 |

[1]DDGS supplementation levels = The experimental treatments were DDGS (**0% BW**), DDGS at 0.02% BW (**0.02% BW**), DDGS at 0.04% BW (**0.04% BW**), and DDGS at 0.06% BW (**0.06% BW**).
[2]Standard error of treatment means; n = 4 heifers per treatment.
[3]Probabilities for the linear (L), quadratic (Q), and cubic (C) effects of level of DDGS.

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# USE OF DRIED DISTILLERS GRAINS TO EXTEND RANGE CAPACITY

K. H. Gustad, J. A. Musgrave, L. A. Stalker, T. J. Klopfenstein, W. H. Schacht, D. C. Adams, and J. D. Volesky

University of Nebraska, Lincoln, NE

**ABSTRACT**: Twenty-four, 1 ha paddocks were used to evaluate dried distillers grains with solubles (DDGS) as a substitute for grazed forage under heavy stocking rates. The experiment was conducted at the University of Nebraska Gudmundsen Sandhills Laboratory for 60 d from mid-June to mid-August each yr for two yr. Paddocks were randomly assigned to one of three treatments: control (CON) at the recommended stocking rate (1.48 AUM/ha in yr 1 and 1.06 AUM/ha in yr 2), double stocked (2X) where paddocks were divided in half with no supplementation, or double stocked with 2.27kg•hd$^{-1}$•d$^{-1}$ DM of DDGS (SUP). There were two replications and paddocks were rotationally grazed. In yr 1, 42 June-born spayed yearling heifers (242 ± 15 kg BW) and in yr 2, 24 June-born yearlings (14 spayed heifers and 10 steers; 229 ± 17 kg BW) were stratified by weight and assigned randomly to treatment paddocks. Forage utilization and standing crop were determined by clipping twenty, 0.25-m$^2$ quadrats pre- and post- grazing in late June, mid-July and early August (paddocks 2, 4, and 6 of rotation). Clipped samples were sorted by live grass, standing dead grass, forbs, and litter. Dietary IVOMD and CP were determined from masticate samples collected using esophageally fistulated cows mid-way through the grazing period. Average daily gains were similar for CON and 2X yearlings ($P$ = 0.44; 0.48 and 0.45 kg•hd$^{-1}$•d$^{-1}$, respectively); however, SUP yearlings gained more (1.14 kg•hd$^{-1}$•d$^{-1}$; P < 0.0001) than un-supplemented groups. Forage utilization was not different between SUP (58%) and 2X (62%; P = 0.15); however, utilization was lower for CON group (36%; P < 0.001). Increased stocking rate decreased IVOMD (55.73, 55.20, and 53.07% for CON, SUP, and 2X respectively; P = 0.03) which showed a quadratic decline over time (P < 0.001). Dried distillers grains with solubles is effective at increasing ADG of calves grazing native Sandhills range; however, DDGS did not substitute for grazed forage such that stocking rate could be increased twofold.

**KEY WORDS:** Dried Distillers Grains with Solubles, Grazing, Stocking Rate, Supplementation, Range Utilization

## Introduction

The improvement in performance of grazing yearlings when supplemented with DDGS has been documented (Klopfenstein, et al., 2007) and studies using harvested feeds to directly measure intake have reported forage replacement rates from 27% to 62%. Findings of these studies suggest that DDGS supplementation may allow for maintained or improved animal performance at increased stocking rates. The objective of this study was to evaluate the effect of DDGS supplementation on growth performance and forage intake of yearlings in heavily stocked situations.

## Materials and Methods

This experiment was conducted at the University of Nebraska Gudmundsen Sandhills Laboratory (GSL) near Whitman, NE. Twenty-four 1 ha paddocks were assigned randomly within two blocks to one of three treatments: 1) control (CON) at the recommended stocking rate (Stubbendieck and Reece, 1992; 1.48 AUM/ha in yr 1 but reduced to 1.06 AUM/ha in yr 2 because of drought) with no supplementation, 2) double stocked (2X) where paddocks were divided in half with no supplementation, or 3) double stocked with 2.27kg·hd$^{-1}$·d$^{-1}$ DM of DDGS (SUP). The DDGS supplement was 88% DM, 28% CP, and 11.2% fat.

Paddocks were rotationally grazed once each year for 60 days from mid-June to mid-August, with days of grazing per paddock adjusted for stage of plant growth. The order which pastures were grazed was rotated between years to maximize recovery. Due to drought in yr 2, stocking rate was reduced and put-and-take of yearlings were used to maintain forage removal similar to yr 1.

In yr 1, 42 summer-born spayed yearling heifers (242± 15 kg BW) and in yr 2, 24 June-born yearlings (14 spayed heifers and 10 steers) (229 ±17 kg BW) were stratified by BW and assigned randomly to treatment paddocks. In addition, six similar yearlings were maintained in yr 2 for put-and-take. Yearlings were limit-fed meadow hay at 2% of BW for five days at the beginning and end of the trial and weighed for three consecutive days.

Paddock botanical composition was determined prior to grazing each year using step-point analysis (Owensby 1973) (Table 1). Forage use and standing crop were determined by clipping twenty, 0.25-m$^2$ quadrats pre- and post-grazing in late June, mid-July and early August (paddocks 2, 4, and 6 of rotation).

Data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, N.C.). Yearling performance was analyzed as a randomized complete block design with treatment, year, and block analyzed as fixed effects.

Standing crop data were analyzed with treatment, order grazed, block, year, and clip type (pre or post) as fixed variables, and pasture as a random effect. Orthogonal contrasts were constructed between the control and both double stocked treatments, and between supplemented and un-supplemented double stocked treatments. Least square means were separated using the Least Significant Difference Method when a significant ($P < 0.05$) f-test was detected. Significance of interactions was determined at the $P < 0.1$ level.

## Results

No ($P > 0.1$) year by treatment interaction occurred for yearling performance (Figure 1). There was no difference between the CON and 2X yearlings ($P = 0.44$); however, the SUP yearlings gained (1.13 kg/day) more ($P < 0.001$) than the un-supplemented groups (0.45 kg/day). There was also a difference in ADG between the two years (Figure 1). This may be a direct result of a lower stocking rate in yr 2. While the goal was to maintain similar forage remaining after grazing between the groups, at times there may have been less grazing pressure in yr 2 because visual appraisal was used to determine when put-and-take yearlings were added or removed.

Because stocking rate differed between the CON and 2X groups, forage intake, and therefore energy intake, should have been different. The lack of difference in ADG between CON and 2X treatments implies energy was not the first limiting nutrient in un-supplemented yearlings. The lower than anticipated ADG for the CON yearlings likely was a result of a metabolizable protein (MP) deficiency. This experiment used young growing yearlings with a high MP requirement. The NRC (1996) model, using 120% NE adjusters and the average IVOMD and CP for the grazing period, suggests CON and 2X yearlings were deficient in MP by 147 g/day and had an energy allowable ADG of 0.77 kg. In contrast, the supplemented yearlings had a 145 g/day MP excess, and energy allowable ADG of 1.18 kg, which was very near their actual gain. This further supports our hypothesis that digestible undegradable protein was the first limiting nutrient in these yearlings, and some of the response to DDGS supplementation was likely a response to undegradable protein.

Use of live standing crop is presented in Table 2. Due to significant interactions between years, the standing crop data are presented by year. We did not expect differences in the standing crop components of yr 1 pre-graze standing crop as paddocks had been rested for 8 years; however, even though paddocks were assigned randomly to treatments, some differences existed at the onset of the trial. There were significant interactions between order grazed, treatment, and block ($P < 0.001$) in the amount of live grass. These interactions are likely caused by variation among pastures, lack of precision in measurement, and low number of replications.

These paddocks consisted of primarily warm-season grasses; therefore, peak yield of grasses did not occur until late in the summer. Live grass standing crop was lower across treatments after grazing. Across all paddocks, CON paddocks had more standing live grass and forbs following grazing ($P < 0.001$) than either of the double-stocked treatments.

Across all treatments, standing crop was lower in yr 2, but this is likely caused by decreased precipitation and not prior treatment. There was no effect of treatment in live grass ($P = 0.49$); however, order grazed did impact standing crop ($P < 0.001$). Across both years, use averaged 36.4%, 58% and 62% for CON, SUP and 2X treatments, respectively.

Contrary to our hypothesis, no significant reduction in forage removal was caused by the supplementation of DDGS in comparison to the CON or 2X treatments. Klopfenstein et al. (2007) reported a forage replacement rate of nearly 50% when DDGS was supplemented to yearlings fed harvested feeds. The DDGS forage substitution in our study is likely quite similar to that seen when DDGS is supplemented to cattle consuming harvested feeds. Extrapolation of harvested forage data from Klopfenstein et al. (2007) to ours suggests that at our rate of supplementation (2.27 kg daily), only 1.13 kg daily of forage would have been replaced. If this is accurate, forage replacement may have indeed occurred, but not at a level that could be detected in the design and sampling procedure of this study.

Supplementation of DDGS to yearlings grazing native Sandhills range increased ADG even when stocking rate was doubled. No apparent reduction in voluntary forage intake was detected in this study due to DDGS supplementation. Some of the laboratory data and visual observations suggest some level of replacement may occur early in the grazing period, but is not sustained throughout a grazing period at these stocking rates. Increasing stocking rate can have detrimental impacts on range condition over time. While the duration of the study was not sufficient to measure this decline, visual appraisals of the double stocked paddocks, along with previous research, suggest the double stocked treatments could decrease range condition. The findings of our study show DDGS supplementation is an effective tool in increasing ADG of yearlings grazing native Sandhills range; however, forage was not replaced such that stocking rate could be increased twofold.

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**Table 1**. Botanical composition of paddocks at the initiation of the trial.

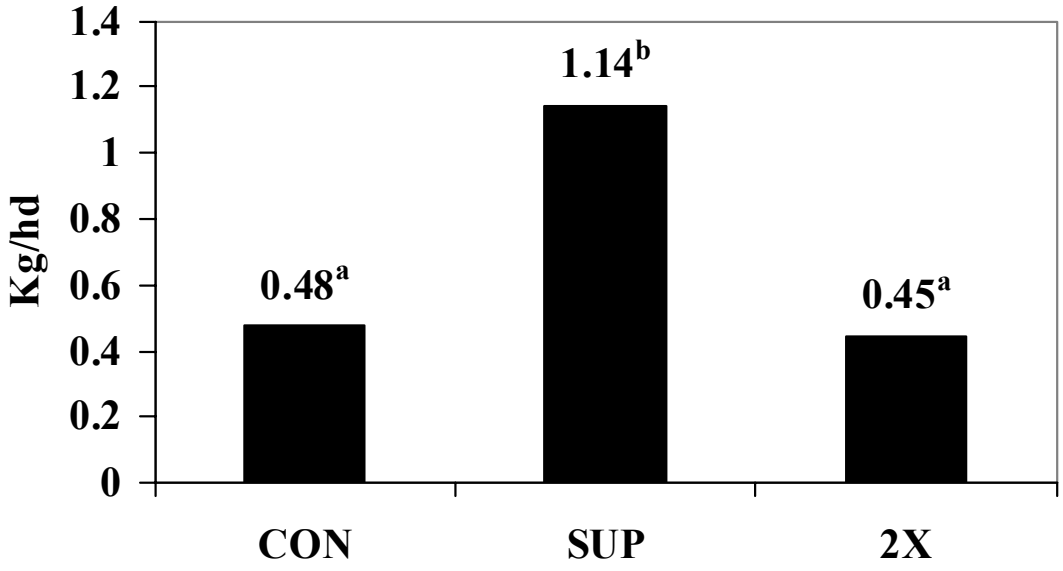
| Species | Block | |
|---|---|---|
| | West (%) | East (%) |
| Sedge (*Carex* spp.) | 25 | 23 |
| Prairie sandreed (*Calamovilfa longifolia*) | 19 | 19 |
| Needleandthread (*Hesperostipa comata*) | 10 | 15 |
| Little bluestem (*Schizachrium scoparium*) | 8 | 6 |
| Switchgrass (*Panicum virgatum*) | 7 | 6 |
| Prairie junegrass (*Koeleria macrantha*) | 3 | 4 |
| Sand dropseed (*Sporobolus cryptandrus*) | 3 | 3 |
| Blue grama (*Bouteloua gracilis*) | 5 | 4 |
| Hairy grama (*Bouteloua hirsuta*) | 4 | 3 |
| Sand bluestem (*Andropogon hallii*) | 2 | 3 |
| Western ragweed (*Ambrosia psilostachy*) | 6 | 6 |
| Stiff sunflower (*Helianthus pauciflorus*) | 3 | 3 |
| Other | 5 | 5 |
| **Total** | 100 | 100 |

**Table 2**. Live standing crop (kg/ha) and utilization of pastures stocked at the recommended stocking rate (CON), twice the recommended stocking rate with 2.27 kg per day dried distillers grains supplement (SUP), and twice the recommended stocking rate without supplement (2X).

| *Order:* | *Late June* | | | *Mid July* | | | *Mid August* | | |
|---|---|---|---|---|---|---|---|---|---|
| *Treatment:* | *CON*[a] | *SUP*[b] | *2X*[c] | *CON* | *SUP* | *2X* | *CON* | *SUP* | *2X* |
| **Year 1** | | | | | | | | | |
| *Pre-graze* | 1240 | 1216 | 1095 | 1376 | 1260 | 962 | 1511 | 1402 | 1343 |
| *Post-graze* | 845 | 544 | 472 | 844 | 480 | 264 | 775 | 464 | 457 |
| *% Utilization*[d] | 31.9 | 55.2 | 95.8 | 38.7 | 62.0 | 72.5 | 48.7 | 66.9 | 66.0 |
| **Year 2** | | | | | | | | | |
| *Pre-graze* | 979 | 941 | 1054 | 1264 | 1212 | 1274 | 1203 | 1172 | 1167 |
| *Post-graze* | 827 | 660 | 663 | 786 | 376 | 381 | 670 | 369 | 349 |
| ***% Utilization***[d] | 15.6 | 30.0 | 37.1 | 37.8 | 68.9 | 70.1 | 44.3 | 68.5 | 70.1 |

[a] Control, 1.48 AUM/hc.
[b] 2.96 AUM/hc plus 2.27 kg daily.
[c] 2.96 AUM/hc.
[d] Control different from SUP ($P < 0.001$).

**Figure 1. Calf average daily gain at recommended stocking rate, 2X recommended stocking rate and 2X plus distillers dried grains supplement. Means with unlike superscripts differ.**



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# AGE AT PUBERTY IN BEEF HEIFERS: CRIOLLO CATTLE VERSUS BRITISH CROSSBRED CATTLE

**S. I. Valverde-Saenz[1], J. D. Walsh[1], C. M. Gardner[1], J. T. Mulliniks[1], S. B. Schilling[1], D. M. Hallford[1], A. L. Gonzales[2], E. L. Fredrickson[2], K. K. Kane[1], and D. E. Hawkins[1]**

[1] Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM 88003
[2]ARS-USDA Jornada Experimental Range

**ABSTRACT:** Age at puberty is an important factor in estimating the potential productivity of the bovine female. A study was conducted at the ARS-USDA Jornada Experimental Range in 2006 and 2007 to compare onset of puberty, BW, and serum insulin in Criollo and Angus-Hereford crossbred heifers. In 2006, 7 Criollo and 5 crossbred heifers were used to determine length of the estrous cycle and insulin concentrations. Heifers grazed the same pasture during the study with free access to water and mineral supplement. Blood samples were collected twice weekly for both years. In 2007, 15 Criollo and 15 crossbred heifers were used to determine age at puberty (2 or more consecutive progesterone concentrations > 1 ng/mL), length of the estrous cycle, and insulin concentrations. Initial BW in both 2006 and 2007 crossbred heifers were heavier *(P* < 0.01), when compared to Criollo heifers (251.7 and 166.4 ± 12.9 kg; 236.2 and 158.8 ± 4.7 kg, respectively). In 2006, BW gain did not differ (*P* < 0.01) between breeds (80.5 and 71.1 ± 4.4 kg for crossbred and Criollo heifers, respectively). In 2007, crossbred heifers gained more BW than Criollo heifers during the study (*P* < 0.01; 128.2 and 91.2 ± 4.1 kg, respectively). In 2007, BW at puberty (*P* < 0.01) was greater for crossbred heifers than Criollo heifers (323.8 and 213.9 ± 6.6 kg, respectively). However, Criollo heifers tended (*P* = 0.15) to reach puberty earlier than crossbred heifers (363.5 and 376.7 ± 6.3 d, respectively). Estrous cycle length in crossbred and Criollo heifers in both 2006 and 2007 did not differ (*P* ≥ 0.53; 18 and 19 ± 1 d; 18 and 19 ± 1 d, respectively). Serum insulin concentrations were also greater (*P* < 0.01) for Criollo compared to crossbred heifers in both 2006 and 2007 (0.67 and 0.54 ± 0.02 ng/mL; 0.68 and 0.58 ± 0.02 ng/mL, respectively). This study suggests that Criollo heifers may reach puberty earlier than British breeds.

**Key words:** beef cattle, Criollo cattle, puberty.

## INTRODUCTION

Criollo cattle came from the Canary Islands of the north-west coast of Africa (Primo, 1992). These cattle were introduced into the New World by Christopher Columbus in 1493, on his second trip (Rouse, 1977). Criollo cattle spread to the southwest and southeast United States by Spanish missions and ranches with Spanish influence (Sponenberg and Olson, 1992). It is believed that fewer than 300 were transported to the new world (Olson, 1988). This type of cattle was one the main food supplies not only for the Spanish conquistadors but also to the natives. Through the years, the Criollo cattle brought to the New World adapted to different environments (Portillo et al. 2006) until new breeds were introduced, changing the initial purpose of the Criollo cattle for food supply to entertainment purposes such as rodeo. Russell et al. (2000) compared the genetic material present in Criollo cattle with Angus, Hereford, Charolais, and Simmental, finding the Charolais breed as the most genetically similar to the Criollo breed and the Angus breed as the most different. Many efforts have been made in order to keep the breed as a potential genetic source for lands with limited forage production or extreme environments. Because reproduction is the most important factor that influences the productivity of the animal industry, it is crucial to understand all the physiological events involved. Puberty is the process of acquiring reproductive competence and the age at which mature gametes are first produced (Foster and Nagatani, 1999; Senger, 2003). Age at puberty in British breeds of cattle has been well established by many researchers (Day et al. 1987; Rodrigues et al., 2002; Gasser et al., 2006abc). However, age at puberty in Criollo cattle has not been well established. The objective of this study was to evaluate the onset of puberty between Criollo cattle and British crossbred cattle.

## MATERIALS AND METHODS

### *Location*

A 2-yr study was conducted at the Jornada Experimental Range, operated by the Agricultural Research Service of the U.S. Department of Agriculture, and located 37 Km. north of Las Cruces, NM. at an elevation of approximately 1,188 m. The annual precipitation in this area is 247 mm, beginning July 1 to September 30 when 53% of the annual rainfall occurs. The vegetation present in the area of study was Honey mesquite (*Prosipis g*landulosa), Soaptree yucca (*Yucca elata*), Broom Snakeweed (*Gutierriza sorothrae*), Black gramma (*Bouteloua eriopoda*), and Tarbrush (*Artriplex canescens*).

### *Animals*

In 2006, 7 Criollo heifers and 5 British crossbred heifers were used to determine length of the estrous cycle and insulin concentrations. The mean BW of the groups was 166 kg and 251 kg (at trial start, Sep. 1, 2006), respectively. Both groups of heifers were maintained in the same pasture under the same environmental conditions. In 2007, 15 Criollo and 15 British crossbred heifers were used to determine age at puberty (2 or more consecutive progesterone concentrations > 1 ng/mL),

length of the estrous cycle, and insulin concentrations. The mean BW was 159 kg and 236 kg (at trial start, Apr. 18, 2007) for Criollo and crossbred heifers respectively.

***Sample Collection***

Serum samples were collected for progesterone and insulin concentration analysis twice weekly to determine onset of estrus. Heifers were gathered and bled by caudal venipuncture for five mo. Blood was collected in 9 mL Corvac serum separator tubes (Kendall Healthcare, Mutansfield, MA) with 20 gauge x 2.54 cm needles (BD Vacutainer Systems, Franklin Lakes, NJ). Blood was transported to Las Cruces and centrifuged at 2000 x g for 20 min at 4°C. Serum was decanted and stored at -20°C prior to subsequent assay for progesterone and insulin concentration. The progesterone assay was conducted at NMSU Endocrinology Laboratory, using a commercial RIA kit (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) modified for use in ruminant serum as established by Scheneider and Hallford (1996). The average CV for progesterone within assay was 9.3% and between assays was 11.3%. For insulin analysis, samples were composited for 2-wk week intervals. The insulin assay was performed, using a solid phase RIA (DPC kit, Siemens Medical Solutions Diagnostics, Los Angeles, CA) as established by Reimers et al. (1982). The average CV for insulin was 6.8% within assays and 18% between assays .

***Statistical Analysis***

Data were analyzed as completely randomized design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) for estimation of age to puberty. For analysis of BW, cycle length, and insulin concentrations, a completely randomized design, using the MIXED procedure of SAS was performed.

## RESULTS AND DISCUSSION

Initial BW of crossbred heifers in both 2006 and 2007 were heavier ($P < 0.01$), when compared to Criollo heifers (251.7 and 166.4 ± 12.9 kg; 236.2 and 158.8 ± 4.7 kg, respectively). In 2006, BW gain did not differ ($P < 0.01$) between breeds (80.5 and 71.1 ± 4.4 kg for crossbred and Criollo heifers, respectively) during the 151 d study. In 2007, crossbred heifers gained more BW than Criollo heifers during the 147 d the study ($P < 0.01$; 128.2 and 91.2 ± 4.1 kg, respectively).

In 2007, BW at puberty ($P < 0.01$) was greater for crossbred heifers than Criollo heifers (323.8 and 213.9 ± 6.6 kg, respectively; Figure 1). However, Criollo heifers tended ($P = 0.15$) to reach puberty earlier than crossbred heifers (363.5 and 376.7 ± 6.3 d, respectively; Figure 2). Criollo cattle are considered in the range of the breeds with lighter BW at puberty and early puberty when compared with the breeds used by Laster et al. (1976, 1979). This comparison with other breeds is an option to maintain reproduction in places with limited forage available, keeping animals with low BW at puberty, but capable of reach puberty earlier with limited access to feed.

Estrous cycle length between crossbred and Criollo heifers in both 2006 and 2007 did not differ ($P \geq 0.53$; 18 and 19 ± 1 d; 18 and 19 ± 1 d, respectively; figure 3). Serum insulin concentrations were also greater ($P < 0.01$), for Criollo compared to crossbred heifers in both 2006 and 2007 (0.67 and 0.54 ± 0.02 ng/mL; 0.68 and 0.58 ± 0.02 ng/mL, respectively). These concentrations appear higher than those reported by Amstalden et al. (2000) and Giacomini (2006). Insulin plays a very important role in controlling metabolic factors that are critical to the reproductive axis in cattle and also is an indicator of the nutritional status of the animal (Hess et al., 2005). These data suggest that while Criollo heifers were lighter than crossbred heifers in BW at puberty, the nutritional status of the Criollo heifers determined by insulin levels appear optimum for reproductive functions.

## IMPLICATIONS

Reproduction is a major factor in the livestock industry. The use of breeds capable of achieving puberty sooner, allow producers to breed heifers as soon as possible and extend the productive life of the animals. This study suggests that Criollo heifers may reach puberty earlier and at lighter BW than British breeds.

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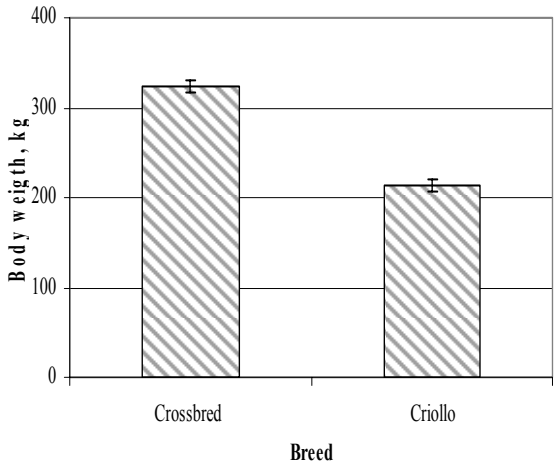


**Figure 1.** Weight at puberty ($P < 0.01$) in crossbred heifers (n=30) and Criollo heifers (n=30).

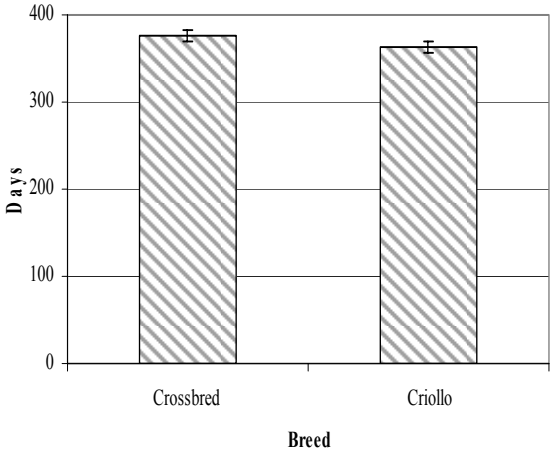


**Figure 2.** Days to puberty ($P = 0.15$) in crossbred heifers (n=30) and Criollo heifers (n=30).

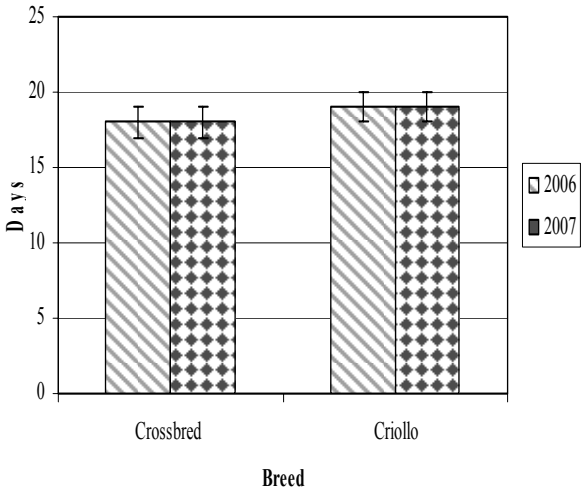


**Figure 3**. Estrous cycle length ($P \geq 0.53$) in crossbred heifers in 2006 and 2007 (n=5 and n=30, respectively) and Criollo heifers (n=7 and n=30, respectively).

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# NEUROENDOCRINE FUNCTION IN HEIFERS CONSUMING LOW QUALITY DORMANT FORAGE

**J.D. Walsh*, C.M. Gardner, S.I. Valverde-Saenz, M.B. Horvath, J.T. Mulliniks, D.M. Hallford, S.L. Lodge-Ivey, K.K. Kane, and D.E. Hawkins**

Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM 88003

**ABSTRACT:** This study examined effects of low quality dormant forage on neuroendocrine function. Eighteen, 2-yr-old, rumen-cannulated, cyclic crossbred heifers (428 ± 44 kg) were stratified by BW and randomly assigned to 1 of 3 dietary treatments (n = 6) fed on a DM basis. Treatment 1 (Trt 1) was a 50% buffalo straw – 50% old world bluestem mix (CP% = 5.1, NDF% = 74.6). Treatment 2 (Trt 2) was composed of sudan grass (CP% = 9.5, NDF% = 57.2). Treatment 3 (Trt 3) was a 50% Trt 1 – 50% Trt 2 mix (CP% = 8.1, NDF% = 63.3). Heifers were allowed ad libitum access to treatments fed at 0700 with refusals recorded at 1900. Feeding began during the luteal phase of the first cycle, proceeded through one ovulation, and heifers were slaughtered when the second dominant follicle reached maximum size (mean = 12 $mm^2$), estimated by previous cycle length and follicle size. Blood samples were collected (caudal venipuncture) before the study for 3 d and every other day during the study until d 25 when it was collected daily until slaughter. Blood serum and anterior pituitary glands were collected at slaughter. Serum was stored at -20°C and anterior pituitary glands were snap frozen in liquid nitrogen and stored at -80°C. Serum was quantified for insulin, IGF-I, and LH (RIA). Supernatant from homogenized anterior pituitary glands was quantified for LH content (RIA). Mean insulin did not differ between treatments ($P$ = 0.35. A day*treatment interaction ($P$ = 0.02) and a trend ($P$ = 0.06) for mean serum insulin to differ between baseline and d 25 samples (0.47 vs. 0.56 ± 0.03 ng/mL) were observed. Serum IGF-I concentration was not different among treatments ($P$ = 0.27). Mean LH concentration and anterior pituitary gland LH content was similar among treatments at $P$ = 0.60 and $P$ = 0.78 respectively. Under the conditions of this study, consumption of low quality forage had no effect on neuroendocrine function.

Keywords: anterior pituitary, heifer, nutrient stress

## INTRODUCTION

Cattle producers in the arid regions of the southwestern United States, rely on low-input systems of production in order to maintain economic viability. Hess et al. (2005) estimated that reproductive disease or failure costs beef cattle producers $441 to $502 million in lost income per year and that the financial cost associated with feed was the greatest factor influencing profit of commercial beef cow operations, accounting for more than 63% in the variation of total cow costs. Furthermore, the detrimental effects of nutritional restriction on reproduction may not only induce anestrus in some farm animals, but it may also compromise fertility in those animals that do exhibit estrus and ovulate (Mackey et al., 1999). Thus the importance of nutrition as related to reproductive success has been recognized by the livestock industry.

Many times in the southwestern United States, cattle graze dormant forage. When cows or heifers graze dormant native vegetation, often diet quality is low, and the preferred ruminant gluconeogenic substrate, proprionate, is inadequate (Hawkins et al., 2000). In long-term studies where heifers experienced chronic nutrient restriction, no effect on neuroendocrine function, as measured by LH secretion during the estrous cycle, was observed (Stagg et al., 1995; Rhodes et al., 1995; Bossis et al., 1999). However, the effects of acute nutrient restriction in cattle are not well defined.

Nutrient restriction in the grazing beef female may compromise gonadotropin concentrations (Mackey et al., 1999) and neuroendocrine function. Feeding a ration containing levels of energy or energy and protein below maintenance has been shown to adversely affect anterior pituitary gland – ovarian function in ruminants (McCann and Hansel, 1986).

Metabolic mediators, including glucose, insulin (**INS**), GH, IGF-I, and insulin-like growth factor binding proteins may modulate nutrient restriction effects on the hypothalamic-pituitary-ovarian-axis (Kiyma et al., 2004). Physiological concentrations of INS are required for normal follicular steroidogenesis to occur and nutrient restriction reduces circulating concentrations of INS in the beef female (Bossis et al., 1999). Infusion of INS into energy-deprived beef heifers caused an increase in both the diameter of the dominant follicle and ovulation rates (Webb et al., 2004). Insulin-like growth factor-I is involved in the proliferation, differentiation, and hypertrophy of several kinds of cells and stimulates synthesis of DNA and progesterone in ovarian tissues (Vandehaar et al., 1995). Concentrations of IGF-I in cattle decrease during restriction of dietary energy and it has been implicated as a mediator of the adverse effects of nutrient restriction in ovarian tissue (Vandehaar et al., 1995).

Frequent pulses of LH from the anterior pituitary gland are needed for maturation and ovulation of pre-ovulatory follicles (Bossis et al., 1999). A diet limited in energy can influence secretion of LH in the bovine female. Imakawa et al. (1986b) showed that mean concentrations of LH in serum were reduced in cyclic beef heifers losing BW as a result of dietary energy restriction. Fasting-induced LH suppression is believed to be a consequence of reduced release of hypothalamic GnRH, as seen when fasted animals show LH pulses that are similar in frequency and

magnitude to fed animals when administered exogenous GnRH (Cunningham et al., 1999).

## MATERIALS AND METHODS

The study was conducted at New Mexico State University Livestock Research Center, Las Cruces, NM, and all animal procedures were approved by the NMSU Institutional Animal Care and Use Committee.

### *Animals and Treatments*

Eighteen, 2-yr-old, rumen-cannulated, cyclic crossbred heifers (428 ± 44 kg) were stratified by BW and randomly assigned to 1 of 3 dietary treatments (n = 6/ treatment) fed on a DM basis. Treatment 1 (**Trt 1**) was a 50% buffalo straw – 50% old world bluestem mix (CP% = 5.1, NDF% = 74.6). Treatment 2 (**Trt 2**) was composed of sudan grass (CP% = 9.5, NDF% = 57.2). Treatment 3 (**Trt 3**) was a 50% Trt 1 – 50% Trt 2 mix (CP% = 8.1, NDF% = 63.3). Prior to receiving treatments, heifers were group-fed an alfalfa diet together in pens. Heifers were halter broke prior to the start of the study. During the study heifers were housed, fed individually, and allowed ad libitum access to treatments (between 0700 and 1900) and water. Treatments were fed at 0700, refusals were pulled 1900 and recorded. Treatments were initially fed at 2% BW then immediately adjusted to ad libitum intake and maintained at ad libitum intake for the duration of the study. The study period (≥ 25 d) consisted of 2 estrous cycles and heifers were slaughtered when the dominant follicle (cycle 2) reached maximal size (mean = 12 $mm^2$) which was estimated by estrus detection, previous maximal follicle size (cycle 1) and previous cycle length (cycle 1).

### *Blood and Tissue Collection*

Blood samples were collected by caudal venipuncture before the study for 3 d for baseline measurements and every other day during the trial until d 25 when it was collected daily until slaughter. Cyclicity of heifers was determined by twice weekly caudal venipuncture samples with serum progesterone concentrations greater than 1 $ng \bullet mL^{-1}$. Heifers were slaughtered by captive bolt stunning and exsanguination. Blood samples during the study were collected by caudal venipuncture in serum separator tubes (Corvac, Sherwood Medical, St. Louis, MO) for quantification of serum INS, LH and IGF-I. Immediately following slaughter, blood samples and anterior pituitary glands were obtained for quantification of serum INS, LH, and IGF-I, and anterior pituitary LH content. Blood samples collected during the trial and at slaughter were allowed to coagulate for 20 m at ambient temperature and were centrifuged at 1,300 x g (25 min, 4°C) and serum was stored at -20°C. Following collection, anterior pituitary glands were snap frozen in liquid $N_2$ and stored at -80°C. In order to evaluate changes in BW, heifers were weighed at the beginning of the study and on the day of slaughter. Weights prior to the study were obtained before feeding at 0630 on d 1 and at the time of slaughter.

### *Serum and Anterior Pituitary Tissue Analysis*

Serum insulin and progesterone concentrations were measured using solid phase RIA kits (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA). Serum insulin was quantified utilizing the method described by Reimers et al. (1982) with an intra- and inter-assay CV of 5.9% and 9.6% respectively. Progesterone analysis was conducted as per Schneider and Hallford (1996) with an inter-assay CV of 4.8%. Serum IGF-I concentrations were quantified via RIA as per Berrie et al. (1995) with an intra- and inter-assay CV of 8.1% and 7.7% respectively. Serum LH was quantified and compared in 2 different periods during the study. These 2 periods (cycle 1 and cycle 2) were localized around the appearance of a preovulatory follicle. The 5 serum samples closest to the ovulation day during the study, as determined by ultrasonography, and in the last 5 samples prior to slaughter were used for analysis. Anterior pituitary gland LH was extracted for analysis of anterior pituitary gland LH content by homogenizing 100 mg anterior pituitary tissue samples in 1 mL of 0.01 M phosphate buffered saline (PBS, pH 7.4). Homogenates were centrifuged at 29,500 x g (30 min, 4°C) and supernatant collected and frozen at -20°C. Serum and pituitary homogenate samples were analyzed for LH by RIA using procedures described by Hoefler and Hallford (1987) with intra-assay CV of 6.3% and 9% respectively.

### *Statistical Analysis*

Serum progesterone, LH, INS and IGF-I were analyzed by analysis of variance (ANOVA) using the MIXED procedure of SAS (SAS 9.1 Inst. Inc. Cary, NC) for a completely randomized design. Heifer BW, feed intake and anterior pituitary gland LH content were analyzed by ANOVA using the PROC GLM procedure of SAS (SAS Inst. Inc. Cary, NC) for a completely randomized design. Heifer BW, feed refusals, serum progesterone, and anterior pituitary gland LH content was analyzed as a one-way ANOVA. Serum LH, INS, and IGF-I were analyzed using split-plot ANOVA with treatment in the main plot, heifer(treatment) as the error term, and time and time x treatment in the subplot. Observed level of significance was set at $P < 0.05$.

## RESULTS AND DISCUSSION

There was no difference in BW loss/gain (Table 1) among treatments ($P = 0.27$). Although no differences in BW change were observed, heifers assigned to Trt 1 had a numerical trend ($P = 0.26$) to lose weight (-8.8 kg) from the beginning of the study to the end, but still were not different from Trt 2 or Trt 3. No observed differences in BW loss may be due to the fact that a percentage of the heifers maintained growth and development throughout the study, while others may have had adverse effects on BW from consumption of diets. Also, the diet may not have been restrictive enough to cause significant changes on BW change.

There were statistically significant differences in feed intake among all treatments ($P > 0.01$). Heifers assigned to Trt 1 had an intake much lower (Table 2) than heifers that received Trt 2 or 3. A difference in intake, as seen, would

suggest that there was energy restriction taking place that would be severe enough to cause an effect on circulating hormone concentrations and anterior pituitary gland LH content. However, as discussed later, there was no significant difference observed, therefore, even though there was difference in DM intake, energy intake was still sufficient enough to meet maintenance requirements and not severe enough to cause detrimental effects on neuroendocrine function.

Mean serum INS did not differ among treatments ($P$ = 0.35). A day*treatment interaction ($P$ = 0.02) and a trend ($P$ = 0.06) for mean serum insulin to differ (Table 3) between baseline samples (0.47 ng/mL) and d 25 samples (0.56 ng/mL) were observed. The day*treatment interaction further validates the increased mean serum INS levels between the baseline and d 25 samples.

Serum IGF-I concentration was not different among treatments ($P$ = 0.27). The lack of difference in IGF-I concentration among treatments may be due to the fact there were minimal differences in the treatment diets, alluding to no significant change in IGF-I concentration as a result of dietary effect. However, mean serum IGF-I concentrations differed ($P$ < 0.01) between the baseline samples (121.1 ng/mL) and the slaughter d samples (103.4 ng/mL).

Mean LH concentration was similar among treatments over time ($P$ = 0.60). However there was a trend ($P$ = 0.12) for mean LH concentrations to differ among all treatments (Table 4) between the first (0.19 ng/mL) and the second (0.24 ng/mL) cycle observed during the study. Cunningham et al. (1999) stated that acute changes in an animal's energetic status can result in modulation of the hypothalamic-pituitary-gonadal axis which would cause adverse effects to circulating LH concentrations. As previously stated, a dietary induced decrease in LH concentration is most probably due to inadequate signaling from the hypothalamus via GnRH. As Mackey et al. (2000) noted, in similar studies (Bossis et al., 1999; Rodes et al., 1995) LH concentrations among treatments had no difference and cattle seemed to be either slow to respond to nutritional deprivation or, alternatively, the degree of feed restriction was not severe enough to induce earlier effects. LH concentrations observed in this study may be due to the fact that dietary treatments were not restrictive enough to cause significant effects on LH concentration.

Anterior pituitary LH content ($P$ = 0.78) was not different among treatments (Figure 1). Since heifers were slaughtered at an experimentally-determined maximal follicle size based on previous estrous cycle, there was a chance anterior pituitary glands were collected prior to the pre-ovulatory release of LH. Also, significant dietary effect on the neuroendocrine system is most likely due to insufficient GnRH production (Dunn and Moss, 1992), which was not quantified in this study. Inadequate nutrition seems to inhibit reproduction by actions ultimately exerted on hypothalamic neurons responsible for release of GnRH (Dunn and Moss, 1992). Therefore, because there was no significant difference in serum LH concentration, it is logical that there were no observed differences in LH content of the anterior pituitary gland.

## IMPLICATIONS

Under the conditions of this study, ad libitum consumption of low quality dormant forage had no effect on neuroendocrine function in beef heifers.

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**Table 1**. Mean body weight loss of heifers from the beginning to the end of the study between treatments.

| | Body weight loss/gain | | |
|---|---|---|---|
| **Treatments[1]** | **Weight, kg** | **SEM[2]** | ***P-Value*** |
| Trt 1 | -8.8 | 7.52 | 0.26 |
| Trt 2 | 6 | 7.52 | 0.43 |
| Trt 3 | 7.3 | 7.52 | 0.36 |

[1]Treatments: Trt 1 = 50% buffalo straw – 50% old world bluestem mix (CP% = 5.1, NDF% = 74.6); Trt 2 = sudan grass (CP% = 9.5, NDF% = 57.2); Trt 3 = 50% Trt 1 – 50% Trt 2 mix (CP% = 8.1, NDF% = 63.3).
[2]SEM = Standard error of the mean.

**Table 2.** Average DM intake by treatment.

| | Average daily DM intake | | |
|---|---|---|---|
| **Treatments[1]** | **DM Intake, kg** | **SEM[2]** | ***P-Value*** |
| Trt 1 | 6.3 | 0.16 | < 0.01 |
| Trt 2 | 8.7 | 0.16 | < 0.01 |
| Trt 3 | 9.3 | 0.16 | < 0.01 |

[1]Treatments: Trt 1 = 50% buffalo straw – 50% old world bluestem mix (CP% = 5.1, NDF% = 74.6); Trt 2 = sudan grass (CP% = 9.5, NDF% = 57.2); Trt 3 = 50% Trt 1 – 50% Trt 2 mix (CP% = 8.1, NDF% = 63.3).
[2]SEM = Standard error of the mean.

**Table 3**. Comparison of mean serum insulin (INS) concentrations between baseline and day 25 samples among all treatments.

| | Serum INS | | | |
|---|---|---|---|---|
| **Item** | **BL[1]** | **D 25[2]** | **SEM[3]** | ***P-Value*** |
| Serum INS, ng/mL | 0.47 | 0.56 | 0.03 | 0.06 |

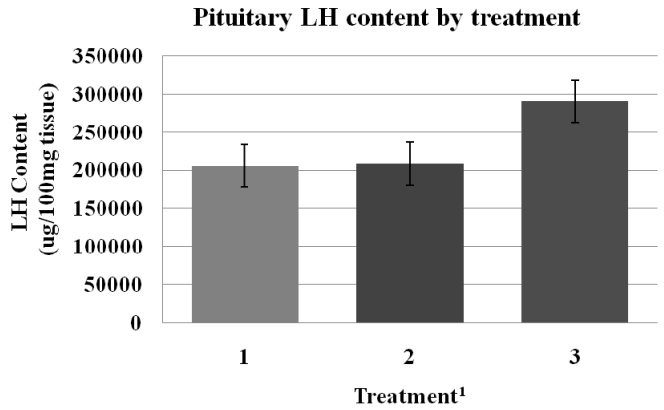
[1]BL = Baseline.
[2]D 25 = Day 25.
[3]SEM = Standard error of the mean.

**Table 4.** Comparison of mean serum LH concentrations between the first and second cycle observed during the study period among all treatments.

| | Serum LH | | | |
|---|---|---|---|---|
| **Item** | **C 1[1]** | **C 2[2]** | **SEM[3]** | ***P-Value*** |
| Serum LH, ng/mL | 0.19 | 0.24 | 0.03 | 0.12 |

[1]C 1 = Cycle #1.
[2]C 2 = Cycle #2.
[3]SEM = Standard error of the mean.

**Figure 1**. Pituitary LH content by treatment.




[1]Treatments: Trt 1 = 50% buffalo straw – 50% old world bluestem mix (CP% = 5.1, NDF% = 74.6); Trt 2 = sudan grass (CP% = 9.5, NDF% = 57.2); Trt 3 = 50% Trt 1 – 50% Trt 2 mix (CP% = 8.1, NDF% = 63.3).
*No differences observed at P = 0.78.

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# DIFFERENTIAL EXPRESSION OF THE IGF-I GENE IN ENDOMETRIUM OF EWES FASTED DURING THE LUTEAL PHASE OF THE ESTRUS CYCLE

**A. Breton, K. J. Austin, E. A. Van Kirk, G. E. Moss, and B. M. Alexander**
**Department of Animal Science, University of Wyoming, Laramie, Wyoming**

**ABSTRACT:** Short-term fasting during the luteal phase of the estrous cycle perturbs circulating concentrations of progesterone and estradiol causing a delay in the onset of the surge release of LH. Although ovulation rate is not affected by fasting in this model, numbers of lambs born are decreased. Fasting may affect fertility by decreasing ova quality and/or altering the endometrium/oviduct environment. The objective of this study was to elucidate differences in gene expression in the endometrium near the time of expected ovulation in fed and fasted ewes. Estrous cycles were synchronized using $PGF_2\alpha$. Control ewes were given ad libitum access to grass hay throughout the experiment. Ewes in the fasted group were withheld from feed on d 7 through d 11 of the estrous cycle (d 0 = first day of estrus). On d 12 all ewes were treated with $PGF_2\alpha$, and fasted ewes were returned to ad libitum feed. Endometrial tissue was collected from fasted and fed ewes during the expected periovulatory period (72 hr following $PGF_2\alpha$ and realimentation of fasted ewes). At 72 hr, ewes were euthanized and sections of endometrium were dissected and snap frozen for RNA analysis. Semi-qualitative Real Time PCR was used to examine gene expression within the endometrium of ewes which had ovulated. Data was analyzed using GLM procedures of SAS. Gene expression for IGF-I was up-regulated ($P < 0.01$) in the endometrium of fasted compared to fed ewes. Gene expression for IGF-II, IGFBP1, IGFBP3, IGFBP6, estrogen receptor α, estrogen receptor β, and progesterone receptor did not differ ($P \geq 0.4$) among fasted and fed ewes. Although estrogen can stimulate IGF-I synthesis, serum concentrations of estrogen did not differ ($P = 0.7$) between groups at 0, 24, 48, and 72 hours following realimentation. Fasting during the luteal phase of the estrous cycle preceeding proestrus influences uterine expression of IGF-I which may influence peri-implantation embryo survival.
**Key Words:** Fasting, Endometrium, Sheep, IGF

## Introduction

Limited feed resources can decrease reproductive efficiency to an extent dependent on the degree (Mackey et al., 2000) and reproductive status (Smith, 1988) at the time of feed restriction. Short-term fasting of mature ewes during diestrus results in increased serum concentrations of progesterone and a delayed pre-ovulatory surge release of LH (Alexander et al., 2007).

Short-term fasting during the luteal phase of the estrous cycle decreased serum concentrations of FSH (Alexander et al., 2007) and affected numbers of small and medium sized follicles. Although the numbers of large follicles did not differ (Alexander et al., 2007), estradiol was decreased in fasted ewes, during the 24 h period prior to the anticipated surge-release of LH. Serum concentrations of insulin and IGF-I were decreased by fasting, while serum concentrations of GH remained similar among fasted and fed ewes (Kiyma et al. 2004). Although ovulation rate did not differ between fasted and fed ewes (Kiyma et al., 2004), numbers of lambs born tended to be decreased in fasted ewes (unpublished observation). Therefore, the objective of this study was to determine if feed withdrawal during the luteal phase preceding proestrus cause changes in mRNA expression in the endometrium which may detrimentally affect embryo survival.

## Materials and Methods

*Animals*

Estrous cycles of 39 mature (≥3 yr old) Western White-Faced ewes in moderate body condition (BCS= 5 to 7) were synchronized with two 100 mg doses of $PGF_2\alpha$ (Lutalyse, Pharmacia & Upjohn Co., Kalamazoo, MI) on d 1 and 10 of the estrous cycle. Estrous behavior was monitored in the presence of two vasectomized rams at 0600 and 1800 for 4 d following the second injection of $PGF_2\alpha$. Twenty ewes with synchronized estrous cycles were selected and randomly allotted to control or fasted (n=10/group treatments) groups. Ewes were housed by treatment in separate, adjacent pens. Control ewes were given ad libitum access to grass hay throughout the experiment. Ewes in the fasted group were withheld from feed on d 7 through 11 of their estrous cycle (d 0 = first day of estrus). Ewes in both groups had ad libitum access to water. On d 12, all ewes were treated with 100 mg of $PGF_2\alpha$, and fasted ewes were returned to ad libitum feed. Blood samples were collected by jugular venipuncture prior to administration of $PGF_2\alpha$ and at 24, 48, and 72 hours post administration. Endometrial tissue was collected from fasted and fed ewes during the expected periovulatory period (72 hr following $PGF_2\alpha$ and realimentation of fasted ewes). At 72 hr, ewes were euthanized and sections of endometrium were dissected and snap frozen for RNA analysis.

*RIA*

Blood samples were allowed to clot overnight at 4 °C. Serum was separated by centrifugation at 1500g for 20 min and stored at – 20 °C until analysis for concentrations of estradiol. All samples were assayed in duplicate in a single assay for estradiol with intra-assay coefficients of variation <10% .

*RNA Isolation and cDNA synthesis*

Total Cellular RNA was isolated using TRI Reagent (Sigma Chemical; St. Louis, MO). Briefly, 100 mg endometrial tissue was homongenized in 1 mL of Tri Reagent and allowed to sit at room temperature for 5 min before adding 0.2 mL chloroform. After 10 min, the homogenate was centrifuged for 15 min at 4 °C at 12,000 rpm. The aqueous layer was transferred to a new tube and the RNA was precipitated with 0.5 mL of isopropanol by centrifuging for 10 min at 12,000 rpm. The RNA pellet was washed once with 70% ethanol and suspended in 100 µL RNase free water. Concentration of RNA was determined using a NanoDrop spectrophotometer and 10 µg was further purified using RNEASY (Qiagen Inc; Santa Clara, CA) with on-column DNase digestion. Approximately 2.0 µg of RNA was mixed with 4 µL reverse transcription buffer (5X) and 1 µL of IScript reverse transcriptase (Bio-Rad Laboratories, Richmond, CA). The mixture was placed in a thermocycler for 5 minutes at 25 °C, 30 min at 42 °C, 5 min at 85 °C and held at 4 °C. The cDNA was diluted with 100 µL nuclease free water and stored at -20 °C until quantitative PCR was performed.

*Semi-Qualitative Real Time PCR*

Diluted cDNA (10 µl) was used as a template for semi-qualitative Real Time PCR amplification in 25 µL reactions consisting of 12.5 µL SYBR Green Supermix (Bio-Rad Laboratories), 0.5 µL $H_2O$ and 1 µL each forward and reverse primer. Ovine GAPDH, IGF-I, IGF-II, IGFBP1, IGFBP3, IGFBP6, estrogen receptor α (ERα), estrogen receptor β (ERβ), progesterone receptor (PR), ADP ribosylation factor-1 (Arf1), hysdroxysteroid dehydrogenase-17-beta-1 (Hsd17β1), pre-proghrelin (PPG), and tumor necrosis factor alpha (TNFα) primers were designed using Primer 3 software to generate ~100bp amplicons. Semi-quantitative RT-PCR was performed using 40 cycles of 95 °C for 30 sec and 62 °C for 30 sec. Following amplification, cDNAs were melted (melting curve analysis) to ensure quality of amplification by incubating RT-PCR products for 10 sec at each step with increase in temperature by 0.5 °C from 55 °C to 95 °C in each cycle. Messenger RNA levels were quantified and reported relative to GAPDH.

*Statistical analysis*

All mRNA data were analyzed by GLM procedures of SAS (Version 9.0). Type III sums of squares were used to examine mean differences in the average fold change of mRNA expression within the endometrium of fasted as compared to fed ewes.

**Results and Discussion**

At 72 hr following administration of $PGF_2\alpha$, some ewes had not ovulated, as serum concentrations of estradiol remained elevated. Ewes selected for mRNA analysis had ovulated by 72 hrs, and serum concentrations of estradiol were low. Since numbers of large follicles and ovulation rate were unaffected, early embryo survival must be affected by fasting. Fasting may effect embryo quality, but may also alter the uterine environment adversely affecting embryo survival.

Expression of mRNA for IGF-I was up-regulated ($P < 0.01$) in the endometrium of fasted compared to fed ewes (Fig. 1). Gene expression for IGF-II, IGFBP1, IGFBP3, IGFBP6, ERα, ERβ, PR, Arf1, Hsd17β1, PPG, and TNFα did not differ ($P \geq 0.4$) among fasted and fed ewes (Table 1). Although estrogen can stimulate IGF-I synthesis, serum concentrations of estrogen did not differ ($P = 0.7$) among treatments, nor was there a time by treatment interaction ($P = 0.54$; data not shown). Differences in serum concentrations of estrogen may be masked by the limited number of blood samples collected.

Nutritional influences on reproduction may effect reproductive efficiency indirectly through the liver IGF-I system or of affect IGF synthesis directly in the reproductive tissues (Roberts et al., 2001). While mRNA levels of IGF-I within the endometirum were up-regulated in fasted ewes, serum concentrations of IGF-I was decreased and concentrations did not return to levels comparable to those of control ewes until 72 hr following realimentation (Kiyma et al., 2004), suggesting differential regulation of liver and tissue IGF synthesis.

Receptors for IGF-I are found in several tissues throughout the body. IGF-I and IGF-II stimulate hormone synthesis and secretion in ovarian granulosa and theca cells (Jones and Clemmons, 1995). The IGFs are also responsible for stimulation of DNA synthesis and cell replication, by affecting the cell cycle (Jones and Clemmons, 1995). Depending on the location of the embryo within the reproductive tract IGF may contribute to the preparation status of the endometrium for embryo implantation and survival. The decrease in serum concentration of IGF-I may contribute to the decreased numbers of follicles in fasted ewes (Alexander et al., 2007).

IGF-I is under the direct influence of estrogen and acts as a mediator for some of the actions of estrogen on the uterus (Stevenson et al., 1994). In the present study there were no differences in serum concentrations of estradiol, yet IGF-I was up-regulated in the endometrium of fasted ewes. In other studies decreased serum concentrations of estradiol were reported in fasted compared to fed ewes during the 24 h before the anticipated surge-release of LH (Alexander et al. 2007). Discrepancy in results could be attributed to the decreased sampling frequency in the present study.

In conclusion, fasting during the luteal phase of the estrous cycle preceeding proestrus influences uterine expression of IGF-I which may detrimentally affect peri-implantation embryo survival.

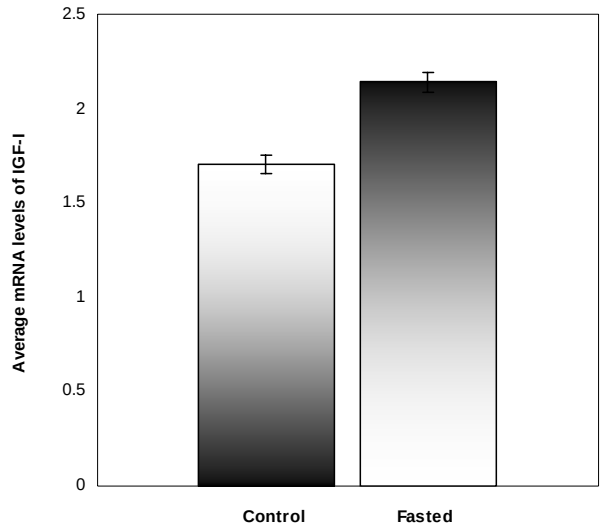


Figure 1. Expression of IGF-I mRNA within the endometrium of fed and fasted ewes which had ovulated.

Table 1. Relative concentrations of mRNA present within endometirum of fasted as compared to fed ewes using semi-quantitative PCR

| Gene | Fold Change [a] | P value |
|---|---|---|
| IGF-II | 0.84 | 0.52 |
| IGFBP1 | 1.13 | 0.92 |
| IGFBP3 | 1.25 | 0.50 |
| IGFBP6 | 0.91 | 0.40 |
| Estrogen receptor α | 1.03 | 0.93 |
| Estrogen receptor β | 1.60 | 0.47 |
| Progesterone receptor | 1.28 | 0.47 |
| Arf1 | 0.91 | 0.72 |
| HSD17β1 | 1.01 | 0.99 |
| PPG | 1.48 | 0.21 |
| TNFα | 0.88 | 0.70 |

**a** Fold change represents up or down-regulation of mRNA in fasted compared to fed ewes.

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# PREGNANCY RATES AND SERUM INSULIN-LIKE GROWTH FACTOR-I, TRIIODOTHYRONINE, AND PROGESTERONE PROFILES IN RAMBOUILLET EWES TREATED WITH RECOMBINANT BOVINE SOMATOTROPIN BEFORE BREEDING

**L. E. Camacho, J. M. Benavidez, and D. M. Hallford**

**New Mexico State University, Las Cruces, NM 88003**

**ABSTRACT:** Twenty three Rambouillet ewes (68.8 ± 1.5 kg) were used to determine the influence of bovine somatotropin (bST) on serum concentrations of IGF-I, triiodothyronine (T3), progesterone (P4) and pregnancy rates. Before beginning a fall breeding period, ewes received an intravaginal insert containing 0.3 g of P4 to synchronize onset of estrus. After 12 d, inserts were removed (d 0) and ewes (stratified by BW and age) received either 0 (control, n = 11) or 250 (n = 12) mg of recombinant bST (Posilac, Monsanto, s.c.). Ewes were joined with fertile rams 24 h after insert removal. Blood samples were collected daily from d 0 to 20 after insert removal. Serum IGF-I concentrations were 315 and 437 (± 58) ng/mL in control and bST-treated ewes 2 d after receiving bST ($P = 0.02$) and remained elevated ($P < 0.03$) in bST- treated ewes through d 13 ($P < 0.05$). Peak IGF-I values in bST-treated ewes (511 vs 193 ± 44 ng/mL for controls) occurred on d 8. Serum T3 concentrations were similar ($P > 0.20$) between treatments from d 0 through 8. On d 9, T3 values were 0.88 and 0.76 (± 0.03) ng/mL for control and bST-treated ewes ($P = 0.01$), respectively. Controls continued to have greater ($P < 0.04$) serum T3 concentrations than did treated ewes until d 18 at which time values were 0.73 and 0.62 (± 0.02) ng/mL ($P = 0.003$), respectively. Serum P4 was similar ($P > 0.10$) in control and bST-treated ewes from d 0 through 3 but was elevated ($P < 0.05$) from d 4 to d 8 in control ewes. Serum P4 was again similar ($P > 0.10$) between treatments from d 9 to 20. Pregnancy rates were determined by serum P4 values greater than 1 ng/mL on d 20. Eight of 11 (72%) control ewes were pregnant whereas 8 of 12 (67%) bST-treated ewes were pregnant ($P = 0.75$). Treatment with bST at a synchronized estrus results in increased serum IGF-I and decreased T3 and P4 compared with control values. Pregnancy rates were not affected by bST treatment.

**Keywords:** growth hormone, reproduction, sheep

## INTRODUCTION

Early embryonic loss is a main cause of reproductive failure, particularly in repeat-breeding cows, where close to 50% of embryos die during the first 16 d after fertilization (Morales-Roura et al., 2001). In sheep, 20 to 30% of embryos die during the first 13 d after fertilization (Carrillo et al., 2006). Somatotropin is a pituitary hormone that controls many aspects of animal growth, nutrient metabolism, and reproduction of animals (Lucy, 2000). Bovine somatotropin **(bST)** treatment in sheep increased the proportion of embryos reaching advanced stages of development at the time of embryo recovery and improved the percentage of transferable embryos in superovulated ewes (Folch et al., 2001). Culturing bovine embryos in the presence of GH or IGF-I accelerated embryo development by d 8 after fertilization and increased the number of cells per embryo (Santos et al., 2004). Moreira et al. (2002) reported that bST treatment of superovulated donor cows reduced the number of unfertilized oocytes, increased the number of embryos that developed to the blastocyst stage, and increased the number of transferable embryos. Administration of bST resulted in increased IGF-I concentrations in serum and in the uterus and stimulated embryonic growth and differentiation, as well as influencing protein synthesis, such as interferon-tau (Morales-Roura et al., 2001). Carrillo et al. (2006) found that a single administration of bST 5 d before exogenous progestin withdrawal increased the proportion of ewes with multiple pregnancies, lambing rate of mature ewes, and prolificacy. Effects were associated with an increase in serum IGF-I. The objective of this experiment was to determine the influence of recombinant bST administered to ewes immediately before estrus on pregnancy rates and serum concentrations of IGF-I, triiodothyrone **(T3)**, and progesterone **(P4)**.

## MATERIALS AND METHODS

All procedures involving animals were approved by the New Mexico State University Institutional Animal Care and Use committee.

***Animals and Treatments***. Twenty three Rambouillet ewes (68.8 ± 1.5 kg) were maintained under ambient conditions during a fall breeding season at the West Sheep Unit on the main campus at New Mexico State University. Ewes were fed alfalfa hay at 1.6 kg daily and had free access to water, salt, and shade. Before initiating the fall breeding period, ewes received a progesterone-impregnated intravaginal insert (CIDR, 0.3 g P4) to synchronize onset of estrus. The CIDR was removed after 12 d and ewes were joined with fertile Rambouillet rams. Ewes were stratified by BW and age and randomly assigned to 1 of 2 treatments on the day of CIDR removal (d 0). Eleven control ewes received a s.c. injection containing 0.5 mL of saline immediately after CIDR removal. The second

group of 12 ewes received 250 mg of prolonged release bST (Posilac, Monsanto Co.).

***Blood Collection and Analysis***. Beginning on d 0 and continuing through d 20, blood was collected daily from ewes by jugular venipuncture into serum separator tubes (Corvac, Kendall Health Care, Sr. Louis, MO) and allow to clot at room temperature for 30 min. Samples were centrifuged at 4°C for 15 min. at 1,500 x g and serum was stored frozen in plastic vials until assayed. Serum IGF-I was quantified by double antibody RIA as described by Berrie et al. (1995). Serum T3 (Wells et al., 2003) and P4 (Schneider and Hallford, 1996) were also quantified by solid phase RIA using components of commercial kits (Coat-A-Count Siemens Medical Solutions Diagnostics; Los Angeles, CA). Within and between assay CV were less than 15% for all hormones.

***Statistical Analysis***. Effects of bST on IGF-I, T3, and P4 during the treatment period were examined by split-plot analysis of variance using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Treatment with bST was the main plot factor and time and the treatment by day interaction were subplot factors. When significant treatment by day interactions were detected, treatment effects were examined within day of sampling. Pregnancy rate was determined by a serum P4 value greater than 1.0 ng/mL on d 20 and treatment effects were determined using the Freq procedure of SAS.

## RESULTS AND DISCUSSION

Serum IGF-I values in treated and control ewes are presented in Figure 1. Serum IGF-I concentrations were 315 and 437 (± 58) ng/mL in control and bST-treated ewes 2 d after receiving bST ($P = 0.02$) and remained elevated ($P < 0.03$) in bST- treated ewes through d 13 ($P < 0.05$). Peak IGF-I values in bST-treated ewes (511 vs 193 ± 44 ng/mL for controls) occurred on d 8. These data demonstrate that bST induces IGF-I secretion in ewes. In agreement, Capuco et al. (2001) showed that serum concentrations of IGF-I were greater during bST than control periods in lactating dairy cows. Likewise, Carrillo et al. (2006) showed a similar IGF-I response to exogenous bST in Pelibuey ewes.

Serum T3 concentrations (Figure 2) were similar ($P > 0.20$) between treatments from d 0 through 8. On d 9, T3 values were 0.88 and 0.76 (± 0.03) ng/mL for control and bST-treated ewes ($P = 0.01$), respectively. Controls continued to have greater ($P < 0.04$) serum T3 concentrations than did treated ewes until d 18 at which time values were 0.73 and 0.62 (± 0.02) ng/mL ($P = 0.003$), respectively. Previous research has shown that treatment with bST did not alter serum T3 concentrations in lactating cows (Capuco et al., 2001). However, Flores et al. (2008) demonstrated an increase in serum thyroxine values in bST-treated beef cows.

Serum P4 (Figure 3) was similar ($P > 0.10$) in control and bST-treated ewes from d 0 through 3 but was elevated ($P < 0.05$) from d 4 to d 8 in control ewes. Serum P4 was again similar ($P > 0.10$) between treatments from d 9 to 20. Lucy et al. (1994) found that 25 mg/d of bST from d 0 to 21 of an estrous cycle did not affect cross-sectional area of the CL or plasma P4 concentration in older Holstein heifers. In addition, Gong et al. (1993) found that administration of bST beginning 14 d before ovulation did not increase the concentration of P4 in the subsequent estrous cycle in heifers. In contrast, administration of bST to lactating cows increased weight of the CL on d 17 and P4 concentration in plasma (Schemm et al., 1990).

Pregnancy rates were determined by serum P4 values greater than 1 ng/mL on d 20. Eight of 11 (72%) control ewes were pregnant whereas 8 of 12 (67%) bST-treated ewes were pregnant ($P = 0.75$). Previous studies have shown that pregnancy rates were increased in cyclic, lactating dairy cows when bST was injected at initiation of the Ovsynch protocol or near the TAI (Moreira et al., 2000, 2001; Morales-Roura et al., 2001; Santos et al., 2004). Carrillo et al. (2006) showed that bST administration to ewes 5 d before CIDR removal resulted in an increased number of lambs born compared with controls.

## IMPLICATIONS

Treatment of ewes with 250 mg of recombinant bovine somatotropin at the time of exogenous progesterone removal resulted in elevated serum IGF-I concentrations from day 2 (approximate day of conception) through day 13 (shortly before maternal recognition of pregnancy). Although pregnancy rate on day 20 did not differ from controls, the effect of treatment on lambs born awaits actual lambing data.


## ACKNOWLEDGEMENTS

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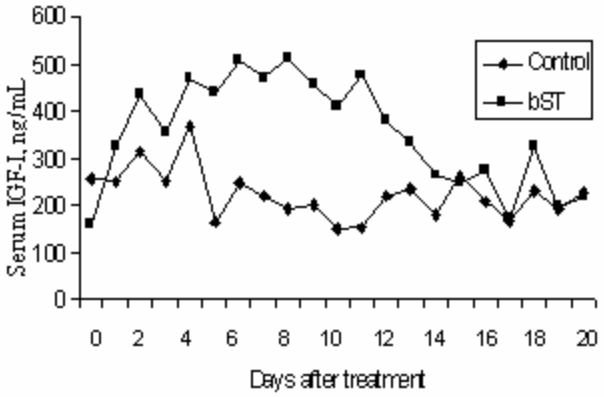


Figure 1. Serum IGF-I in Rambouillet ewes (n = 23 ewes) receiving 0 (control, n = 11) or 250 mg (bST, n = 12) of bovine somatotropin (bST) beginning on the day after insert removal (d 0).

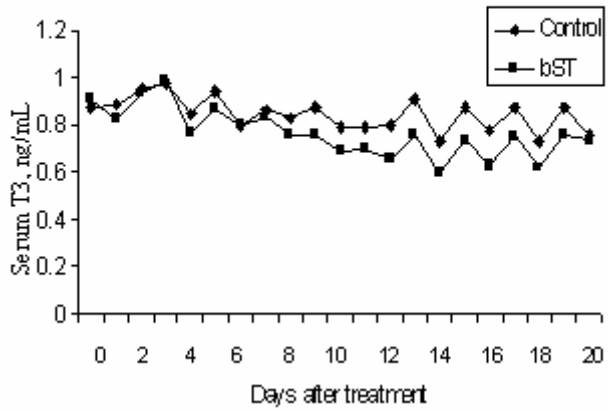


Figure 2. Serum triiodothyronine (T3) in Rambouillet ewes (n = 23 ewes) receiving 0 (control, n = 11) or 250 mg (bST, n = 12) of bovine somatotropin (bST) beginning on the day after insert removal (d 0).

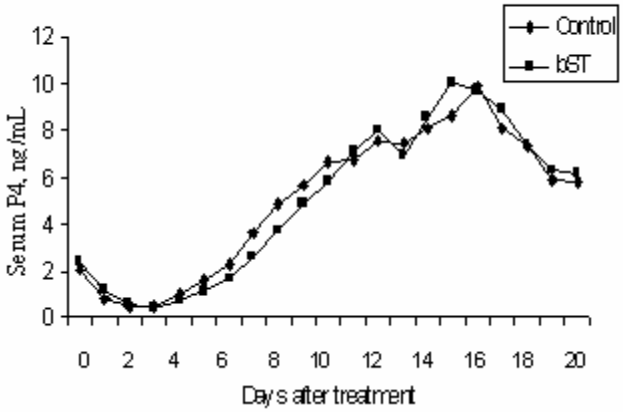


Figure 3. Serum progesterone (P4) in Rambouillet ewes (n = 23 ewes) receiving 0 (control, n = 11) or 250 mg (bST, n = 12) of bovine somatotropin (bST) beginning on the day after insert removal (d 0).

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# OVARIAN FUNCTION IN HEIFERS CONSUMING LOW QUALITY DORMANT FORAGE

**C. M. Gardner, J. D. Walsh, S. I. Valverde-Saenz, M. B. Horvath, J. T. Mulliniks, D. M. Hallford, S. L. Lodge-Ivey, K. K. Kane, and D. E. Hawkins**

Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM 88003

**ABSTRACT:** Dormant forages are the primary diet available to beef cows in the western United States. This study investigated effects of low quality forage on hormone concentrations and follicular dynamics in beef heifers. Eighteen, 2-yr old, rumen cannulated, cyclic crossbred heifers (428 ± 44 kg) were stratified by BW and randomly assigned to 1 of 3 dietary treatments (n = 6). Treatment 1 (Trt 1) was composed of 50% buffalo straw-50% old world bluestem (CP% = 5.1, NDF% = 74.6), treatment 2 (Trt 2) was Sudan hay (CP% = 9.5, NDF% = 57.2), and treatment 3 (Trt 3) was a mixture of 50:50 Trt 1 and Trt 3, (CP% = 8.1, NDF% = 63.3). Individually fed heifers were allowed ad libitum access to treatment between 0700 and 1900, when orts were recorded. Feeding began during the luteal phase of the first cycle, proceeded through 1 ovulation, and was concluded when the second dominant follicle reached maximum size as estimated by previous cycle length and follicle size (mean = 12 mm²). Serum samples were collected and follicles measured via ultrasonography every other day for the first 25 d and then daily until the end of the study. Serum insulin, IGF-I, and LH were quantified. The second estrous cycle, prior to the conclusion of the study, was analyzed for length, number of follicular waves, and maximal follicle size. Treatment had no effect on mean number of follicular waves ($P$ = 0.33), however 2 of 6 heifers in Trt 2 had 4-wave cycles while no heifers in Trt 1 or 3 had more than 3 waves. Treatments had no effect ($P$ > 0.30) on maximum follicle size or cycle length. Neither serum IGF-1 nor insulin differed among treatments ($P$ > 0.30) but mean insulin tended to increase across all treatments over time ($P$ = 0.06). Serum LH was not affected by treatment ($P$ = 0.59). Under the conditions of this study, slight nutritional differences between low quality forages fed for a short period of time, had no effect on follicular waves, dominant follicle size, or cycle length in cyclic beef heifers.

Key Words: heifer, dormant forage, ovary

## INTRODUCTION

In much of New Mexico, cows graze dormant forage 40 wks of the year (Forbes and Allred, 2001). Therefore, many spring calving cow herds are required to calve and rebreed while grazing dormant forage. As supplementation becomes more expensive, it is increasingly important to efficiently utilize dormant forage in range situations. However, reproductive efficiency is still imperative to maintain profitability for cow/calf producers. Understanding when dormant forage combined with body reserves is adequate to meet the nutrient demands of the animal to maintain reproductive efficiency with minimal supplementation is vital.

The specific mechanisms by which dormant forage affect reproduction are not fully understood. In long-term studies, with chronically nutrient-deprived heifers, LH was not affected, even when heifers lost BW and size of dominant follicle decreased (Rhodes et al., 1996, Bossis et al., 1999). The concentration of LH seems to only decrease in the estrous cycle prior to the onset of anestrus (Bossis et al., 1999). However, in these studies heifers were fed significantly below maintenance, with energy being the most limiting nutrient over a long period of time. Similarly, heifers fed 40% of maintenance for a short period of time (< 20 d) had decreased maximum follicle size in 2 consecutive follicular waves, without any change in LH compared to heifers fed at or above maintenance (Mackey et al., 2000). Energy was once again the limiting nutrient.

Insulin and IGF-1 may be signals indicative of nutritional status. Both insulin and IGF-1 have been shown to be involved in follicular growth and ovulation without changes in gonadotropins (Simpson et al., 1994; Griffin and Ojeda, 1996; Gong et al., 2002).

The aim of this study was to determine the effects of low quality, dormant forages, differing in NDF and CP, on ovarian follicular dynamics and on LH secretion when fed ad libitum. Furthermore, the study determined changes in insulin and IGF-1 over time due to poor quality diets and examined if these hormones may be the link to changes in follicular dynamics without changes in LH.

## MATERIALS AND METHODS

This study was conducted at the New Mexico State University Livestock Research Center in Las Cruces. Prior to being placed on study, heifers were fed chopped alfalfa once daily. After being placed on study, heifers were individually fed and provided ad libitum access to water and mineral supplement. All animal handling and experimental procedures were in accordance with guidelines set by the New Mexico State University Institutional Animal Care and Use Committee.

Eighteen, 2-yr-old, rumen cannulated, cyclic crossbred heifers (428 ± 44 kg) were stratified by BW and randomly assigned to 1 of 3 dormant forage, dietary treatments (n = 6, each) to simulate different dormant range situations. Each diet represented a different quality of dormant forage, with differences in CP and NDF. Treatment 1 was composed of 50% buffalo straw-50% old world bluestem (**Trt** 1:CP% = 5.1, NDF% = 74.6),

treatment 2 was Sudan hay (**Trt 2**:CP% = 9.5, NDF% = 57.2), and treatment 3 was a mixture of 50:50 BS-OW and S, (**Trt 3**:CP% = 8.1, NDF% = 63.3). Heifers were allowed ad libitum access to treatment between 0700 when fresh feed was placed in bunks, and 1900, when orts were collected and weighed to ensure ad libitum intake throughout the study. Heifers were fed for a minimum of 28 d. Heifers were placed on the diet during the mid luteal phase of an estrous cycle. While on study, heifers proceeded through 1 ovulation and another entire estrous cycle. The study was concluded with heifer slaughter by stunning with a captive bolt gun and puncturing the jugular for exsanguination, to collect tissues for associated studies. Heifers were slaughtered when the dominant follicle reached maximum size, but prior to ovulation, as estimated by previous dominant follicle size (mean = 12 mm²) and previous estrous cycle length. Therefore, heifers were on study different number of days, depending on the length of their estrous cycle. This allowed for observation of one ovulation during the study and observation of ovarian follicular activities via ultrasonography during an entire estrous cycle. Serum samples were collected, via caudal venipuncture and stored at -20ºC (Corvac, Sherwood Medical, St. Louis, MO). Serum samples were collected on 2 alternating days before being placed on the diet (baseline), every other day for the first 25 d on diet, and daily until the conclusion of the study. Collection was performed prior to morning feeding to allow for a 12-h fasting time. Heifers were weighed weekly, prior to morning feeding, to assess BW change.

Prior to heifers being placed on study, ultrasound examination was performed every other day for 3 wk to confirm cyclicity and to determine when each heifer would be placed on a treatment. During the study, transrectal ultrasonography was performed on days when serum was collected. Ultrasonography was performed using a real time B mode, equipped with a 7.5 MHz, linear-array, intrarectal transducer. Follicles were measured to determine maximum dominant follicle size for both ovulatory and anovulatory follicles. The data were used, with progesterone assay data, to determine length of estrous cycle. Number of follicular waves were counted using methods described by Ginther (1993).

Serum progesterone was assayed by RIA to confirm cyclicity (Schneider and Hallford, 1996) with an intra assay CV of 4.8%. Serum insulin was quantified by RIA in all samples using methods described by Reimers (et al., 1982) with an intra assay CV of 5.9% and an inter assay CV of 9.6%. In the first five serum samples, including both baseline samples, and in the last five samples prior to the conclusion of the study, IGF-I was quantified using methods described by Berrie et al. (1995) with intra and inter assay CV of 8.1 and 7.7, respectively. Lutenizing hormone was quantified and compared in 2 periods during the study using RIA method described by Hoefler and Hallford (1987). These 2 periods were localized around the appearance of a preovulatory follicle. The 5 serum samples closest to the ovulation day during the study, as determined by ultrasonography, and in the last 5 samples prior to the conclusion of the study, which was determined by the appearance of a preovulatory follicle, were assayed for LH. The intra assay CV for LH was 6.3%.

Length of estrous cycle, insulin, IGF-1, and LH concentration were analyzed by analysis of variance (ANOVA) using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC) for a completely randomized design. Number of follicular waves per estrous cycle was analyzed using Chi-square model in SAS. Maximum follicle size for last follicular wave, the last preovulatory follicle and change in follicle size from the first preovulatory follicle to the second preovulatory follicle, intake, and BW change were analyzed using the GLM procedure of SAS. Each heifer was considered an experimental unit. Variables were analyzed over time and between treatments.

## RESULTS AND DISCUSSION

Heifers fed Trt 1 lost BW during the study while heifers on Trt 2 and Trt 3 gained weight with change in BW of -8.8, 6.0, and 7.3 kg, respectively. Also, 5 of 6 heifers fed Trt 1 lost BW, while only 5 of 12 heifers on Trt 2 and Trt 3 lost BW. Heifers fed Trt 1 tended to lose more weight than heifers fed Trt 2 or Trt 3 ($P = 0.11$). There was no difference in BW change between heifers fed Trt 3 and Trt 2 ($P = 0.90$). Heifers consumed less of Trt 1 than Trt 3 or 2 and less of Trt 3 than Trt 2 ($P < 0.01$) consuming 6.3, 8.7, and 9.3 kg, respectively. Although Trt 3 was a 50:50 mixture of Trt 1 and Trt 2, feed analysis, intake and the performance of the heifers indicate Trt 2 and Trt 3 were similar.

There was no differences between mean number of follicular waves per cycle ($P = 0.33$). However, 2 of 6 heifers fed Trt 2 had 4 wave cycles while no heifers fed Trt 1 or Trt 3 had more than 3 follicular waves. There was no difference in estrous cycle length between treatments ($P = 0.65$). According to Mackey et al., (2000) heifers fed 0.4 of maintenance had smaller maximum follicle size compared to heifers fed 1.2 and 2.0 of maintenance the first follicular wave after initiation of diets. However, mean dominant follicle size in the last follicular wave prior to the conclusion of the study did not differ ($P = 0.64$) among treatments (Trt 1 = 9.1 ± 3.0 mm², Trt 3 = 10.1 ± 1.8 mm², Trt 2 = 11.0 ± 4.7 mm²). Also, treatment had no effect ($P > 0.17$) on maximum follicle size at the conclusion of the study (Trt 1 = 10.1 ± 2.7 mm², Trt 3 = 13.2 ± 3.9 mm², Trt 2 = 11.9 ± 3.0 mm²) or change in maximum follicle size from the estrous period during the study to the estrus period at the conclusion of the study (Trt 1 = -1.1 ± 3.8 mm², Trt 3 = +3.3 ± 4.8 mm², Trt 2 = +2.4 ± 3.2 mm²). Heifers were slaughtered when it was determined that the preovulatory follicle was at a maximum size as determined by previous maximum preovulatory follicle size and cycle length, however error in this determination could effect both maximum follicle size at the conclusion of the study and change in maximum follicle size from the estrus cycle during the study to the estrus cycle at the end of the study. One heifer fed Trt 1 became anestrus which may be due to her refusal to consume the diet.

Treatment had no affect on mean serum insulin concentration among treatments ($P = 0.35$). However, insulin tended to be higher at the end of the study versus the

beginning across all treatments ($P$ = 0.06). This was not expected and may be due to heifers being individually fed an ad libitum diet and therefore increasing total intake from the alfalfa diet fed before the start of the study. Mean serum IGF-1 concentrations were not different among treatments ($P$ = 0.27) and did not change from the beginning to the end of the study ($P$ = 0.14). Mean LH concentration was not different among treatments ($P$ = 0.45) or periods ($P$ = 0.12). According to Mackey et al., (2000) when heifers were fed 0.4 of maintenance for a short period (< 20 d) there was no dietary effect on LH characteristics but Bossis et al. (1999) reported depressed LH concentrations the estrous cycle prior to nutritionally-induced anestrus. The dormant forage diets, fed ad libitum were adequate to maintain cyclicity and therefore we would not expect to see a change in LH. This may also account for the lack of change in the ovarian follicular dynamics.

## IMPLICATIONS

In non-pregnant, non-lactating beef heifers, maintenance demands are low thus, short term stress due to dormant forage low in CP, had no adverse effect on circulating hormone concentration or ovarian follicular dynamics. If beef heifers, with low nutrient requirements due to physiological state, are allowed to maximize intake, low quality dormant forage may be sufficient to meet their requirements.

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**Table 1.** Body weight and follicular changes between treatments.

| | Treatments [a] | | | | |
|---|---|---|---|---|---|
| Variable | Trt 1 | Trt 2 | Trt 3 | SEM [b] | *P*-value |
| BW change, kg [c] | -8.8 | 6.0 | 7.3 | 7.5 | 0.27 |
| Last wave maximum follicle, $mm^2$ [d] | 9.1 | 11 | 10.1 | 4.7 | 0.64 |
| Preovulatory maximum follicle, $mm^2$ [e] | 10.1 | 11.9 | 13.2 | 3.9 | 0.28 |
| Follicle change, $mm^2$ [f] | -1.1 | 2.4 | 3.3 | 4.8 | 0.17 |

[a] Treatments: Trt 1 = 50% Buffalo straw, 50% Old world bluestem; Trt 2 = Sudan grass; Trt 3 = 50% Trt 1, 50% Trt 2
[b] Most conservative standard error
[c] BW change from d 1 of the study to end of study
[d] Last wave before the appearance of final preovulatory follicle
[e] Maximum preovulatory follicle size prior to slaughter
[f] Change in maximum follicle size from preovulatory follicle during the treatment period to preovulatory follicle prior to slaughter

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# ASSESSMENT OF INSULIN-LIKE GROWTH FACTOR-1 AS AN INDICATOR OF COMPETENCE FOR REBREEDING IN FIRST CALF HEIFERS [1]

**A. J. Roberts**
USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT

**ABSTRACT:** Objective of this research was to evaluate whether concentrations of IGF-1 in circulation, BW and BCS measured before calving, after calving and immediately before breeding could be used as indicators of nutritional competency for time of rebreeding in first calf heifers. Heifers were artificially inseminated at about 14 mo of age and then exposed to bulls for the remainder of a 53-d breeding season. This breeding season resulted in a 71-d calving period that began on March 8. Blood samples, BW and BCS were obtained at 3 time points from 74 cows: February 25, which was 12-d prior to beginning of the calving period, 0 to 14 d after calving (date varied in relation to calving), and again on May 27 (before a 52-d breeding season). Separate regression analyses were run for each time point, using a model that included concentration of IGF-1, cow BW, cow BCS and day of calving as independent variables, with day of second calving as the dependant variable. For precalving data, birthday of second calf was influenced ($P = 0.01$; $R^2 = 0.17$) by birthday of previous calf ($P = 0.01$; b = 0.31 ± 0.12) and circulating concentration of IGF-1 ($P = 0.001$; b = -0.20 ± 0.06), but not by BW ($P = 0.4$) or BCS ($P = 0.9$). Regression analyses with post-calving ($P = 0.14$; $R^2 = 0.10$) and prebreeding ($P = 0.25$; $R^2 = 0.07$) data accounted for less variation in birthday of second calf than precalving measurements of IGF-1. However, similar trends toward a negative relationship between IGF-1 and second calving day were observed with post-calving ($P = 0.06$; b = -0.08 ± 0.04) or prebreeding ($P = 0.09$; b = -0.07 ± 0.04) measures of IGF-1. Results indicate precalving concentrations of IGF-1 may be a useful indicator of nutritional competency for rebreeding in first calf heifers. In contrast, measures of BW and BCS at the 3 time points evaluated in this study did not appear to be indicative of time to rebreeding.

Key Words: Body weight, IGF-1, Pregnancy

## Introduction

A major challenge for cattle producers is to get cows rebred after calving. This is especially true for young cows after their first and second calf due to the fact that these cows are still growing which adds to their nutritional requirements. In many production settings, forage conditions may not be adequate to support the combined requirements of growth and gestation in the last trimester of pregnancy, and growth and lactation after calving to allow for young cows to resume cycling in sufficient time to be rebred in the subsequent breeding season. This limitation can be overcome by providing additional supplemental feed resources; however this can substantially increase cost of production. Thus, producers are faced with the challenge of balancing feed resources to achieve optimal reproductive performance, while minimizing costs of feed inputs.

Visual appraisal of cow BCS has traditionally been used to assess nutritional status of cattle. Guidelines established several decades ago recommend that cows be a BCS of 5 to 6 at calving to help ensure optimal numbers of cows will be nutritionally competent to rebreed. However, nutritional status is dynamic and substantial change and lag in time may be required to detect changes by visual appraisal of BCS.

Circulating concentrations of IGF-1 fluctuate in response to level of nutrient intake (McGuire et al., 1992), and appear to provide objective indicators of nutritional status in dairy (Ronge et al., 1988; Spicer et al., 1990) and beef (Nugent et al., 1993; Roberts et al., 1997, 2005) cattle. Because circulating concentrations of IGF-1 are associated with nutritional status, there is potential to use measurements of this hormone as an indicator of nutritional competency for rebreeding. Objective of this research was to evaluate whether concentrations of IGF-1 in circulation, BW and BCS measured before calving, after calving or immediately before breeding could be used as indicators of nutritional competency for time of rebreeding in first calf heifers.

## Materials and Methods

All research protocols used in this study were approved by our institutional Local Animal Care and Use Committee. Cows used in this study were a stable composite population (**CGC**; ½ Red Angus, ¼ Charolais, ¼ Tarentaise In the year preceding the study, heifers were artificially inseminated and then exposed to bulls for the remainder of a 53-d breeding season. This breeding season resulted in 87 heifers giving birth to live calves in a 71-d calving period that began on March 8. Heifers were calved in small pastures, with access to ~ 10 to 11 kg of hay/d. Date of calving, sex and BW of calf were recorded at birth. After calving, cows and calves were placed on native range.

Blood samples, BW and BCS were obtained from

[1]USDA-ARS is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA or the author and does not imply its approval to the exclusion of other products that may be also suitable. Assistance of Brooke Shipp with sample collection and laboratory analysis is greatly appreciated.

each cow at 3 time points: February 25 (12-d prior to beginning of the calving period), 0 to 14 d after calving (date varied in relation to calving), and again on May 27 (17 d before a 52-d breeding season). For the last 5 cows that calved, the post-calving measurements were made on May 27. These measurements were included in the analyses for both post-calving and prebreeding measurements.

Blood samples were collected from the tail vein and serum from these samples was used to determine circulating concentrations of IGF-1. Concentrations of IGF-1 were determined by RIA after acid-ethanol extraction, as described previously (Roberts et al., 1997) using AFP4892898 as the primary antiserum. Inter-assay and intra-assay (n = 2) CV for IGF-1 were 12 and 15%, respectively.

On June 7, 87 cows were subjected to a CO-Synch + CIDR protocol to synchronize estrus, with timed AI of heifers not detected in heat by 48 h after CIDR removal and an i.m. injection of PGF on June 14 (74 d after average date of calving). After AI, cows were placed on native range and exposed to bulls for the remaining duration of a 52-d breeding season. Of the 87 cows entering the breeding season, 7 did not become pregnant and 6 were culled for various reasons or lost pregnancies, leaving 74 that calved the following year. Measures of BW were recorded for both the calf and cow (n = 86) at weaning to provide an indication for level of production. Day of second calving was recorded for 74 cows and BW at birth was recorded for 73 calves.

Data were analyzed with the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Separate regression analyses were run for each time point, using a model that included concentration of IGF-1, cow BW, cow BCS and day of calving as independent variables, with day of second calving as the dependant variable. Independent variables with F values < 1 were removed from the analyses in a stepdown approach to simplify models when appropriate. Simple correlations were determined between variables considered to be indicative of nutritional status and variables associated with production status.

## Results and Discussion

Descriptive statistics for variables measured in the study are presented in Table 1. Regression analyses with models including effects of day of first calving, IGF-1, BW and BCS on day of second calving revealed that precalving measurements accounted for more of the variation in birthday of second calf ($P = 0.01$; $R^2 = 0.17$) than post-calving ($P = 0.14$; $R^2 = 0.10$) or prebreeding ($P = 0.25$; $R^2 = 0.07$) measurements when the full models were compared. Birthday of previous calf and circulating concentration of IGF-1 accounted for variation in birthday of second calf in regression models for each time period, but BW or BCS did not (F values < 1). Regression coefficients for the reduced models after removal of BW and BCS measurements are shown in Table 2. Previous studies have documented negative associations among circulating concentration of IGF-1 at various times after calving and duration of postpartum anestrus (Nugent et al., 1993; Roberts et al., 1997). The present study extends these previous findings by demonstrating that subsequent calving date is also negatively associated with circulating concentrations of IGF-1 at different times during the postpartum period. In addition, the present study provides evidence that measures of IGF-1 prior to calving may be a more robust indicator of postpartum rebreeding performance than measures of IGF-1 after calving.

The reason that association among IGF-1 and date of second calving was greater for precalving measures of IGF-1 than post-calving and prebreeding measures was not identified in the present study. Concentrations of IGF-1 after calving may be confounded by time after calving, level of milk production and nutritional status. Circulating concentrations of IGF-1 decline precipitously at parturition and gradually increase over time (Ronge et al., 1988; Vega et al., 1991). Magnitude of the decline after parturition and rate of increase over time vary in response to level of milk production (Ronge et al., 1988; Schams et al., 1991), level of dietary intake (Ronge et al., 1988; Spicer et al., 1990; Nugent et al., 1993), breed (Spicer et al, 2002) and dietary intake by breed interactions (Roberts et al., 2005). Including the number of days from calving to time when post-calving measurement was made as an independent variable ($P = 0.17$) in the regression analysis for this time period resulted in minimal improvement in the model ($P = 0.052$; R-square = 0.11). In addition, regression models including changes in IGF-1, BW and BCS between the different time points did not improve the ability to predict day of second calving ($P$ exceeded 0.24 for change in any measure between any possible time point comparison; data not shown). Thus, relationship of IGF-1 and ability to rebreed may be more evident when IGF-1 is measured prior to calving because of the absence of physiological factors affecting IGF-1 that occur after parturition.

Pearson correlation coefficients among variables are shown in Table 3. Day of first calving was positively associated ($P < 0.1$) with pre- and post-calving measures of IGF-1, post-calving and prebreeding BW, and BCS at all time points. The interpretation of these associations is that nutritional environment in the calving pasture, when cows were fed hay, was favorable compared to conditions in pastures used before and after calving. Thus, the longer a heifer was in the calving pasture the greater time with access to hay. Associations among day of calving and precalving measures of IGF-1 and BCS may reflect lower nutritional status of heifers at later stages of gestation when the measurement was made due to greater nutritional requirements associated with the later stages of gestation. The impact that the nutritional conditions occurring during this study may have on the results should be considered when extrapolating to other settings.

Measures of IGF-1, BW and BCS at each time point were correlated ($P < 0.001$) with their respective measures at other time points (Table 3). Correlations between measures of BW at different times were larger in magnitude than correlations between measures of IGF-1 or BCS. Measurers of IGF-1 before calving were correlated with pre and post-calving BCS, but not the prebreeding BCS, whereas both post-calving and prebreeding measures of IGF-1 were correlated with BCS at these two times. The lack of significant association of precalving IGF-1 with prebreeding BCS is

consistent with discussion above concerning the results from regression. Heifer BW before calving was correlated with precalving BCS, but not BCS at subsequent measurements. In contrast measures of BW after calving and at prebreeding were correlated with all measures of BCS. Measures of BW at the three time points were also correlated ($r > 0.81$) with cow BW at weaning and BW of second calf ($r > 0.36$), consistent with genetic correlations among these traits, which is a limitation of using BW as an indicator of nutritional status. Correlations were not observed between measures of BW and IGF-1. This is in contrast to previous results where positive correlations were observed between BW and IGF-1 at different times after calving in mature cows (Roberts et al., 2005), which may reflect a difference between animals that have and have not reached mature BW. Measures of IGF-1 at all 3 time points were negatively associated with calf BW at weaning (Table 3). Direction of this association is consistent with trends for negative associations between IGF-1 in the postpartum period and peak milk production in beef cows (Roberts et al., 2005) and strong negative associations between circulating IGF-1 during the peripartum period and level of milk production in dairy cows (Ronge et al., 1988). The fact that this association exists with IGF-1 measured prior to parturition indicates that any nutritional connection can be prior to initiation of lactation or that the association may be independent of nutrient status.

## Implications

Failure of cows to rebreed after calving is a major factor influencing economic viability beef cattle operations. This is especially true for young cows after their first and second calf due to the fact that these cows are still growing which adds to their nutritional requirements. Industry has adapted the use of BCS as a tool for producers to evaluate competency of their cows to resume cycling after calving. Results from the present research indicate that circulating concentrations of IGF-1 during the peripartum period were better indicators of rebreeding performance of first calf heifers than either BCS or BW. Results from the present study also indicate that associations of IGF-1 and rebreeding were stronger when IGF-1 was measured prior to calving, rather than after calving.

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Table 1. Descriptive statistics for variables measured

| Variable | N | Mean | SD | Min | Max |
|---|---|---|---|---|---|
| First calving | | | | | |
| Calving day | 87 | 24 | 16 | 0 | 72 |
| Precalving IGF-1, ng/mL | 87 | 67 | 33 | 11 | 210 |
| Post-calving IGF-1, ng/mL | 86[a] | 78 | 52 | 10 | 240 |
| Prebreeding IGF-1, ng/mL | 87 | 93 | 53 | 10 | 281 |
| Precalving BW, kg | 87 | 420 | 40 | 288 | 494 |
| Post-calving BW, kg | 87 | 388 | 41 | 270 | 475 |
| Prebreeding BW, kg | 87 | 379 | 39 | 262 | 464 |
| Precalving BCS | 87 | 5.0 | 0.5 | 4.0 | 6.0 |
| Post-calving BCS | 87 | 4.6 | 0.5 | 3.5 | 6.0 |
| Prebreeding BCS | 87 | 4.5 | 0.5 | 3.0 | 5.5 |
| Calf BW at Birth, kg | 87 | 31 | 5 | 20 | 51 |
| Calf BW at wean, kg | 86 | 178 | 25 | 115 | 235 |
| Cow BW at wean, kg | 86 | 410 | 45 | 277 | 514 |
| Second calving | | | | | |
| Calving day | 74 | 25 | 17 | 1 | 63 |
| Calf BW at Birth, kg | 73 | 34 | 5 | 20 | 47 |
| Calf BW at wean, kg | 67 | 209 | 25 | 149 | 273 |

[a]Lost one sample

Table 2. Regression equations for effects of day of first calving and circulating concentrations of IGF-1 measured at one of three times on day of second calving (n = 74)[1]

| Measurement | *P* model | R-square | Intercept (*P* = ) | | Calving day (*P* = ) | | IGF-1 (*P* = ) | |
|---|---|---|---|---|---|---|---|---|
| Precalving | 0.002 | 0.164 | 32.11 ± 4.37 | (0.001) | 0.29 ± 0.12 | (0.017) | -0.20 ± 0.06 | (0.001) |
| Post-calving | 0.054 | 0.080 | 26.69 ± 4.09 | (0.001) | 0.23 ± 0.13 | (0.074) | -0.08 ± 0.04 | (0.033) |
| Prebreeding | 0.078 | 0.069 | 28.13 ± 4.65 | (0.001) | 0.18 ± 0.12 | (0.146) | -0.07 ± 0.04 | (0.051) |

[1]Inclusion of measures of BW and BCS in these regressions did not account for variation in day of second calving.

Table 3. Simple correlations among measures of IGF-1, BW, BCS prior to calving (Feb), after calving (post) and prior to breeding (May), day of birth (DOB) for 1st and 2nd calf, BW at birth for 1st and 2nd calf, and BW of 1st calf and cow at weaning

| | | Feb IGF-1 | Post IGF-1 | May IGF-1 | Feb BW | Post BW | May BW | Feb BCS | Post BCS | May BCS | Calf BW birth | Calf BW wean | Cow BW wean | DOB 2nd calf | Calf #2 BW birth |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n | | 87 | 86 | 87 | 87 | 87 | 87 | 87 | 87 | 87 | 87 | 86 | 86 | 74 | 73 |
| DOB | r = | **0.36** | **0.29** | 0.11 | -0.08 | **0.24** | 0.18 | 0.18 | **0.36** | **0.28** | 0.05 | **-0.65** | 0.15 | 0.13 | 0.10 |
| 1st Calf | *P* = | 0.01 | 0.01 | 0.30 | 0.44 | 0.03 | 0.09 | 0.09 | 0.01 | 0.01 | 0.68 | 0.01 | 0.17 | 0.26 | 0.42 |
| Feb | r = | | **0.57** | **0.41** | -0.06 | 0.06 | 0.02 | **0.24** | **0.35** | 0.14 | -0.10 | **-0.25** | 0.02 | **-0.31** | -0.11 |
| IGF-1 | *P* = | | 0.01 | 0.01 | 0.57 | 0.57 | 0.82 | 0.03 | 0.01 | 0.19 | 0.37 | 0.02 | 0.88 | 0.01 | 0.35 |
| Post | r = | | | **0.63** | -0.16 | -0.02 | 0.00 | 0.09 | **0.22** | **0.22** | -0.06 | **-0.24** | 0.02 | -0.19 | 0.00 |
| IGF-1 | *P* = | | | 0.01 | 0.13 | 0.88 | 0.97 | 0.43 | 0.04 | 0.04 | 0.60 | 0.03 | 0.87 | 0.10 | 0.99 |
| May | r = | | | | -0.12 | -0.02 | 0.04 | 0.14 | 0.17 | **0.32** | -0.14 | **-0.21** | 0.07 | -0.20 | 0.06 |
| IGF-1 | *P* = | | | | 0.29 | 0.88 | 0.68 | 0.20 | 0.11 | 0.01 | 0.20 | 0.06 | 0.51 | 0.08 | 0.62 |
| Feb | r = | | | | | **0.88** | **0.86** | **0.31** | 0.08 | 0.13 | **0.47** | 0.20 | **0.81** | 0.07 | **0.37** |
| BW | *P* = | | | | | 0.01 | 0.01 | 0.01 | 0.48 | 0.22 | 0.01 | 0.07 | 0.01 | 0.56 | 0.00 |
| Post | r = | | | | | | **0.94** | **0.31** | **0.31** | **0.29** | **0.37** | -0.06 | **0.86** | 0.05 | **0.45** |
| BW | *P* = | | | | | | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.57 | 0.01 | 0.66 | 0.01 |
| May | r = | | | | | | | **0.30** | **0.24** | **0.38** | **0.34** | -0.10 | **0.92** | 0.05 | **0.47** |
| BW | *P* = | | | | | | | 0.01 | 0.02 | 0.01 | 0.01 | 0.34 | 0.01 | 0.70 | 0.01 |
| Feb | r = | | | | | | | | **0.49** | **0.41** | -0.04 | -0.15 | **0.28** | -0.06 | 0.03 |
| BCS | *P* = | | | | | | | | 0.01 | 0.01 | 0.70 | 0.17 | 0.01 | 0.63 | 0.80 |
| Post | r = | | | | | | | | | **0.51** | **-0.25** | **-0.38** | 0.20 | -0.10 | -0.11 |
| BCS | *P* = | | | | | | | | | 0.01 | 0.02 | 0.01 | 0.06 | 0.39 | 0.34 |
| May | r = | | | | | | | | | | 0.04 | **-0.33** | **0.41** | -0.07 | 0.02 |
| BCS | *P* = | | | | | | | | | | 0.68 | 0.01 | 0.01 | 0.53 | 0.85 |

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# COMPARISON OF SALIVARY AND SERUM CORTISOL CONCENTRATION IN RESPONSE TO ACTH CHALLENGE IN SHEEP

**D. T. Yates, T. T. Ross, D. M. Hallford, L. J. Hill, and R. L. Wesley**
New Mexico State University, Las Cruces, NM

**ABSTRACT:** Cortisol concentration in blood serum is routinely used as a physiological marker of stress in animals. However, blood sampling is an invasive procedure and may itself be a stressor. The objective of this study was to determine if salivary cortisol concentration reflects serum cortisol concentration in ewes. In each of 2 studies, 6 Suffolk ewes were administered ACTH (100 IU, i.v.), while 6 others received saline. Serum and saliva samples were taken, and cortisol concentration was measured in each (RIA). Collection interval was 15 min for study 1 and 3 min for study 2. Cortisol concentration was similar ($P > 0.05$) between control and ACTH-treated ewes for serum area under the curve, time to peak serum cortisol, and time to peak saliva cortisol, and between saliva and serum for time to peak and return to baseline in both studies. Both saliva and serum cortisol concentration rose in response to both ACTH and saline injection, and rate of cortisol release was similar in both responses. Salivary area under the curve was greater ($P = 0.016$) for ewes receiving ACTH than control ewes in study 1, but this trend only approached significance ($P = 0.067$) in study 2. Return of serum cortisol to baseline was longer ($P < 0.05$) for ACTH-treated ewes in both studies, as was return of saliva cortisol to baseline ($P < 0.05$) for ACTH-treated ewes. Serum to saliva cortisol correlation analysis revealed close correlation between the 2 fluids in both studies (r = 0.88 and 0.73 for studies 1 and 2, respectively; $P < 0.0001$), and regression analysis indicated salivary to serum cortisol coefficients of b = 0.86 and 0.47 for studies 1 and 2, respectively ($P < 0.001$). These data indicate that salivary cortisol concentration is closely linked to concentrations in blood, and is suitable as a noninvasive substitute to serum collection for the measurement of cortisol in animals.

Keywords: ACTH challenge, saliva collection, salivary cortisol

## INTRODUCTION

Cortisol concentration has for decades been used as a physiological marker of stress in animals. The degree upon which this method is relied in animal welfare evaluation is evident by the wide array of species for which cortisol release has been successfully indexed: from non-human primates (Kuhar et al., 2005) to dolphins (Pedernera-Ramano et al., 2006). Blood plasma cortisol concentration is used extensively in livestock research, where importance of stress management is governed by financial considerations. However, an interest in development of easier and quicker sampling methods coupled with an understanding that invasive blood sampling for stress evaluation can cause stress led to investigation of saliva as an alternative physiological medium for cortisol measurement. Cook and Jacobson (1995) described a method of saliva collection for sheep in which a brief oral swab resulted in 1 to 2 mL of assayable fluid. Others found success with suction/aspiration protocols (Fell et al., 1985). Despite a general consensus of greater ease and reduced stress of salivary sampling compared to venipuncture, published opinions are divided on the value and reliability of salivary cortisol concentration when compared to that of blood. Fell and Shutt (1986, 1988) twice reported increases in salivary cortisol in sheep that corresponded directly to the introduction of stress. In the latter publication, the authors described similarity in the patterns of plasma and saliva cortisol concentrations. A year after proclaiming saliva collection a less stressful procedure than blood sampling (Cook and Jacobson, 1995), Cook (1996) showed a definite release of cortisol in response to shock stress. Others have examined salivary cortisol measurement (Kuhar et al., 2005; Pedernaer-Ramano et al., 2006). Conversely, Blackshaw and Blackshaw (1989) expressed concern over the difficulty of the salivary method in pigs, and Morméde et al. (2007) suggested that high variation in salivary cortisol may lead to less assay sensitivity, and that saliva to plasma cortisol correlation was weakened in periods of low stress and subsequent cortisol output. Lefcourt et al. (1993) warned of ultradian (incremental) pulsatility in plasma cortisol of lactating dairy cows, a condition that may or may not exist in ovine saliva. In an effort to further the understanding of salivary cortisol measurement and its use as a stress marker, repeated saliva and blood serum samples were obtained concurrently from ACTH-challenged sheep in 2 studies.

## MATERIALS AND METHODS

All procedures and methods were approved by the New Mexico State University Institutional Animal Care and Use Committee (2007-016) before animal use.

***Animals and Care.*** For each of 2 studies, 12 Suffolk ewes were selected for inclusion in the experiment. Animals were examined for physical soundness, weighed, and shorn about the neck 24 h before use. In the first study, yearling ewes averaging 64 ±1.2 kg were used. Due to (noninvasive) prior use, ewes were

halter-broken and extensively acclimated to handling and human contact. Feed was withheld for 24 h before experimental onset and for the duration of the sampling period, but clean water was offered *ad libitum*. Ewes included in the second study were adolescent lambs, approximately 6 mo of age, chosen at random from a contemporary pool. Ewe lambs averaged 45 kg, and had little previous exposure to human interaction or restraint. Feed was withheld for 24 h and both feed and water were withheld during sampling. Animals were monitored closely during and after experimental processing for signs of illness, injury, or overexertion. At the conclusion of each experiment, all ewes used received an antibiotic injection (5 ml LA-200 @ 200 mg/mL, s.c.) to prevent infection.

***Experimental Design and Treatment.*** Ewes in the first study were confined in 2 by 2m shaded wooden pens 1 h before experimentation, with 3 animals randomly assigned to each pen (pen assignment was independent of treatment assignment). Animals remained in the pens until the conclusion of sampling. For the second study, ewe lambs were haltered and tethered to an immovable outdoor panel fence 30 to 60 min before starting the sampling period. Approximately 30 cm of halter slack was allowed for each ewe lamb. Ewe lambs were required to stand for the duration of sampling, but were allowed physical contact with ewe lambs adjacently restrained. Blood samples were collected in both studies via jugular venipuncture with Vacutainer® hypodermic needles and 10 ml Corvac® serum-separator tubes. For each blood sample, a simultaneous saliva sample was taken via 30 to 45 s oral swab using a 1 by 2 cm cotton strip held in surgical forceps. Baseline samples for the first study were collected in 30-min intervals for 2 h just before challenge. At time 0, 6 randomly pre-assigned ewes were administered ACTH (100 IU per 64 kg BW in 5 mL physiological saline, i.v.), while the remaining 6 animals received a placebo injection (5 mL physiological saline, i.v.). Sampling interval was reduced to 15 min for the first 2 h following ACTH challenge, but was returned to 30 min for the final 3 h of the study. For the second study, baseline collections were taken in 3-min intervals for 15 min. At time 0, ewe lambs received ACTH (100 IU per animal in 5 mL physiological saline, i.v.) or saline placebo, depending upon randomly prescribed treatment. Post-challenge samples were taken in 3 min intervals for a total of 1 h.

***Sample Analysis.*** Blood samples were allowed to stand in serum-separator tubes at room temperature for 30 min after collection, then centrifuged (2,500 rpm, 4° C) in a Sorvall RT-6000 refrigerated centrifuge (Thermo Scientific Inc., Waltham, MA) for 15 min. Serum was poured into 5 mL plastic vials while cool and immediately frozen (-80° C) for storage. Cortisol concentration was measured via radioimmunoassay (Kiyma et al., 2004). Between assay coefficients of variation were 8.6% and 3.2% for studies 1 and 2, respectively, and within assay coefficient were 10.3 and 11.3, respectively. Saliva samples were placed in Sarstedt salivette® tubes (Numbrecht, Germany) and cooled in ice immediately after collection, then centrifuged along with blood samples. Samples were frozen (-80° C) for storage in salivette® tubes while still cool. Cortisol concentration was determined via radioimmunoassay along with serum samples.

***Statistical Analysis.*** All data were analyzed by SAS (SAS Inst. Inc, Cary, NC). Cortisol concentration data were analyzed for serum cortisol area under curve and salivary cortisol area under curve (GLM procedure of SAS), time to peak serum cortisol and time to peak salivary cortisol (Mixed procedure of SAS), time to serum cortisol return to baseline and time to salivary cortisol return to baseline (Mixed procedure of SAS), and serum cortisol to salivary cortisol correlation (Correlation procedure of SAS) and regression (Regression procedure of SAS). Comparisons were made between challenged and control ewes for each sample medium, and between saliva and serum within treatment. Comparisons were not made between studies. Both studies were treated independently as split-plot designs, with the experimental unit represented by the individual animal.

## RESULTS AND DISCUSSION

Analysis of total cortisol (Tables 1 and 2) revealed no difference ($P > 0.05$) in serum cortisol area under the curve (Figures 1 and 3) between ACTH-treated and control animals in either study. Salivary cortisol area under the curve was greater ($P = 0.02$) for treated ewes in the first study (Figure 3), and the trend approached significance in the second study ($P = 0.07$) (Figure 4). These data indicate a difference in biological treatment by the body of cortisol in the bloodstream and that passing into the saliva. Excess cortisol production results in binding protein-ligand saturation in the blood and appearance of non-bound or "free" cortisol. This unbound cortisol is subject to both metabolic inactivation and whole-steroid excretion as means of elimination from the blood (Griffin and Ojeda, 2004). The adrenal response of control ewes to saline injection, although less severe than ACTH-challenged counterparts, may have been sufficient to reach binding protein saturation. Because no binding protein is present in saliva, salivary cortisol assays measure only free cortisol arising from excessive production and passing into saliva, a presumably more controlled and exact measurement of cortisol excess.

Time to peak serum cortisol tended to be hastened ($P = 0.06$) in ACTH-treated ewes in study 1, but no difference ($P = 0.44$) was found between treatments in the second trial. Similar results were found ($P = 0.06$ and 0.40, respectively) in time to peak cortisol in saliva in studies 1 and 2. The differences between studies 1 and 2 are most likely contributable to divergences in experimental protocol and nature of animals used between trials. Study 1 results followed expectations of adrenal stimulation differences between ACTH and saline injection, but greater cortisol baseline values coupled with use of restraint in study 2 may have contributed to inconsistently induced cortisol release. In both studies, time at which cortisol returned to baseline level was greater ($P < 0.05$) for ACTH-challenged ewes than

controls in both serum and saliva. Considering adrenal stimulation from placebo injection was mild compared to ACTH challenge, persistence of cortisol elevation seemed to follow patterns of expected adrenal stimulation. Interestingly, this observation was much more pronounced than time for cortisol to peak, suggesting finite response time of the adrenal cortex. Comparison of peak and baseline return between sample media showed that serum and salivary cortisol levels peaked at similar times ($P > 0.05$) in both studies. Likewise, serum and salivary cortisol return to baseline levels were not different ($P > 0.05$). Cortisol concentration in saliva was closely correlated to serum concentration in both studies (r = 0.88 and 0.73 for studies 1 and 2, respectively; $P < 0.001$), and a serum to salivary cortisol regression model indicated that cortisol concentration in saliva is an accurate predictor of serum cortisol concentration (b = 0.86 and 0.47 for studies 1 and 2, respectively; $P < 0.001$).

**Table 1.** Total serum or salivary cortisol area under the curve, time to peak, and time to return to baseline level for ACTH-challenged Suffolk ewes in experiment 1.

| | Treatment | | | |
|---|---|---|---|---|
| Item | Control | ACTH | SE | *P* |
| AUC[1] | | | | |
| Serum | 28,571.5 | 34,914.1 | 4,789.7 | 0.19 |
| Saliva | 1,048.6 | 2,025.2 | 277.3 | 0.02 |
| Time to peak[2] | | | | |
| Serum | 62.5 | 40.0 | 9.5 | 0.13 |
| Saliva | 80.0 | 50.0 | 12.0 | 0.11 |
| Return to baseline[2] | | | | |
| Serum | 170.0 | 270.0 | 17.0 | 0.002 |
| Saliva | 170.0 | 290.0 | 11.4 | 0.001 |

[1]Total AUC units.
[2]Time to peak and return to baseline in min from challenge.

**Table 2.** Total serum or salivary cortisol area under the curve, time to peak, and time for ACTH-challenged Suffolk ewe lambs in trial 2.

| | Treatment | | | |
|---|---|---|---|---|
| Item | Control | ACTH | SE | *P* |
| AUC[1] | | | | |
| Serum | 19,832.5 | 22,490.8 | 1,984.5 | 0.20 |
| Saliva | 1,731.8 | 2,852.3 | 486.0 | 0.07 |
| Time to peak[2] | | | | |
| Serum | 26.5 | 25.5 | 4.8 | 0.88 |
| Saliva | 26.0 | 24.0 | 5.0 | 0.78 |
| Return to baseline[2] | | | | |
| Serum | 33.5 | 51.0 | 4.8 | 0.03 |
| Saliva | 50.5 | 57.5 | 2.6 | 0.09 |

[1]Total AUC units.
[2]Time to peak and return to baseline in min from challenge.

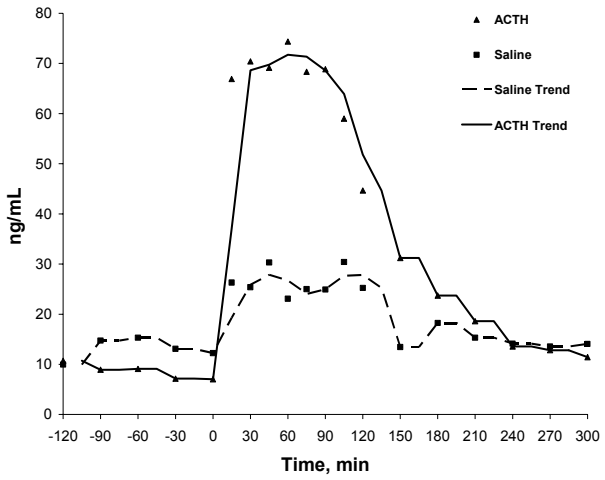


**Figure 1.** Average serum cortisol concentrations of Suffolk ewes in study 1 challenged with ACTH or saline at time 0.

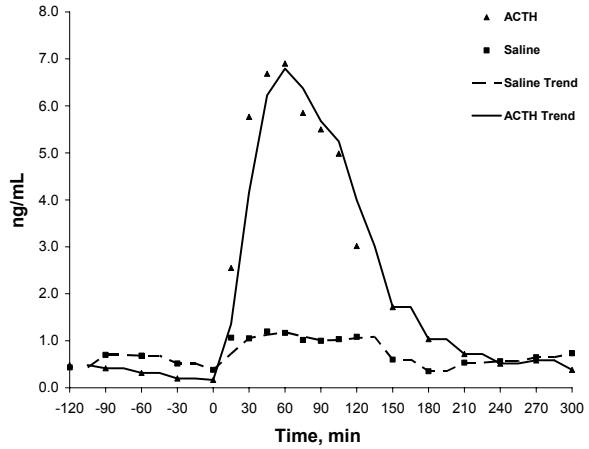


**Figure 2.** Average salivary cortisol concentrations of Suffolk ewes in study 1 challenged with ACTH or saline at time 0.

Data collected from these ACTH challenges indicate salivary cortisol is indeed an accurate and suitable indicator of blood levels of cortisol, a common marker of physiological stress in animals. Ease of saliva sample collection previously reported (Cook and Jacobson, 1995; Kuhar et al., 2005; Pedernera_Ramano et al., 2006) was confirmed and no problems with volume adequacy were encountered, unlike Blackshaw and Blackshaw (1989). Radioimmunoassay of salivary cortisol revealed no indication of reduced sensitivity or increased variation compared to serum cortisol assay as previously suggested by Mormède et al. (2007), and concentration pulsatility reported by Lefcourt et al. (1993) did not seem to affect stimulation patterns noticeably. Strong support was lent to previous studies suggesting similarity in salivary and blood cortisol concentrations (Fell and Shutt, 1988; Cook and Jacobson, 1995). Although cortisol release may vary greatly between animals and situations and should not be considered a tool for small scale stress changes, it is linked to stress perception and response in animals. Current findings

coupled with previous reports indicate that measurements of cortisol from salivary samples are no less meaningful than those from blood samples.

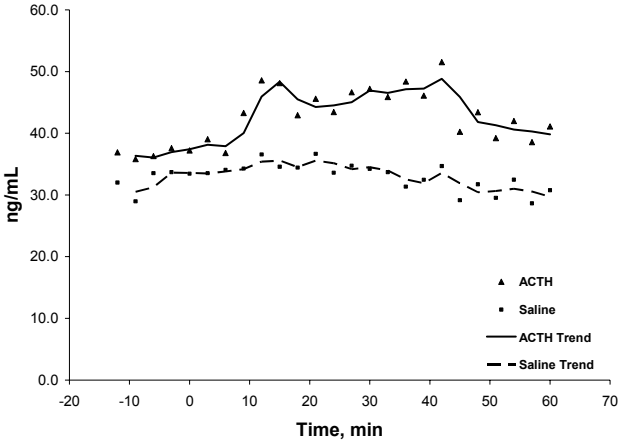


**Figure 3.** Average serum cortisol concentrations of Suffolk ewe lambs in study 2 challenged with ACTH or saline at time 0.

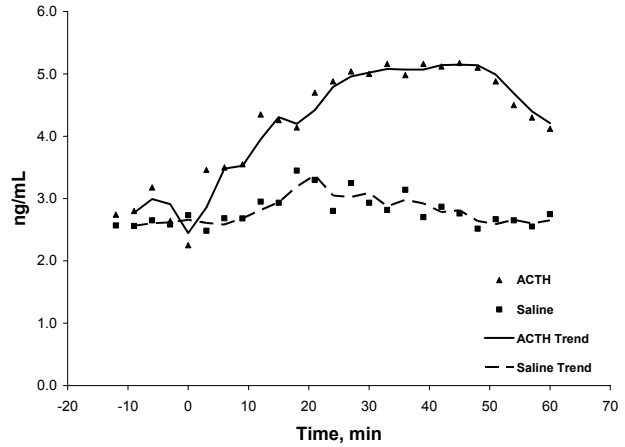


**Figure 4.** Average salivary cortisol concentrations of Suffolk ewe lambs in study 2 challenged with ACTH or saline at time 0.

## IMPLICATIONS

Results from two independent ACTH-challenges in young Suffolk ewes suggest that salivary cortisol is a suitable marker of adrenal cortex stimulation comparable to blood cortisol. Salivary sampling alleviates invasiveness and technical difficulty associated with blood sampling and may be a viable alternative in many stress determinations.

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# Seasonal and pregnancy-associated changes in energy-related metabolites and hormones in Elk

**J. G. Berardinelli, R. Garrott, S. A. Tauck, J. R. C. Wilkinson, and J. R. Olsen**
Montana State University, Bozeman 59717

**ABSTRACT:** There is a lack of data related to metabolites and metabolic hormones in pregnant elk (*Cervus elaphus*) and their association with season of the year. Thus, the objective of this study was to evaluate effects of season and pregnancy on energy-related metabolites and hormones in female elk of the Greater Yellowstone Ecosystem. The null hypotheses were that serum concentrations of progesterone (P4), glucose, NEFA, cortisol, thyroxine (T4), tri-iodothyronine (T3), T3:T4 ratios, and insulin do not differ between season (late fall or mid-spring) or pregnancy status (pregnant or non-pregnant) in female elk. Blood samples were obtained by venepuncture from radio-collared animals. The technique employed a very low-stress immobilization procedure that included stalking, and sedation with a cocktail of ketamine and xylazine. A total of 111 samples were collected in the late fall (n = 75) and mid-spring (n = 36) between 1997 and 2007. Glucose and NEFA concentrations were determined by colorimetric assays. Progesterone, cortisol, total T3, and total T4 concentrations were assayed by RIA, and insulin assayed with an ELISA. All assays were validated for elk serum. A P4 concentration of > 2.0 ng/mL was used as the criterion for classifying pregnancy. Season had no affect ($P$ > 0.10) on P4 concentrations. Cortisol, T3, T4, and insulin concentrations, and T3:T4 ratios were not influenced ($P$ > 0.10) by season or pregnancy status. However, glucose concentrations were greater ($P$ < 0.05) in samples collected in fall than in spring. There was an interaction ($P$ < 0.05) between season and pregnancy status for NEFA concentrations. Concentrations of NEFA tended to ($P$ = 0.08) in pregnant elk between fall and spring; whereas, NEFA concentrations decreased ($P$ < 0.05) by more than 30% between fall and spring in non-pregnant elk. The results indicate that glucose concentrations decrease in both pregnant and non-pregnant elk from the end of breeding season to near the time of calving. However, it would appear that pregnant elk draw heavily upon available adipose tissue to meet the demands of late pregnancy and impending parturition.

**Key words**: cervids, hormones, metabolites

## INTRODUCTION

Elk (*Cervus elaphus nelsoni*) are an important faunal component of the Greater Yellowstone ecosystem. Pregnant elk in the Yellowstone are exposed to predation pressures, severe weather fluctuations, and declining quality and quantity of forage during gestation from late November to early April (White et al. 2008). These factors place much stress upon pregnant females. The "level" of stress and how females accommodate these stressors are important to the success of pregnancy, parturition, and lactation.

Presently, there are little or no baseline data regarding energy-related metabolites and endocrinological changes that occur in free-ranging pregnant elk to sustain pregnancy in the face of nutritional and predation stresses. Thus, the objective of this study was to evaluate the effects of season and pregnancy on energy-related metabolites and hormones in free-ranging female elk in Yellowstone National Park. The null hypotheses were that serum concentrations of progesterone (P4), glucose, NEFA, cortisol, thyroxine (T4), tri-iodothyronine (T3), T3:T4 ratios, and insulin do not differ between season (late fall or mid-spring) or pregnancy status (pregnant or non-pregnant) in female elk.

## MATERIALS AND METHODS

### *Animals and Blood Sampling*

Blood samples were obtained by venepuncture from free-ranging, radio-collared female elk, located in Yellowstone National Park. The technique for obtaining samples employed a very low-stress immobilization procedure that included stalking and i.m. sedation with a cocktail of ketamine and xylazine delivered in a darting syringe. Blood samples (20 mL) were allowed to clot and centrifuged within 6 h of after collection. Serum was harvested for each sample and stored at −20°C until assayed for metabolites and hormones. A total of 111 samples were collected in the late fall (n = 75) and mid-spring (n = 36) between 1997 and 2007.

### *Metabolite and Hormone Assays*

Progesterone (P4) concentration were assayed using solid-phase RIA kits (Diagnostic Products Corp., Los Angeles, CA) validated for bovine serum in our laboratory (Custer et al., 1990). Percent recoveries for elk serum were determined by adding the middle progesterone standard (1.5 ng/mL) to a serum pool and assaying this pool at three different volumes. Percent recoveries were ranged from 96 to 101%. Intra- and inter-assay CV for a serum pool that contained 1.0 ng/mL were < 10%. The criterion for pregnancy was a concentration of P4 that was > 1.8 ng/mL.

Glucose concentrations was determined in duplicate 10 µL aliquots by an end-point enzymatic assay using a commercially available glucose assay kit (Infinity™ Glucose Hexokinase liquid reagent,

ThermoElectron Corporation, Waltham, MA). Standard curves were generated using reagent grade D-glucose (Sigma-Aldrich, Corp., St. Louis, MO). Percent recoveries for serum were determined by adding the middle glucose standard (80 mg/dL) to an elk serum pool and assaying this pool at three different volumes. Percent recoveries were ranged from 96 to 101%. Sensitivity of the assay using elk serum was 12.5 mg/dL. Intra- and inter-assay coefficients of variation were < 10% elk serum pools of that contained 180 and 60 mg/dL of glucose.

Concentrations of NEFA were quantified with a commercially available enzymatic-colorimetric assay (HR Series NEFA – HR [2]; Wako Diagnostics, Richmond, VA). Standard curves were generated using oleic acid provide with the kits. Percent recoveries for elk serum were determined by adding oleic acid standard (0.125 mmol/L) to an elk serum pool and assaying this pool at three different volumes. Percent recoveries were ranged from 98 to 103%. Sensitivity of the assay using elk serum was 0.062 mmol/L. Inter- and intra-assay CV were 5.8 and 4.6% for a serum pool that contained 0.415 mmol/L; and, 14 and 5.6%, respectively, for a serum pool that contained 0.100 mmol/L.

Cortisol concentrations were assayed using solid-phase RIA kits (Diagnostic Products Corp., Los Angeles, CA) validated for bovine serum in our laboratory. Percent recoveries for elk serum were determined by adding cortisol standard (7.81 ng/mL) to an elk serum pool and assaying this pool at three different volumes. Percent recoveries were ranged from 94 to 100%. Sensitivity of the assay using elk serum was 1.95 ng/mL. Intra- and inter-assay CV for a serum pool that contained 8.0 ng/mL were 5.8 and 11%, respectively; and for a pool that contained 30.0 ng/mL were 7.0 and 8.4%, respectively.

Total thyroxine (T4) and triiodothyronine (T3) concentrations were assayed using solid-phase RIA kits (Diagnostic Products Corp., Los Angeles, CA). The intra-assay CV for elk serum pools that contained 66.3 and 29.8 ng/mL of T4 were 5.8 and 4.9%, respectively. The intra-assay CV for elk serum pools that contained 54.3 and 7.8 ng/mL of T3 were 9.2 and 14.5%, respectively.

Concentrations of serum insulin were assayed using ELISA assay kits specific for bovine insulin (Mercodia AB, Uppsala, Sweden). Percent recoveries for elk serum were determined by adding bovine insulin standard (1.0 ng/mL) to an elk serum pool and assaying this pool at three different volumes. Percent recoveries were ranged from 97 to 102%. Sensitivity of the assay using elk serum was 0.125 ng/mL. Intra- and inter-assay CV for a serum pool that contained 0.18 ng/mL were 6.6 and 14.3%, respectively.

*Statistical Analyses*

Table 1 shows the number of female elk that were considered pregnant if the concentration of P4 exceed 1.5 ng/mL and non-pregnant if it was at or below 1.5 ng/mL in samples collected from elk between late October and early December (Fall), and late March and early April (Spring). Data for glucose, NEFA, cortisol, T4, T3, T3:T4 ratios, and insulin were analyzed by separate ANOVA using PROC GLM of SAS (SAS Inst. Inc., Cary, NC) for a completely random design arranged in a 2 x 2 factorial. The model included pregnancy status (pregnant or non-pregnant), season (Fall or Spring), and the interaction between pregnancy status and season. Means were separated by Bonferroni multiple comparison tests.

## RESULTS

Glucose concentrations were greater ($P < 0.05$) in samples collected from female elk in Fall than in Spring (Table 2). There was an interaction ($P < 0.05$) between season and pregnancy status for NEFA concentrations. Concentrations of NEFA tended ($P = 0.08$) to increase in pregnant elk between Fall and Spring (Fig.1); whereas, NEFA concentrations decreased ($P < 0.05$) by more than 30% between Fall and Spring in non-pregnant elk (Fig. 1).

Cortisol, T3, T4, and insulin concentrations, and T3:T4 ratios were not influenced ($P > 0.10$) by season, pregnancy status or the interaction between season and pregnancy status (Tables 3 through 7).

## DISCUSSION

The purpose of this study was to evaluate changes in concentrations of the energy metabolites, glucose and NEFA, and metabolic hormones that may impact their availability and utilization in free-ranging pregnant and non-pregnant elk during two seasons of the year in Yellowstone National Park. We used serum concentrations of progesterone that exceeded of 1.5 ng/mL as the criterion for establishing the numbers of pregnant and non-pregnant elk for use as treatments in the statistical analyses of the data. However, using a single blood sample to determine pregnancy with P4 does have limitations in cervids. Serum P4 concentrations were found to be higher in pregnant than in non-pregnant elk during the late breeding season, with the accuracy of P4 for determining pregnancy status at this time being 85.8% (Willard et al., 1994). On the other hand, single samples obtained from female elk in March yielded an accuracy of > 95%. Furthermore, adrenal release of progesterone may contribute to misdiagnosis of pregnancy in elk, especially during the Fall season in response to sampling stress (Jopson et al., 1990). Thus, there is the possibility that some percentage of the elk judged to be pregnant in the present study may have indeed been non-pregnant. Nevertheless, we feel that use of this criterion yielded an accuracy of > 90% overall, and interpretation of the data based upon the numbers of elk pregnant and non-pregnant elk in each season would seem to be valid.

Glucose concentrations obtained in the elk of this study were within the range of those reported for free-ranging and captive female elk in the Rocky Mountains of the U.S. (Vaughn et al., 1973). We found that glucose concentrations decreased by approximately 20% from late October and early December to late March and early April

independent of pregnancy status. This result is most probably due to a lack of availability of nutrients in the forage and a decrease in body condition caused primarily by winter snow-pack (White et al., 2008).

A rather significant finding in this study was the interaction of pregnancy status and season on NEFA concentrations in female elk. Concentrations of NEFA did not differ between pregnant and non-pregnant elk soon after the breeding season. However, by late March-early April, concentrations of NEFA increased somewhat in pregnant elk but decreased by more than 32% in non-pregnant elk. It is well known that catabolism of adipose tissue results in the release of NEFA from adipocytes. NEFA is the portion of the total fatty acid pool that circulates in immediate readiness for metabolic needs whenever insufficient quantities of glucose limit the usual carbohydrate energy source. Generally, glucose and NEFA concentrations are inversely related; as glucose increases NEFA decreases and vice versa (see Hadley and Levine, 2007). Changes in concentrations of these metabolites are regulated by a host of metabolic hormones. In the present study, we measured cortisol, T3 and T4, and insulin; all of which can affect circulating concentrations of these energy-related metabolites. Based upon the statistical analyses of these hormones it does not appear that any of them can explain the seasonal change in glucose or the interaction of pregnancy status and season on NEFA. Nevertheless, pregnant elk appear to respond well to the seasonal change glucose by mobilizing fat reserves needed to sustain pregnancy. The mechanism for this effect may be related to other metabolic hormones that were not measured in this study.

## IMPLICATIONS

The results indicate that glucose concentrations decrease in both pregnant and non-pregnant elk from the end of breeding season to near the time of calving. However, it would appear that pregnant elk draw heavily upon available adipose tissue to meet the demands of late pregnancy and impending parturition. Further investigations are necessary to explain how pregnant elk utilize adipose depots late in gestation. Knowledge of this relationship might be used for management of elk in the greater Yellowstone ecosystem

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**Table 1.** Number of female elk that were considered pregnant or not pregnant based on the criterion of > 1.5 ng/mL of progesterone for serum samples collected between late Oct. and early Dec. (Fall), and late Mar. and early Apr. (Spring)

| | Treatment | | |
|---|---|---|---|
| Treatment | Pregnant | Non-pregnant | Total |
| Fall | 49 | 25 | 74 |
| Spring | 18 | 18 | 36 |
| Total | 67 | 43 | 110 |

**Table 2.** Least squares means for glucose concentrations (mg/dL) of female elk that were considered pregnant or not pregnant based on the criterion of > 1.5 ng/mL of progesterone for serum samples collected between late Oct. and early Dec. (Fall), and late Mar. and early Apr. (Spring)[1]

| | Treatment | | |
|---|---|---|---|
| Treatment | Pregnant | Non-pregnant | Main effect |
| Fall | 148 | 142 | 145 [a] |
| Spring | 116 | 121 | 119 [b] |
| Main effect | 133 | 132 | |

[1] Pooled standard error of the mean = 30.1 mg/dL.

[a,b] Main effects means that lack a common superscript differ ($P < 0.05$).

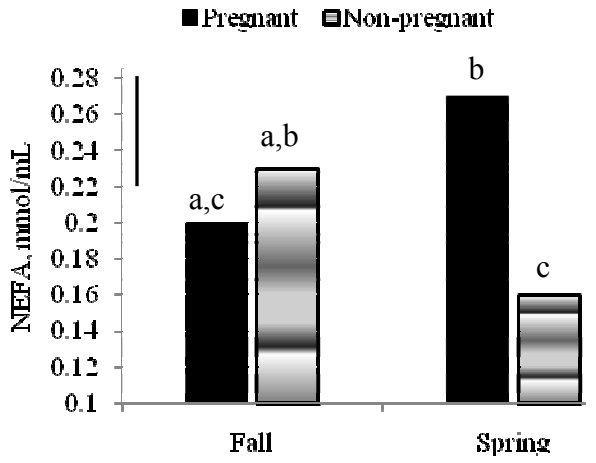


**Figure 1.** Least squares means for NEFA concentrations (mmol/mL) of female elk that were considered pregnant or not pregnant based on the criterion of > 1.5 ng/mL of progesterone for serum samples collected between late Oct. and early Dec. (Fall), and late Mar. and early Apr. (Spring). Interaction of pregnancy status and season ($P < 0.05$). Means that lack a common letter differ ($P < 0.05$). The vertical line represents the SEM; 0.061 mmol/mL.

**Table 3.** Least squares means for cortisol concentrations (ng/mL) of female elk that were considered pregnant or not pregnant based on the criterion of > 1.5 ng/mL of progesterone for serum samples collected between late Oct. and early Dec (Fall), and late Mar. and early April (Spring)[1]

| Treatment | Treatment | | Main effect |
|---|---|---|---|
| | Pregnant | Non-pregnant | |
| Fall | 45.6 | 29.8 | 37.7 |
| Spring | 29.6 | 30.4 | 30.0 |
| Main effect | 37.6 | 30.1 | |

[1] Pooled standard error of the mean = 25.2 ng/mL.

**Table 4.** Least squares means for T3 concentrations (ng/mL) of female elk that were considered pregnant or not pregnant based on the criterion of > 1.5 ng/mL of progesterone for serum samples collected between late Oct. and early Dec (Fall), and late Mar. and early April (Spring)[1]

| Treatment | Treatment | | Main effect |
|---|---|---|---|
| | Pregnant | Non-pregnant | |
| Fall | 18.0 | 13.3 | 15.6 |
| Spring | 11.3 | 8.3 | 9.8 |
| Main effect | 14.6 | 10.8 | |

[1] Pooled standard error of the mean = 17.6 ng/mL.

**Table 5.** Least squares means for T4 concentrations (ng/mL) of female elk that were considered pregnant or not pregnant based on the criterion of > 1.5 ng/mL of progesterone for serum samples collected between late Oct. and early Dec (Fall), and late Mar. and early April (Spring)[1]

| Treatment | Treatment | | Main effect |
|---|---|---|---|
| | Pregnant | Non-pregnant | |
| Fall | 54.9 | 52.3 | 53.6 |
| Spring | 50.9 | 63.5 | 57.2 |
| Main effect | 52.9 | 57.9 | |

[1] Pooled standard error of the mean = 41.7 ng/mL.

**Table 6.** Least squares means for T3:T4 ratios of female elk that were considered pregnant or not pregnant based on the criterion of > 1.5 ng/mL of progesterone for serum samples collected between late Oct. and early Dec (Fall), and late Mar. and early April (Spring)[1]

| Treatment | Treatment | | Main effect |
|---|---|---|---|
| | Pregnant | Non-pregnant | |
| Fall | 0.519 | 0.373 | 0.446 |
| Spring | 0.249 | 0.256 | 0.252 |
| Main effect | 0.383 | 0.315 | |

[1] Pooled standard error of the mean = 0.536.

**Table 7.** Least squares means for insulin concentrations of female elk that were considered pregnant or not pregnant based on the criterion of > 1.5 ng/mL of progesterone for serum samples collected between late Oct. and early Dec (Fall), and late Mar. and early April (Spring)[1]

| Treatment | Treatment | | Main effect |
|---|---|---|---|
| | Pregnant | Non-pregnant | |
| Fall | 0.39 | 0..57 | 0.48 |
| Spring | 0.47 | 0.51 | 0.49 |
| Main effect | 0.43 | 0.54 | |

[1] Pooled standard error of the mean = 0.46 ng/mL.

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# Histological Examinations of Major Organs from Sprague-Dawley Female Rats Fed Ruminaly Digested Snakeweed and Undigested Snakeweed

**L. J. Hill[1], T.T. Ross[1], R.F. Taylor DVM MS[2], R.A. Halalsheh[1], and D.T. Yates[1]**

[1]Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM 88003
[2]New Mexico Department of Agriculture Veterinarian Diagnostic Services, Albuquerque, NM 87196

**ABSTRACT:** Limited data are available on histological changes of major organs in animals consuming snakeweed (SW). The objective of this study is to examine effects of SW on major organs in rats and compare differences between rats fed 15 % digested SW (DSW), 20% DSW, and 15% undigested SW (USW). Thirty-six female rats were used in each of two trials and randomly assigned to one of the three SW levels or as a pair-fed control. Treatment diets included 15% DSW, 20% DSW, or 15% USW. The remainder of the diet was Laboratory 5001 Rat Chow®. A mixture of corn meal and Laboratory 5001 Rat Chow® was fed to the pair-fed controls, so that energy and protein intake was equal among treatment and control pairs. Rats were fed for 10 days, then euthanized and necropsied to harvest organ samples. Samples were placed in neutral buffer formalin and processed for histological analysis, which was performed by New Mexico Department of Agriculture Veterinary Diagnostic Services in Albuquerque, NM. In trial 1, intake was similar ($P$ >0.05) between treatments during the 10d trial. However, in Trial 2, intake was greater ($P$ < 0.05) for rats offered 15% DSW compared to 20% DSW on all days, and greater ($P$ <0.05) than rats offered 15% USW on days 3, 5, and 6. Intake was also greater ($P$ <0.05) in rats receiving 15% USW compared to 20% DSW on all days. Body weight changes were similar ($P$ >0.05) among SW levels in both trials. Histological effects were evaluated by notable pathological changes in cells. Tissues were scored on a scale of 1 (none) to 3 (very notable). No differences ($P$ >0.05) were found among treatment levels for histology score in liver, spleen, kidney, pancreas, GI tract, lymph node, ovary, oviduct, uterus, stomach, or adrenal gland. This study indicates there no common reliable characteristic histological lesion among these rats due to a SW toxicity.

**Key Words**: Histology, Snakeweed, Tissue lesions

## INTRODUCTION

Broom Snakeweed (*Gutierrezia sarothrae*) and Threadleaf Snakeweed (*Gutierrezia microcephala*) are perennial plants that have toxic effects on ruminant animals when ingested. Snakeweed (SW) invades rangelands throughout western North America, including northern Mexico and western Canada. Snakeweed quickly overtakes grasslands during times of drought, overgrazing, and other soil disturbances because of its ability to reproduce rapidly. One SW plant can produce 21,000 seeds that may lay dormant and viable in the soil for years (Parker, 1984). Average life span of a single SW plant is 2.42 years (Pieper and McDaniel, 1989) and the species is known to exhibit cyclical population growth. Snakeweed is among the first rangeland plants to become green in early spring and is thus a prime grazing target for gestating livestock during this time (Smith et al., 1991). For most animals, SW is less than 10% of total dietary intake under rangeland conditions (Pieper, 1990), but even seemingly low levels of SW may cause reproduction failure, decrease overall performance, and lead to small birth weights and retained placentas (Dollahite and Anthony 1957). Unfortunately, previous research has failed to specify the toxic principles of SW. Chemical analysis of SW has revealed presence of saponins, flavonols, and terpines (Lucero et al., 2006), yet none of these toxins have been directly implicated as the causative agent for SW toxicity. Saponins are believed to possess abortive ability, a common result of SW toxicity (Kingsbury 1964; Cheeke and Shull 1985). Saponins have lipophilic and sugar-like portions that can combine to form a glycoside (Smith et al., 1989) which may be steroidal in nature. If introduced into the bloodstream, these steroid-like compounds could pose a vast threat to proper reproductive function, however, toxins associated with SW maybe altered by ruminant digestion (Hernandez et al. 2004). Additionally, ruminant microbial hydrolysis of saponins may release easily absorbable lipophilic sapogenins, furthering the toxic effect (McDonald et al. 2002).

Because SW toxicosis includes abortion and general reproductive inefficiency, it is of utmost economic concern. Minimizing industrial loss in agriculture is a primary goal of veterinary diagnostic laboratories. Currently, pathologists must eliminate any alternative causes of abortion via fetal necropsy to conclude SW toxicosis, and although histological examination of major organs is a widely used diagnostic tool, little information is available on histological markers associated with SW toxicity, although eosinophil concentrations have been implicated as markers of endophyte toxicity in cattle grazing fescue (Oliver et al., 2000). Diagnostic laboratories are often limited to fetal and placental tissue, which have been shown to exhibit normalcy even after suspected SW toxicity. In an effort to further the mechanistic understanding of SW toxicity, two feeding trials were conducted to examine effects of SW ingestion on major organ histology, with specific

interest toward landmark changes in lesions and eosinophil activity. Because previous work with SW has yet to examine microbial changes to SW in the ruminal environment, comparison of histological changes in major organs will include a consideration of differently digested SW levels.

## MATERIALS AND METHODS

All procedures and protocols described below were approved by the New Mexico State University Institutional Animal Care and Use Committee (2006-016) prior to initiation of experimentation.

Samples of SW were gathered during pre-bloom via hand clipping. Approximately 5 to 10 cm of the distal portion of the plants were harvested. All SW samples were taken from the Chihuahuan Desert Range Research Center (CDRRC), located 37 km north of Las Cruces, New Mexico, in the summer of 2003. Snakeweed samples were frozen (-20°C) for storage and then finely ground through a 2 mm screen. Dry ice was used in the grinding process to keep SW frozen and moving through the Wiley mill. A total of 5 kg of SW were ground for *in vitro* rumen digestion and feeding.

*In vitro ruminal digestion*. Two salt solutions were used in the in vitro digestion. Salt solution A consisted of 7.3 g $K_2HPO_4*3H_20$/L de-ionized (DI) water. Salt solution B consisted of 6.9 g $KH_2PO_4$, 12.0 g $(NH_4)_2SO_4$, 12.0 g NaCl, 2.5 g $MgSO_4*7H_2O$, and 25 g $CaCl_2*2H_2O$. Forty mL of salt A and 40 mL of salt B were mixed together along with 0.6 g of cestein hydrochloride, 1 mL resazorine (1.0%), and 875 mL DI water to formulate the complete buffer. Buffer was then adjusted to a pH of 7.0, autoclaved and cooled. Finally, 8% $Na_2CO_3$ was added to the buffer, and bubbled with $CO_2$ for 3 min. Rumen fluid was collected from canulated cows at the New Mexico State University farm. Cattle had been fed a diet of sorghum hay *ad libitum* and water. Rumen fluid, buffer, and 40.6 g of SW were combined and incubated for 24 h at 39ºC. The mixture was shaken every 2 h. An estimated 40% recovery rate was obtained from filtration of digested SW. Samples of both digested and undigested SW were analyzed by SDK Laboratories (Hutchinson, Kansas) for moisture, DM, CP, ADF, TDN, EE, Ca, P, and K (Table 1).

**Table 1.** Nutrient analysis of *in vitro* ruminaly digested and undigested SW in dry matter basis.[1]

| | Nutrient (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | DM | CP | ADF | EE | Ca | P | K |
| USW[2] | 61.2 | 11.3 | 26.5 | 11.7 | 0.90 | 0.17 | 1.44 |
| DSW[3] | 23.9 | 10.7 | 37.5 | 11.3 | 1.22 | 0.26 | 0.53 |

[1]analysis by SDK Laboratories, Hutchinson, KS.
[2]undigested snakeweed.
[3]ruminantly digested snakeweed.

*Experimental design and treatment.* In each of two trials, 36 female Sprague-dawley rats weighing 387 +/- 7.6 g were housed individually in 15 by 30 cm plexiglass cages. An adjustment period of 7 d allowed rats to adjust to environment. Laboratory 5001 Rat Chow® was fed free choice during the adjustment period. Rats were individually paired with a male for 24 h, after which males were removed. Female rats were 5 to 10 d into gestation before feeding SW. Three different levels of SW were added to Rat Chow® for treatment rats (n = 6/treatment). Treatment diets were as follows: 15% ruminaly digested SW and 85% Rat Chow®, 20% ruminaly digested SW and 80% Rat Chow®, and 15% undigested SW and 85% Rat Chow®. Each treated rat was assigned a pair-fed control rat (n = 18). To eliminate variation due to nutritional considerations such as decreased intake, control rats were fed a mixture of Rat Chow® and corn meal balanced with associated treatment rat by protein concentration and total digestible nutrients concentration. Intake was measured daily for both control and treated rats.

Live weights were recorded before treatment initiation and again just prior to euthanasia. Rats were euthanized via blow to the head stunning followed by exsanguination via heart puncture. Rats were then necropsied and liver, gastrointestinal tract, spleen, kidney, pancreas, lymph nodes, ovary, oviduct, uterus, and adrenal glands collected. All tissues harvested were fixed in neutral buffer formalin (10%) and submitted to New Mexico Department of Agriculture Veterinary Diagnostic Services (VDS), for histopathology evaluation.

*Statistical analysis.* All data, except intake, were analyzed as a completely randomized design by SAS (Mixed Procedures SAS Institute, Cary, NC), with individual rats as experimental units. Intake was measured as a split plot. For each trial, intake was compared between SW levels for each of the 10 feeding days (Mixed procedure of SAS). Histological findings for lesion presence and eosinophil concentration in liver, spleen, kidney, pancreas, gastrointestinal tract, lymph node, ovary, oviduct, uterus, stomach, and adrenal gland tissues were analyzed by chi-square test (Frequency procedure of SAS).

## RESULTS AND DISCUSSION

Data from the first trial indicated no difference ($P > 0.05$) in intake among treatments. However, in trial 2 (Table 3) rats consuming 20% DSW ate less feed ($P < 0.05$) than those consuming 15% DSW or 15% USW on all days. On d 3, 5, and 6, rats consuming 15% USW ate less feed ($P < 0.05$) than rats offered 15% DSW diet. Seemingly, little or no effect on intake factors such as palatability was obtained by digestion. This was surprising assuming hydrolysis of saponins through digestion, as reported by MacDonald et al. (2002), should have led to reduced adverse post-ingestive feedback. Increasing concentration of DSW decreased intake, as expected.

Changes in BW over the 10 d feeding period (Table 4) were not different ($P > 0.05$) between treatments and controls or among SW treatments in trial 1, but BW changes were different ($P < 0.05$) between rats fed SW and control rats in the second trial, despite equal CP and energy offered to SW-fed and control animals.

Body weight changes were not different ($P > 0.05$) between SW levels in trial 2. High animal-to-animal variability most likely superceded nutritional availability, resulting in a wide variety of BW responses.

**Table 3.** Intake (g) of Sprague-Dawley rats offered *ad libitum* diets containing 15% USW, 15%DSW, or 20% DSW in trial 2.

| Day | SW Treatment | | | SE |
|---|---|---|---|---|
| | 15% USW[1] | 15% DSW[2] | 20% DSW[2] | |
| 1 | 22.5[a] | 29.7[a] | 2.8[c] | 3.6 |
| 2 | 24.3[a] | 33.0[a] | 11.0[c] | 3.3 |
| 3 | 24.9[a] | 33.8[b] | 7.1[c] | 2.8 |
| 4 | 23.5[a] | 33.0[a] | 9.0[c] | 3.4 |
| 5 | 27.4[a] | 34.4[b] | 9.3[c] | 2.4 |
| 6 | 25.8[a] | 33.8[b] | 17.3[c] | 2.6 |
| 7 | 26.8[a] | 33.7[a] | 17.9[c] | 2.6 |
| 8 | 25.5[a] | 31.2[a] | 16.2[c] | 2.8 |
| 9 | 26.8[a] | 32.6[a] | 26.8[c] | 2.4 |

Means within a row with different superscripts differ ($P < 0.05$).
[1]undigested snakeweed.
[2]ruminantly digested snakeweed.

**Table 4.** Body weight (g) of Sprague-Dawley rats offered *ad libitum* diets containing 15% USW, 15% DSW, or 20% DSW in two trials.

| | Trial 1 | | | | |
|---|---|---|---|---|---|
| | SW Treatment | | | | |
| Item | Control | 15% USW[1] | 15% DSW[2] | 20% DSW[2] | SE |
| Initial BW | 395.8 | 359.7 | 407.4 | 370.1 | 18.1 |
| Final BW | 382.6 | 351.7 | 372.5 | 351.1 | 20.7 |
| Change | -12.2 | -8.0 | -34.8 | -18.9 | 18.5 |

| | Trial 2 | | | | |
|---|---|---|---|---|---|
| | SW Treatment | | | | |
| Item | Control | 15% USW | 15% DSW | 20% DSW | SE |
| Initial BW | 339.6 | 289.7 | 320.8 | 327.0 | 21.4 |
| Final BW | 352.6[a] | 280.0[b] | 295.2[b] | 306.7[b] | 18.9 |
| Change | 12.9[a] | -9.7[b] | -25.6[b] | -20.4[b] | 8.3 |

Means within a row with different superscripts differ ($P < 0.05$).
[1]undigested snakeweed.
[2]ruminantly digested snakeweed.

Although eosinophil activity is not fully understood, it has been reported that changes in eosinophil concentration may be associated with toxicosis in cattle grazing endophyte-infected fescue (Oliver et al., 2000). However, eosinophil concentration was not altered by ingestion of SW at any level ($P > 0.05$) in any of the major organs examined in either of the current trials. Although eosinophils were present in oviduct and uterine tissues, and lymphocytic activity was observed in liver, spleen, and kidney tissues, prevalence was indifferent ($P > 0.05$) between any treatment-control pair or between SW levels. Histopathology analysis did not reveal any specific histological changes of tissue resulting from SW consumption. These findings fail to support the hypothesis of toxin-induced immune response as a mechanism of SW toxicity.

## IMPLICATIONS

Despite previous information linking SW ingestion to toxicosis and other performance problems, no evidence was found from this study suggesting immunological response was the mechanism by which these problems occurred. Investigation into histological changes to major organs due to SW ingestion did not provide characteristic histological changes as indicators of SW toxicosis.

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# EFFECT OF WINTERING SYSTEM, NUTRITION AROUND BREEDING AND PROSTAGLANDIN ON REPRODUCTION AND CALF PRODUCTION OF BEEF HEIFERS

**D.M. Larson[1], R.D. Richardson[2], and R.N. Funston[3]**
[1]University of Nebraska, Lincoln, [2]Rex Ranch, Ashby, NE, and [3] West Central Research and Extension Center, North Platte, NE

**ABSTRACT:** Experiments evaluated heifer development system, level of nutrition around breeding, and prostaglandin on pregnancy rate and calf production. In Exp. 1, 96 heifers grazed winter range (**WR**) or corn residue (**CR**) with supplement (0.45 kg/d) and were then treated similar until 7 d before PG, when half CR and WR received supplement (1.4 kg/d) for 21 d. In Exp. 2, 99 heifers grazed CR with supplement (0.45 kg/d) or were developed in dry lot (**DL**). Seven d before and 12 d after AI, heifers were placed on a high (**HE**) or low (**LE**) energy diet and then exposed to bulls (62 d). After post-weaning WR development, Exp. 3 (n = 1160) evaluated 25 d natural breeding with or without PG 96 hr after bull exposure began. In Exp. 1, CR tended ($P = 0.06$) to have greater BCS compared to WR before calving. Calf birth BW and BD were similar ($P > 0.10$). In Exp. 2, HE tended ($P = 0.08$) to have greater AI pregnancy rate than LE. Calf birth BW and BD were unaffected ($P > 0.10$) by treatment. However, CR that conceived to AI had a longer ($P = 0.05$) gestation length than DL. In Exp. 3, pregnancy rate, calf BD, and sex distribution were unaffected by PG ($P > 0.10$). However, PG increased ($P < 0.01$) birth BW compared to no PG and the percentage heifers calving in the first 21 d of the season. Neither CR development nor supplementation around natural service breeding impact calf production while higher energy nutrition around AI tends to improve AI conception rate. Injection of PG in a 25 d breeding season improves synchrony of calving and increases birth BW.

**Key Words:** Corn residue, Heifer, Supplement

## Introduction

There is increasing interest in lower cost, low gain heifer development systems. Recent data indicate heifers reaching less than 58% of mature body weight by breeding do not display impaired reproductive performance (Funston and Deutscher, 2004; Martin et al. 2008). Furthermore, supplementation offered to nutritionally restricted multiparous females prior to breeding improved embryo survival (Khireddine et al., 1998), but how this type of supplementation may interact with low gain winter development is unknown. Due to hypothesis that lighter heifers may conceive later in the breeding season, synchronization might be beneficial. Prostaglandin injected 96 hours after bull turn-in increased the percentage of cows pregnant in the first nine days of the breeding season (Whittier et al., 1991).

The effects of developing virgin heifers using corn residue are not well characterized. Therefore, the current studies evaluated the effect grazing of corn residue compared to winter range with additional supplementation around the time of breeding on first service conception rate, pregnancy rate, and first calf production characteristics. Additionally, an experiment evaluated a single injection of prostaglandin in a 25 day breeding season on pregnancy rate and calf production characteristics.

## Materials and Methods

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved the procedures and facilities used in this experiment.

***Experiment 1***

Weaned heifer calves (n = 96) were blocked by initial BW (223 ± 3 kg) and assigned randomly to graze either corn residue (**CR**) or dormant native Sandhills range (**WR**). Treatment began 30 d after weaning, from November through March (138 d). A daily supplement was offered (0.45 kg/hd; 28 % CP; 80 mg·animal$^{-1}$·d$^{-1}$ monensin). Subsequently, all heifers were recombined and grazed a common pasture for 48 d with a daily supplement (0.45 kg/hd; 28 % CP; 80 mg·animal$^{-1}$·d$^{-1}$ monensin). At breeding heifers were offered a daily supplement (**Supp**; 1.4 kg/hd; 28 % CP; 240 mg·animal$^{-1}$·d$^{-1}$ monensin) 7 d prior to and for 14 d following PG injection or not offered a supplement (**NSupp**). Two blood samples were collected 26 and 14 d prior to PG injection to assess percentage pubertal via progesterone concentration.

Estrus was synchronized using a single injection of PG (d 0). Five d prior to PG, fertile bulls were turned in with both groups of heifers for a period of 45 d. Pregnancy rate was determined via transrectal ultrasonography 40 d after bull removal. The data were analyzed using the Mixed and Glimmix procedures of SAS.

***Experiment 2***

Weaned heifer calves (n = 99) were blocked by initial BW (258 ± 3 kg) and assigned randomly to graze either corn residue (**CR**) or were developed in a dry lot (**DL**). Treatments were initiated in November following a 30 d weaning period and were continued through March. The DL heifers were offered a common diet following weaning for 143 d (6.4 kg, DMI) that was composed of (DM basis) grass hay (62%), corn silage (20%), dried distiller grain plus solubles (13%), and a supplement (5%) that provided 200 mg·animal$^{-1}$·d$^{-1}$ monensin. During the spring period, after the 143 d winter period and continuing for 45 d, the DL diet (6.8 kg, DMI) was composed of (DM basis) grass hay (57%), corn silage (26%), dried distiller grain plus

solubles (12%), and a supplement (5%) that provided 200 mg·animal$^{-1}$·d$^{-1}$ monensin.

A daily supplement was offered to the CR group while grazing (0.45 kg/hd; 28 % CP; 80 mg·animal$^{-1}$·d$^{-1}$ monensin). The CR heifers grazed for 134 d following a 30 d weaning period and were subsequently moved to the dry lot for 59 d prior to breeding. The dry lot diet (6.4 kg, DMI) for the CR group was composed of (DM basis) grass hay (62%), corn silage (20%), dried distiller grain plus solubles (13%), and a supplement (5%) that provided 200 mg·animal$^{-1}$·d$^{-1}$ monensin.

Heifers were assigned within winter treatment by BW to receive either a high (**HE**; 8.6 kg/d DMI) or low (**LE**; 10.4 kg/d DMI) energy diet 7 d prior to and for 12 d after timed artificial insemination (TAI). The HE diet was composed of (DM basis) grass hay (57%), corn silage (17%), dry-rolled corn (17%), wet corn gluten feed (6%), and a supplement (3%) that provided 200 mg·animal$^{-1}$·d$^{-1}$ monensin. The LE diet was composed of (DM basis) grass hay (74%), corn silage (16%), wet corn gluten feed (6%), and a supplement (4%) that provided 200 mg·animal$^{-1}$·d$^{-1}$ monensin.

Pubertal status was assessed as described in Exp. 1. Estrus was synchronized using an MGA/PG system. Beginning 36 d prior to TAI and continuing for 14 d, MGA was added to the diet. Eighteen and one-half d after MGA withdrawal, a single injection of PG was administered and TAI was performed for both groups 60 hr after PG. Thirteen d after TAI, fertile bulls were turned in with both groups for a period of 60 d. Transrectal ultrasonography was performed 44 d after TAI to determine first service conception rate and again approximately 50 d after bull removal to determine pregnancy rate. The data were analyzed using the Mixed and Glimmix procedures of SAS.

***Experiment 3***

Weaned heifer calves (n = 1160) grazed native Sandhills range at two locations from November through June with a supplement. Seven days prior to PG injection, heifers either received or did not receive a daily supplement (**Supp vs. NSupp**; 1.35 kg/hd; 28 % CP; 240 mg·animal$^{-1}$·d$^{-1}$ monensin) and continued to receive the supplement for 14 d after the PG injection. However, supplementation scheme was completely confounded within location and will not be presented. In addition, half of the heifers in each pasture at each location were injected with prostaglandin (**PGF** vs. **NoPGF**). Prostaglandin was injected 96 hours after the bulls were turned-in with the heifers. There were 11 mixed age bulls and approximately 580 heifers in each breeding pasture. The bulls remained with the heifers for an additional 20 d after PGF injection. Pregnancy rate was determined via transrectal ultrasound 47 days following bull removal. The data were analyzed using the Mixed and Glimmix procedures of SAS.

## Results and Discussion

***Experiment 1***

Heifer performance, reproduction, and calf production data are summarized in Table 1. The CR heifers had a lower ($P \leq 0.002$) ADG prior to breeding compared to WR heifers, but compensated with a greater ADG between the beginning of the breeding season and pregnancy diagnosis ($P < 0.001$). The CR heifers were lighter after grazing ($P < 0.001$), at PG injection ($P < 0.001$), and at pregnancy diagnosis ($P = 0.004$) when compared to the WR heifers, although BCS at pregnancy diagnosis was not different ($P > 0.10$). Supplemental nutrition did not affect ($P > 0.10$) weight or BCS at pregnancy diagnosis. There were a greater ($P < 0.001$) percentage of WR heifers pubertal prior to breeding than CR heifers, although neither winter development nor supplemental nutrition affected ($P > 0.10$) overall pregnancy rate. This is most likely due to the compensatory gain of the CR heifers during the breeding season. Furthermore, Cushman et al. (2007) suggest that more estrous cycles prior to breeding does not necessarily improve pregnancy rate. Thus, even though the CR heifers potentially experienced fewer estrous cycles prior to breeding, system did not affect pregnancy

Neither winter development nor supplemental nutrition affected ($P > 0.10$) heifer BW prior to parturition. However, CR heifers tended to have a greater ($P = 0.06$) BCS than WR heifers at this point. Winter development and supplement treatments did not affect ($P > 0.10$) the percentage of heifers calving in the first 21 days, calf birth weight, calving ease score, or sex distribution. Heifers that originally grazed WR with no supplementation around breeding gave birth to heavier ($P = 0.05$) calves than non-supplemented heifers that grazed CR. Further, calf weaning weight and second season rebreeding rates were unaffected by either treatment ($P > 0.10$), although heifers originally grazing CR were still lighter ($P \leq 0.01$) prior to the second breeding season and at second pregnancy diagnosis.

***Experiment 2***

Heifer performance, reproduction, and calf production data are summarized in Table 2. The CR heifers had a lower ($P < 0.001$) ADG prior to breeding compared to DL heifers. However, the CR heifers compensated with a greater ADG ($P < 0.001$) after being introduced to the dry lot diet and between the beginning of the breeding season and pregnancy diagnosis ($P < 0.001$). The CR heifers were lighter after grazing ($P < 0.001$), at PG injection ($P < 0.001$), and at pregnancy diagnosis ($P = 0.003$) when compared to the DL heifers. The HE diet improved ($P = 0.02$) ADG after breeding. There were a lower ($P < 0.001$) percentage of CR heifers pubertal prior to breeding than DL, yet winter development did not affect ($P > 0.10$) first service conception rate or overall pregnancy rate, much the same as Exp. 1. These data would seem to indicate beef cattle genetics have improved, minimizing the negative effect of nutrition on age at puberty and of age at puberty on pregnancy rates. Increased energy around breeding tended ($P = 0.09$) to improve first service conception rate. As these heifers were time inseminated, all individuals received additional energy for the same period of time. This would indicate that higher energy supplementation at the time of maternal recognition may improve embryo retention. There was, however, no effect ($P > 0.10$) of supplemental nutrition on overall pregnancy rate.

For those heifers that conceived to TAI, CR heifers had a longer ($P = 0.05$) gestation length than the DL heifers. However, neither winter development nor

supplemental nutrition affected ($P > 0.10$) calf birth weight, calving ease score, or sex distribution.

***Experiment 3***

Heifer BW, pregnancy rate and calf production data are summarized in Table 3. Synchronization did not affect heifer BW at pregnancy diagnosis ($P = 0.25$) or pregnancy rate ($P = 0.18$). Calf birth date was similar ($P = 0.16$) between PGF and NoPGF, however NoPGF heifers gave birth to lighter ($P < 0.001$) calves than PGF. Sex distribution was similar ($P = 0.70$) as was the percentage of heifers that experienced dystocia ($P = 0.32$). There was a greater ($P = 0.003$) percentage of PGF heifers calving in the first 21 days of the calving season. This agrees with Whittier et al. (1991) who indicate that synchronization in this manner increases the percentage of cows that conceive in the first 9 days of the breeding season. While this experiment did not directly measure the time of conception, PG injection shortened the calving season. The increased calf birth weight with PG is puzzling due to the shorter gestation length associated with this system.

## Implications

Winter development utilizing CR is a suitable alternative to WR or DL. Offering nutritionally challenged heifers a HE diet around the time of breeding may improve first service conception. While the factors that mediate these effects are unclear, developing heifers using CR does not negatively influence reproductive efficiency.

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Table 1. Effect of winter system and breeding supplementation on gain, reproduction and calf production in heifers, Exp.1

| | Treatment[1] | | | | | *P*-values[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | WR | CR | NSupp | Supp | SEM | Sys | Supp | Sys*Supp |
| n | 48 | 48 | 48 | 48 | | | | |
| Initial BW, kg | 222 | 223 | | | 3 | 0.71 | | |
| Pre-breeding BW, kg | 297 | 270 | | | 4 | <0.001 | | |
| Pregnancy diagnosis BW, kg | 364 | 346 | 353 | 357 | 4 | 0.004 | 0.54 | 0.99 |
| ADG during grazing, kg/d[3] | 0.21 | 0.10 | | | 0.01 | <0.001 | | |
| ADG after grazing, kg/d[4] | 0.99 | 0.83 | | | 0.04 | 0.002 | | |
| ADG from breeding to pregnancy diagnosis, kg/d | 0.71 | 0.81 | 0.75 | 0.77 | 0.03 | <0.001 | 0.35 | 0.27 |
| Pubertal at breeding, % | 73 | 33 | | | | <0.001 | | |
| Yearling pregnancy rate, % | 88 | 85 | 85 | 88 | | 0.77 | 0.77 | 0.77 |
| n[5] | 15 | 13 | 16 | 12 | | | | |
| Pre-calving BW, kg | 449 | 437 | 440 | 445 | 6 | 0.17 | 0.59 | 0.93 |
| Pre-breeding BW, kg | 418 | 397 | 404 | 411 | 5 | 0.004 | 0.40 | 0.62 |
| Second pregnancy diagnosis BW, kg | 458 | 429 | 437 | 450 | 8 | 0.01 | 0.24 | 0.20 |
| BCS before calving | 5.1 | 5.2 | 5.2 | 5.1 | 0.1 | 0.06 | 0.30 | 0.05 |
| Calved in first 21d, % | 74 | 65 | 70 | 69 | | 0.37 | 0.94 | 0.29 |
| Calf birth weight, kg | 32.6 | 33.0 | 32.9 | 32.7 | 0.7 | 0.60 | 0.87 | 0.05 |
| Calf birth date, julian d | 62 | 65 | 63 | 63 | 2 | 0.20 | 0.80 | 0.45 |
| Bull calves, % | 53 | 58 | 65 | 46 | | 0.71 | 0.08 | 0.20 |
| Calving ease score[6] | 1.5 | 1.5 | 1.6 | 1.4 | | 0.63 | 0.44 | 0.18 |
| Two-year old pregnancy rate , % | 87 | 100 | 94 | 92 | | 0.17 | 0.83 | 0.99 |

[1] WR = heifers developed on winter range, CR = heifers developed on corn residue, NSupp = heifers receiving no supplemental concentrate around breeding, Supp = heifers receiving supplemental concentrate

[2] Sys = heifer winter development system; Supp = supplemental concentrate around breeding

[3] ADG during the winter grazing period; [4] ADG after the winter grazing period prior to breeding

[5] Number of heifers/treatment with calving data, subsequent data in table generated from this n

[6] 1= No assistance, 2= easy assist, 3= difficult assist, 4= caesarean section, 5= breech/abnormal presentation

Table 2. Effect of winter system and energy level around breeding on growth, reproduction, and calf production in heifers, Exp. 2

| | Treatment[1] | | | | | *P*-values[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | DL | CR | HE | LE | SEM | Sys | Supp | Sys*Supp |
| n | 49 | 50 | 50 | 49 | | | | |
| Initial BW, kg | 259 | 258 | | | 3 | 0.89 | | |
| Pre-breeding BW, kg | 414 | 366 | | | 5 | <0.001 | | |
| Pregnancy diagnosis BW, kg | 485 | 460 | 475 | 471 | 6 | 0.003 | 0.63 | 0.80 |
| ADG during winter, kg/d[3] | 0.63 | 0.21 | | | 0.01 | <0.001 | | |
| ADG during spring, kg/d[4] | 1.11 | 1.32 | | | 0.03 | <0.001 | | |
| ADG from breeding to pregnancy diagnosis, kg/d | 0.57 | 0.74 | 0.70 | 0.62 | 0.03 | <0.001 | 0.02 | 0.23 |
| Pubertal at breeding, % | 94 | 47 | | | | <0.001 | | |
| AI pregnant, % | 50 | 48 | 58 | 41 | | 0.90 | 0.08 | 0.60 |
| Yearling pregnancy rate, % | 88 | 82 | 86 | 84 | | 0.36 | 0.73 | 0.14 |
| n[5] | 44 | 40 | 43 | 41 | | | | |
| Gestation length, d[6] | 281 | 284 | 282 | 282 | 1 | 0.05 | 0.91 | 0.32 |
| Calved in first 21d, % | 64 | 69 | 70 | 63 | | 0.58 | 0.53 | 0.34 |
| Calf birth weight, kg | 36.1 | 34.8 | 35.8 | 35.1 | 0.8 | 0.22 | 0.47 | 0.20 |
| Calf birth date, julian d | 77 | 78 | 76 | 78 | 2 | 0.68 | 0.52 | 0.10 |
| Bull calves, % | 59 | 54 | 57 | 56 | | 0.64 | 0.95 | 0.20 |
| Calving ease score[7] | 1.5 | 1.4 | 1.5 | 1.4 | | 0.73 | 0.65 | 0.71 |

[1] DL = heifers developed in the dry lot, CR = heifers developed on corn residue, HE = heifers receiving higher energy diet around breeding, Supp = heifers receiving lower energy diet around breeding

[2] Sys = heifer winter development system; Supp = supplemental concentrate around breeding

[3] ADG during the winter period; [4] ADG after the winter period prior to breeding

[5] Number of heifers/treatment with calving data, subsequent data in table generated from this n

[6] Of heifers that conceived to timed AI

[7] 1= No assistance, 2= easy assist, 3= difficult assist, 4= caesarean section, 5= breech/abnormal presentation

Table 3. Effect of prostaglandin synchronization 96 hrs after bull turn-in on reproduction and calf production in heifers, Exp. 3

| Item | PGF | NoPGF | SEM | P |
|---|---|---|---|---|
| n | 570 | 590 | | |
| Weight, kg | | | | |
| Initial BW, kg | 237 | 230 | 4 | 0.20 |
| Pregnancy diagnosis BW, kg | 370 | 360 | 6 | 0.20 |
| Pregnant, % | 80 | 83 | | 0.18 |
| n[1] | 330 | 354 | | |
| Calved in first 21d, % | 78 | 68 | | 0.003 |
| Calf birth weight, kg | 37.4 | 35.8 | 0.3 | <0.001 |
| Calf birth date, julian d | 89 | 90 | 0.5 | 0.16 |
| Bull calves, % | 54 | 52 | | 0.70 |
| Calving ease score[2] | 1.3 | 1.2 | 0.3 | 0.45 |

[1] Number of heifers/treatment with calving data, subsequent data in table generated from this n

[2] 1= No assistance, 2= easy assist, 3= difficult assist, 4= breech/abnormal presentation, 5= caesarean section

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# VARIATIONS IN INTERVAL BETWEEN GNRH AND PROSTAGLANDIN $F_{2\alpha}$ INJECTIONS AND CIDR INSERTION FOR 5 OR 7 DAYS ON PREGNANCY RATE AND FOLLICULAR RESPONSE

**A. Hyland[1], J.C. Whittier[1*], G.E. Seidel Jr.[2], R.M. Enns[1] and R.K. Peel[1]**

[1]Department of Animal Sciences, Colorado State University, Fort Collins
[2]Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins

**ABSTRACT:** Angus-based cows (n=509), over 2 yr, were used to evaluate the effects of 5 or 7 d controlled intravaginal drug releasing (**CIDR**) administration and 5 or 7 d intervals between first GnRH and prostaglandin (**PG**) injection to determine effects on follicle size at insemination and AI pregnancy rate (**PR**). Melengestrol acetate (**MGA**) was fed to all cows for 14 d prior to trt. Cows were randomized into 3 trt groups. Group 1 (**5,5**) received both a CIDR and GnRH 14 d after MGA, then CIDR removed and PG given 5 d later. Group 2 (**7,7**) received a CIDR and GnRH 12 d after MGA, with CIDR removal and PG 7 d later. Group 3 (**7,5**) received a CIDR 12 d after MGA and GnRH 2 d after CIDR insertion, with CIDR removal and PG given 5 d after GnRH. All cows were observed for signs of estrus 2X/d for 72 h following PG injection. Cows detected in estrus were AI approx. 12 h after observed estrus (**EAI**), and cows not observed in estrus by 72 h were time inseminated (**TAI**) at approx. 78 h after PG and given GnRH. Diameter of the largest follicle was determined by trans-rectal ultrasonography (**US**) at AI. PR to AI was determined by US 41-43 d after AI. PR was higher (P<0.01) in 7,5 (69.0%) than the 5,5 cows (56.3%) with the 7,7 cows intermediate (63.0%). The proportion bred on estrus was greater (P<0.01) for the 7,7 cows (50.3%) compared to the 5,5 cows (30.6%). More (P<0.05) 7,5 cows were bred EAI (40.8%) than 5,5 cows. Cows in 7,5 trt had higher (P<0.05) PR to TAI (64.2%) than 5,5 cows (46.8%) or 7,7 cows (45.1%). Cows bred at estrus had similar PR for all trt (77.6%, 76.0%, and 80.7% for the 5,5, 7,5, and 7,7 trt, respectively). Follicle size at breeding was 13.1 mm, and did not differ between trt, but was larger (P<0.05) for 2-yr-olds (13.4 ± .4 mm) than older cows (12.5 ± .2). Unlike other recent work with shorter CIDR and GnRH to PG interval, single, rather than double PG injections were used in this study. We conclude that increased PR can be obtained by shortening the interval between GnRH and PG injections in the presence of CIDR.

**Key Words:** GnRH, CIDR, AI, Beef Cows

## Introduction

Various estrous synchronization programs have been developed to improve the success of timed AI. These programs usually involve a combination of gonadotropin releasing hormone (GnRH) and prostaglandin $F_{2\alpha}$ (PG). Bridges et el. (2008) reported that shortening the duration of P4 exposure and the interval between GnRH and PG injections from 7 to 5 d in the Select Synch + CIDR protocol would improve pregnancy rates. The objectives of this project were to determine the difference of the effects of the treatments on follicular size at breeding and subsequent pregnancy rates.

## Materials and Methods

The experiment used three treatment groups (Figure 1) designated hereafter as follows: **5,5**= CIDR for 5 d and 5 d interval between GnRH and $PGF_{2\alpha}$ injections; **7,7**=CIDR for 7 d and 7 d interval between GnRH and $PGF_{2\alpha}$ injections; or **7,5** = CIDR for 7 d and 5 d interval between GnRH and $PGF_{2\alpha}$ injections. Cows were assigned randomly to the three treatment groups. The experiment consisted of 509 (year 1 n=248; year 2 n=261) Angus-based cows over 2 years of age. All cows were fed a 0.5 mg/hd/day of melengestrol acetate (Ahola et al., 2006) in approximately 1.5 kg of range cube supplement for 14 d. Twelve days after MGA feeding ended (d 0), the cows in groups 7,7 and 7,5 had a CIDR inserted and 7,7 cows received a 100 µg injection of GnRH. On day 2, cows in group 5,5 had a CIDR inserted and 7,5 and 5,5 cows were given a 100 µg injection of GnRH. CIDR inserts were removed from all cows on d 7. At CIDR removal, all cows received a single injection of 25 mg of $PGF_{2\alpha}$. All cows were evaluated for body condition.

Estrus detection was done for 2 h twice daily to the timed AI. All cows detected in estrus were inseminated approximately 12 h after detection (**EAI**). All cows not detected in estrus were time inseminated (**TAI**) and received 100 µg of GnRH at 78 h post $PGF_{2\alpha}$. Ultrasound imaging was used to determine pregnancy to AI at 35-41 days post insemination.

Pregnancy rates, percentages of cows bred on estrus, and BCS where compared using PROC GENMOD (SAS 9.1) adjusting for the factors of experiment year, age (2 yrs vs. older), year by treatment interaction, BCS, AI technician within year, interval from calving to the start of MGA and breed. The factors not showing significant effect at the alpha = .05 level, were removed using backwards elimination and the analysis was repeated. Least squares means were determined by using PROC GLM (SAS 9.1) and the model chosen previously. Interval from PG injection to AI, interval from calving to the start of MGA feeding and BCS were analyzed using PROC GLM (SAS 9.1).

At the alpha = .05 level, there was a significant effect of year but no significant effect of BCS or year by treatment interaction on interval from PG injection to AI. Similarly, there was a significant effect of year on BCS at the alpha = .05 level. The significant effects for both

interval from PG to AI and BCS were retained in the model. The was no effect of year or any interactions with treatment on interval from calving to the start of MGA feeding but there was an effect of age, so age was retained in the model. These results are included in Table 1.

## Results

Pregnancy rate to AI was greater for the 7,5 treatment (69.0%) than the 5,5 treatment (56.3%, P=.01, Table 1). There was no significant difference between the 7,7 (63.0%) and 5,5 treatments (P=.24). Age had no effect on the pregnancy rates within the three treatment groups (P=.97, Table 2). There was a difference in pregnancy rates between the 3+ year old cows in the 5,5 and 7,5 treatments. Within the 3+ yr old cows the 7,5 treatment (68.8%) produced higher pregnancy rates to AI than the 5,5 treatment (56.2%, P=.03).

Pregnancy rates for the three treatment groups were analyzed by those cows bred after exhibiting estrus and cows bred by timed AI (Table 3). The pregnancy rates were greater for the cows bred by timed AI in the 7,5 treatment (64.2%) than those cows bred by timed AI in either the 5,5 (46.8%) or the 7,7 (45.1%) treatments (P<.05). The cows bred by timed AI in the 7,5 treatment had equivalent pregnancy rates to the cows bred after exhibiting estrus in the 5,5 treatment (77.6%) and the 7,5 treatment (76.0%, P<.006). The cows bred after exhibiting estrus for the 7,7 treatment had a higher pregnancy rate (80.7%) than the 7,5 treatment timed AI cows (P=.01) but were equivalent to the 5,5 and 7,5 cows bred after exhibiting estrus (p>.09).

The percentage of cows bred by estrus AI for the three treatment groups were significantly different, overall (P=.001). When analyzed by treatment group the 7,7 treatment had significantly more cows bred on estrus than the 5,5 treatment (P<.001). The percentage of cows bred on estrus was also higher in the 7,5 treatment than the 5,5 treatment (P=.05, Table 1).

BCS did not affect pregnancy rate (data not shown). Cows with a BCS of 4 had a lower pregnancy rate to AI (45.9%) than the cows with a BCS of 5 (64.6%, P= .002). Oppositely, the cows with a BCS of 4 had a larger follicle size at insemination (13.77 mm) than the cows with a BCS of 5 (12.87 mm, P=.04). This contradicts some findings that a larger follicle size at insemination produces higher pregnancy rates. Interval from calving to the start of MGA feeding did not have an effect on treatment pregnancy rate to AI (P=.37) or pregnancy rate by estrus AI or timed AI (P=.47).

## Discussion

Pregnancy rates to AI did not differ between the 5,5 and 7,7 treatment (P=.21). These results are similar to what Bridges et al., (2008) found when a 5d Select Synch + CIDR produced pregnancy rates no different from those of 7d Select Synch + CIDR in multiparious cows. Similarly, Martinez et al. (2002) found no significant difference in pregnancy rate when examining either a 6 or 7 d interval between GnRH and PG injections in the CO-Synch protocol. This study found that the 7,5 treatment produced higher pregnancy rates to AI than the 5,5 treatment (P=.03) but produced similar (P=.36) pregnancy rates to the 7,7 treatment. These results can not be explained by the mean follicle sizes for the three treatments. The mean follicle sizes did not differ between treatments. Contrary to what Day (2004) found when examining the 5d Select Synch + CIDR and 7d Select Synch + CIDR, this study found no difference in pregnancy rates for 2 yr old cows between any of the treatments. There was a difference in the 3+ yr old cows between the 5,5 and 7,5 treatments. The 7,5 treatment produced a higher pregnancy rate to AI than the 5,5 treatment.

Pregnancy rates of the cows bred after exhibiting estrus and those bred by timed AI showed that the 7,5 treatment produced higher pregnancy rates for cows bred by timed AI than either the 5,5 or 7,7 treatments. The 7,5 timed AI pregnancy rates were similar to the pregnancy rates of cows bred after exhibiting estrus in the 5,5 and 7,5 treatments. The 7,7 treatment produced the highest pregnancy rate for those cows bred after exhibiting estrus and was similar to the pregnancy rates of the cows bred after exhibiting estrus in the 5,5 and 7,5 treatments. The 7,5 treatment may be the a better protocol for stand alone timed AI than the 5,5 or 7,5 treatments due to the higher pregnancy rates observed when cows were bred by timed AI.

The proportions of cows bred on estrus might also be used to explain the pregnancy rates to AI. Cows bred on estrus are more likely to become pregnant than those that do not exhibit estrus (Perry et al., 2005). These findings partially explain the higher pregnancy rate for the 7,5 treatment when compared to the 5,5 treatment. There was a tendency (P=.05) for more cows to be bred on estrus in the 7,5 treatment than for the 5,5 treatment. Conversely, there was no significant difference in the pregnancy rates for the 7,7 treatment and the 5,5 treatment (P=.21) but there were more cows bred on estrus for the 7,7 treatment (p<.001) than the 5,5 treatment.

Further explanation of these results can only be done by examining the events that take place during synchronization. Due to ease of management and labor availability, the three treatments were all fed MGA during the same 14 day time period and scheduled for clean-up timed AI on the same day. Therefore, the interval between MGA withdrawal and GnRH injection was 14 days for the 5,5 and 7,5 treatments and 12 days for the 7,7 treatment. Exhibition of estrous occurs approximately 3 days following MGA withdrawal (Day and Geary, 2005). Ovulation occurs at approximately 4 days following MGA withdrawal. This would have placed all cows in the 7,7 treatment at approximately day 9 of their estrous cycle (estrus = day 0) and all cows in the 5,5 treatment would have been at day 11 at the first GnRH injection and CIDR insertion. The cows in the 7,5 treatment had a CIDR implanted at day 9 and a GnRH injection at day 11. Geary et al. (2000) found that only 20% of cows between days 10 and 14 of their estrous cycle respond to a GnRH injection as opposed to 80% responding when they are between days 6 and 9. Thatcher et al (2002) found that GnRH induced turnover of follicles is most efficient if ovulation occurs as a response to the first GnRH injection. These studies are supported by others that recommend that timed AI estrous synchronization protocols are initiated between days 5 and

10 of the estrous cycle (Moreira 2000, Martinez 2002, El-Zarkouny 2004). The insertion of the CIDR 12 days after MGA withdrawal may have resulted in a persistent dominant follicle that was able to respond to the GnRH injection at 14 days after MGA. McDowell et al. (1998) were able to maintain a dominant follicle from day 7 until day 11 of the estrous cycle in 18 of 19 heifers by utilizing a norgestomet implant. Without the CIDR, the dominant follicle would have likely begun regressing and would have been less responsive to the 1st GnRH injection resulting in a lower percentage of cows that ovulated to the injection. Due to the timing of the first GnRH injection, it may not be fair to compare the pregnancy rates for the 5,5 treatment to the 7,7 and 7,5 treatments.

## Implications

As administered, the 7,7 and 7,5 protocols provide acceptable results when combining estrus detection and AI with a clean-up timed AI. The 7,5 treatment protocol provided higher pregnancy rates, but required extra handling of cattle. The 7,5 treatment may also be a better protocol when only timed AI is utilized due to the higher pregnancy rates to timed AI than the other two protocols. To get a fair comparison of the 5,5 treatment versus the others it may be necessary to administer the first GnRH injection the same number of days after MGA withdrawal.

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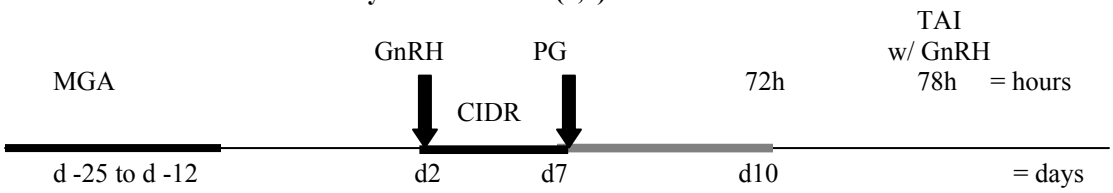
Table 1 – Least squares means of treatment effects for beef cows synchronized for estrus using three treatment protocols

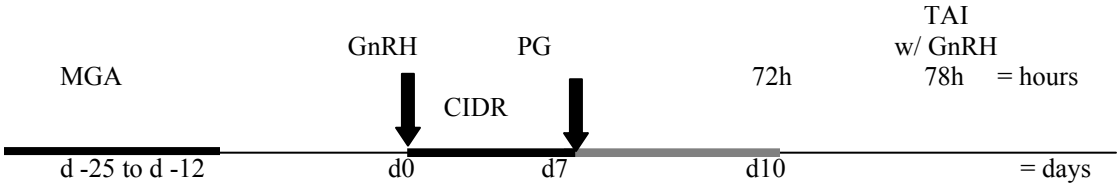
| Treatment | n | Calving to MGA (d)* | BCS* | PG to AI interval (h)* | % EAI | % Pregnant to AI |
|---|---|---|---|---|---|---|
| 5,5 | 160 | 49.5 ± 1.4 | 5.0 ± .05 | 75.9 ± .50 | 30.6% [a] | 56.3% [a] |
| 7,7 | 165 | 49.6 ± 1.4 | 5.1 ± .05 | 75.3 ± .53 | 50.3% [b] | 63.0% [a,b] |
| 7,5 | 184 | 48.8 ± 1.3 | 5.0 ± .05 | 76.6 ± .49 | 40.8% [b] | 69.0% [b] |

* - means within column do not differ between treatments (p>.05)
[a,b] – means within a column lacking a common superscript differ (P<.05)

**"MGA- GnRH- CIDR 5 days- PG- GnRH" (5,5)**




**"MGA- GnRH- CIDR 7 days- PG- GnRH" (7,7)**




**"MGA-CIDR 7 days-GnRH (5d between) PG-GnRH" (7,5)**

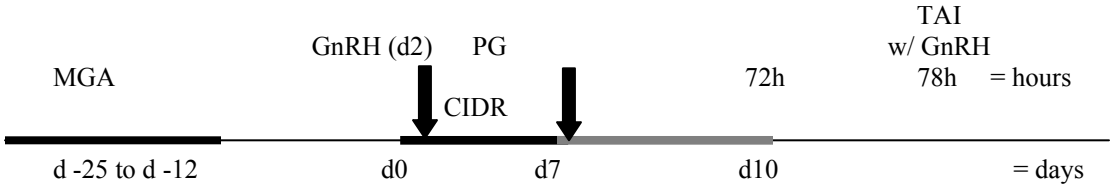



Figure 1 – Diagram of timeline for treatment combinations used to synchronize ovulation in beef cows in this experiment

Table 2 – Least squares means pregnancy rates to AI by age of cow

| Treatment | Age | n | % Pregnant to AI |
|---|---|---|---|
| 5,5 | 2 yrs | 23 | 56.5% [a,b] |
| | 3+ yrs | 137 | 56.2% [a] |
| 7,7 | 2 yrs | 20 | 60% [a,b] |
| | 3+ yrs | 145 | 63.4% [a,b] |
| 7,5 | 2 yrs | 27 | 70.4% [a,b] |
| | 3+ yrs | 157 | 68.8% [b] |

[a,b] – means within a column lacking a common superscript differ (p<.05)

Table 3 – Least squares means for pregnancy rates and follicle sizes by treatment group to estrus AI (EAI) or timed AI (TAI)

| Treatment | EAI or TAI | n | % Pregnant to AI | Follicle Size, mm |
|---|---|---|---|---|
| 5,5 | EAI | 49 | 77.6% a,c | 13.50 ± .52 [a,b] |
| | TAI | 111 | 46.8% b | 12.83 ± .37 [b] |
| 7,7 | EAI | 83 | 80.7% a | 13.85 ± .43 [a] |
| | TAI | 82 | 45.1% b | 12.26 ± .42 [b] |
| 7,5 | EAI | 75 | 76.0% a,c | 14.29 ± .46 [a] |
| | TAI | 109 | 64.2% c | 12.58 ± .36 [b] |

[a,b,c] – means within a column lacking a common superscript differ (P<.05).

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# EFFICACY OF THE PORTASCC® MILK TEST TO ESTIMATE SOMATIC CELL COUNT (SCC) AND EFFECT OF SAMPLING DAY ON CONSTITUENTS IN SHEEP MILK

**E.R. Kretschmer[1*], D.W. Holcombe[2*], G.C.J. Fernandez[*], and D. Redelman[*]**
[*] Department of Animal Biotechnology, University of Nevada, Reno 89557

**ABSTRACT:** The PortaSCC® milk test is an on-farm test which uses a test strip requiring a drop of milk and produces a color change proportional to the somatic cell count (SCC) in cow's milk. The objectives of this study were to assess the effectiveness of the PortaSCC® milk test to estimate SCC and determine the effect of sampling day on sheep milk constituents. Ninety-two Rambouillet x Merino ewes were sampled from each udder side at weaning (89 ± 16 d) and 24 h post-weaning. Milk samples were analyzed by the PortaSCC® milk test, flow cytometry (FC) and the traditional method (TM) using a Bentley 2000 component analyzer. The four color changes produced by the PortaSCC® milk test strip represent SCC ranges for cow's milk of < 200 (< 5.3 $log_{10}$), 200 to 750 (5.3 to 5.9 $log_{10}$), 750 to 2,000 (5.9 to 6.3 $log_{10}$), and > 2,000 x $10^3$ cells/mL (>6.3 $log_{10}$), and indicate udder health status of Healthy, Healthy/Inflammation, Infection/Subclinical Mastitis, and Chronic/Clinical Mastitis, respectively. For both FC and TM, most udder sides that tested in the Healthy and Chronic/Clinical Mastitis (58% and 79%; 86% and 100 %, respectively) categories remained within the SCC ranges for those categories. Udder sides within the Healthy/Inflammation category were 72% and 39% and for the Infection/Subclinical Mastitis category were 9% and 14% (FC and TM, respectively). $Log_{10}$ SCC values differed (P < .0025) between the Healthy, Chronic/Clinical and the two intermediate categories for FC; all four categories differed (P < .0007) for TM. $Log_{10}$ values were greater (P < .0001) and more udder sides tested Chronic/Clinical at 24 h post-weaning than at weaning. Protein, lactose and solids concentrations differed (P < .05) between weaning and 24 h post-weaning in several categories. Our results suggest the PortaSCC® milk test is suitable for distinguishing between Healthy and Chronic/Clinically infected udders but not the intermediate health categories for sheep milk.
Key Words: Mastitis, Milk Constituents

## Introduction

Mastitis, an inflammation of the mammary gland, is a disease of lactating ewes that occurs in all sheep-producing countries (Jones and Watkins, 2000). Mastitis causes losses for the sheep industry through premature culling, a decrease in milk quality and quantity, poor lamb growth, and in severe cases, death (Watson and Buswell, 1984; Torres-Hernandez and Hohenboken, 1979). Acute mastitis can be readily diagnosed either visually or by palpation, but the only means of identifying subclinical mastitis is by measuring SCC in the milk (Keisler et al., 1992).

The PortaSCC® milk test (PortaScience Inc., Moorestown, NJ) is an easy to use on-farm test developed to estimate SCC in bovine milk. The test consists of a disposable test strip that requires one drop of milk and produces a color change proportional to the SCC in milk. The strip produces one of four colors which can be read by visual comparison to a color chart. The objectives of this study were to 1) assess the effectiveness of the PortaSCC® milk test to estimate SCC and detect subclinical mastitis in sheep and 2) determine the effects of cell counting method and sampling day on SCC.

## Materials and Methods

*Animal Management.* Ninety-two Rambouillet x Merino multiparous and primiparous lactating ewes (2-6 yr of age) were used in this study. All ewes were placed in a dry-lot and pen-fed alfalfa pellets a week prior to parturition and remained there through the first 60 d following parturition. Ewes were placed on pasture at 60 d postpartum and remained there until weaning (89 ± 16 d). Milk samples were collected the day of weaning and 24 h post-weaning.

*Milk Sampling and Analysis.* Before sampling, udders were disinfected with isopropyl alcohol and the first ~3 mL of milk from each teat was stripped and discarded. A 40 mL sample was collected from each udder half and used to determine SCC and milk constituent concentrations.

Milk samples were analyzed for SCC by PortaSCC® milk test, flow cytometry (FC) and for SCC and constituents by the traditional method (TM) using a Bentley 2000 component analyzer. For the PortaSCC® milk test 33 µL of milk was pipetted from each sample tube onto the sample window of the PortaSCC® milk test strips along with 100µL of activator solution. The strips were allowed to develop for 1 h, during which time a color reaction took place correlating to the SCC in the milk. The blue color generated by the color reaction was read visually by comparison to the Quick Check Color Chart.

For FC analysis, a 50-µL sample of milk was pipetted from each tube into separate test tubes. Cells in the milk were counted by dilution in a detergent solution that also contained propidium iodide (PI) to label nuclei of lysed cells and beads at a known concentration.

Somatic cell counts and milk constituents were analyzed using a Bentley 2000-Somacount 500 Combi® (TM; Bentley Instruments, Chaska, MN). The upper

SCC limit for the Bentley 2000 is 9,999 x $10^3$ cells/mL, thus actual SCC for seven ewe sides that exceeded this limit were estimated using linear regression.

*Statistical Procedure.* SAS software version 9.1 (SAS Institute Inc., Cary, NC) was used to perform all statistical analyses. The SCC were transformed to the logarithm to the base of 10 which is commonly used to describe SCC associated with mastitis and milk quality (Ali and Shook, 1980). Using SAS PROC MIXED, two-factorial and three-factorial models with variables and their interactions were fitted to determine factors influencing the $\log_{10}$ transformation of SCC. Only statistically significant ($P < .05$) terms were included in the final models. The variables, cell counting method (FC and TM), udder health categories and sampling day (weaning and 24 h post-weaning) were fitted into the factorial models. A two (sampling day) x four (udder health category) factorial arrangement was used to examine the effect of sampling day and udder health category on concentrations of protein, fat, lactose and solids in ewe milk.

## Results

The four color changes produced by the PortaSCC® milk test strip were no color change, light blue, blue and dark blue, and represent SCC ranges for cow's milk of < 5.3, 5.3 to 5.9, 5.9 to 6.3 and > 6.3 $\log_{10}$ cells/mL, respectively. Based on previous studies (Gonzalez-Rodriguez, 1995; Romeo et al., 1996), the SCC range for no color change (<5.3 $\log_{10}$ cells/mL) on the test strip indicated the normal limit for a healthy udder half, and the SCC range for light blue (5.3 to 5.9 $\log_{10}$ cells/mL) indicated a range of healthy to inflamed/infected udder halves. The blue color represents a SCC range of 5.9 to 6.3 $\log_{10}$ cells/mL and indicated infection/subclinical mastitis. The dark blue color represented a SCC range of > 6.3 $\log_{10}$ cells/mL and indicated chronic/clinical mastitis. Therefore, the color changes on the test strip of no color, light blue, blue and dark blue are represented as udder health categories of Healthy, Healthy/Inflammation, Infection/Subclinical Mastitis, and Chronic/Clinical Mastitis, respectively.

The percent of udder sides that fell below (false negative), within, or above (false positive) the projected SCC range for the udder health category in which they tested are shown in Table 1. For FC and TM, 58% and 79%, respectively, of udder sides that tested in the Healthy category fell within the projected SCC range for that category and 42% and 21% had SCC above the projected SCC range for that health category. For FC, 119 of 127 udder sides and for TM 58 of the 64 udder sides above the SCC range for Healthy had SCC below 5.7 $\log_{10}$ cells/mL, indicating within normal limits of a healthy udder. Most udder sides not within the projected SCC range for the Healthy/Inflammation category fell below 5.3 $\log_{10}$ cells/mL. Less than 14% of udder sides in the Infection/Subclinical Mastitis category for FC and TM were within the projected SCC range for that category; most udder sides had SCC below the projected SCC range, some as low as 5.0 $\log_{10}$ cells/mL.

No PortaSCC® test strip category by sampling day by cell counting method was detected ($P = .33$); therefore, the three-way interaction was removed from the statistical model. An interaction between PortaSCC® test strip category and cell counting method was detected ($P < .0001$) and is shown in Table 2. Somatic cell counts in $\log_{10}$ transformation for samples in the Healthy range were greater ($P < .0001$) for the FC method than TM. No difference ($P > .07$) was detected between methods for samples within the Healthy/Inflammation and the Infection/Subclinical Mastitis categories. Somatic cell count values in $\log_{10}$ transformation measured by TM testing Chronic/Clinical Mastitis were greater ($P < .03$) than those measured by FC.

For both methods, $\log_{10}$-transformed SCC values were lower ($P \leq .0025$) for Healthy than the other three categories. For FC, the two intermediate categories did not differ ($P > .08$), and values for Chronic/Clinical Mastitis were greater ($P < .0001$) than the other three categories. Values measured by TM for Healthy/Inflammation, Infection/Subclinical Mastitis and Chronic/Clinical Mastitis differed amongst the categories ($P \leq .0007$), with each representing successively greater SCC, respectively. Somatic cell count values in $\log_{10}$ reported for Infection/Subclinical Mastitis were greater ($P < .05$) than those observed for Healthy and Healthy/Inflammation, but less than ($P < .0001$) Chronic/Clinical Mastitis.

McFarland (2000) and Surian (2001) reported that ewes with SCC 5.6 $\log_{10}$ cells/µL were classified as likely having an infection and above 1 million cells/mL was suggested to indicate subclinical mastitis. By these standards, $\log_{10}$ values measured by TM for healthy and healthy/inflammation remain within normal SCC limits of a healthy udder. Somatic cell count values in $\log_{10}$ transformation reported for Infection/Subclinical mastitis were greater ($P > .05$) than those observed for Healthy and healthy/inflammation, but less than ($P < .0001$) Chronic/Clinical mastitis. The average actual SCC of both methods for Infection/Subclinical was 5.7 ± .10 $\log_{10}$. Any SCC values in this range would be considered to be near the value associated with subclinical mastitis. The $\log_{10}$ transformed values in the Chronic/Clinical Mastitis range (represented at least 2 million cells/mL) had a mean above 7 million cells/mL.

No sampling day by cell counting method or sampling day by PortaSCC® test strip category interaction was detected ($P > .17$) for SCC; however, an overall sampling day effect on SCC was detected ($P < .0001$). The mean SCC values were greater at 24 h post-weaning (5.9 ± .04 $\log_{10}$) than at weaning (5.5 ± .04 $\log_{10}$).

The effect of sampling day and test strip category on % concentrations of protein, fat, lactose and solids is shown in Table 3. No sampling day by test strip category interaction was detected ($P = .76$) for fat concentration in milk. An interaction between sampling day and test strip category was detected ($P \leq .004$) for percentages of protein, lactose and solids. Protein concentration increased from weaning to 24 h post-weaning for samples that tested in the Healthy, Infection/Subclinical Mastitis or Chronic/Clinical Mastitis

categories. Lactose concentrations did not differ ($P > .15$) for samples that tested in the Healthy, Healthy/Inflammation or Infection/Subclinical Mastitis categories, but samples that tested in the Chronic/Clinical Mastitis category decreased ($P < .0001$) from weaning to 24 h post-weaning. Percent solids increased ($P < .0012$) from weaning to 24 h post-weaning for samples that tested in the Healthy and Infection/Subclinical Mastitis categories. However, values did not differ between days for samples in the Healthy/Inflammation category, but decreased ($P < .0001$) from weaning to 24 h post-weaning for samples in the Chronic/Clinical Mastitis category.

At weaning, no difference ($P > .2$) was detected among udder health categories for protein concentration. At 24 h post-weaning values for Healthy, Healthy/Inflammation and Infection/Subclinical Mastitis did not differ ($P > .05$) but were lower ($P < .004$) than those for Chronic/Clinical Mastitis. Lactose concentrations for the first three udder health categories did not differ ($P > .5$) at weaning, but were lower ($P < .0001$) than those for Chronic/Clinical Mastitis. Conversely, at 24 h post-weaning % lactose for udder sides that tested Chronic/Clinical Mastitis was lower ($P < .0001$) than values for the other three categories. At weaning, % solids for Healthy, Healthy/Inflammation and Infection/Subclinical Mastitis did not differ ($P < .05$) but were lower ($P < .0001$) than Chronic/Clinical Mastitis. At 24 h post-weaning, % solids for Healthy and Infection/Subclinical Mastitis were greater ($P < .013$) than for Chronic/Clinical Mastitis, whereas values for Healthy/Inflammation did not differ ($P > .05$) from the other three categories.

## Implications

Our results indicate that the PortaSCC® milk test could be used as an on-farm tool for determining udder health status in sheep as either Healthy or within the Chronic/Clinical mastitis range. Similarly, Berger (2006) reported that the PortaSCC® milk test appears to be a good tool for the detection of either healthy udder sides with a very low SCC (< 5.3 $\log_{10}$ cells/mL) or sides with chronic mastitis (SCC > 6.0 $\log_{10}$ cells/mL). However, this test has not shown to be able to differentiate between udder sides between the Healthy and Infection/Subclinical mastitis ranges (between 5.3 and 6.0 $\log_{10}$ cells/mL). This study indicates that the SCC standards for cattle do not apply to sheep and a SCC of 5.7 $\log_{10}$ cells/mL should be used as the cutoff to determine healthy and infected udder sides. Sampling day affected SCC, indicating that testing at 24 h post-weaning would detect more ewes with udder health problems than at weaning.


## Acknowledgements

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Table 1. The percent of udder sides below (false negative), within and above (false positive) the projected somatic cell count (SCC x $10^3$ cells/mL) range for the four test strip categories as measured by flow cytometry (FC) and the traditional method (TM.

| Udder Health Category[a] (SCC range) | FC | | | TM | | |
|---|---|---|---|---|---|---|
| | False Negative | Within Range | False Positive | False Negative | Within Range | False Positive |
| Healthy (< 5.3 $\log_{10}$) | 0% | 58% | 42% | 0% | 79% | 21% |
| Healthy/ Inflammation (5.3 – 5.9 $\log_{10}$) | 17% | 72% | 11% | 44% | 39% | 17% |
| Infection/ Subclinical Mastitis (5.9 – 6.3 $\log_{10}$) | 91% | 9% | 0% | 73% | 13.5% | 13.5% |
| Chronic/ Clinical mastitis (> 6.3 $\log_{10}$) | 14% | 86% | 0% | 0% | 100% | 0% |

[a] n = 299, 18, 22, and 14 for udder sides that tested in udder health categories: Healthy, Healthy/Inflammation, Infection/Subclinical Mastitis and Chronic/Clinical Mastitis, respectively.

Table 2. Effect of flow cytometry (FC) and the traditional method (TM) on SCC (mean ± confidence interval) in $\log_{10}$ transformation for each PortaSCC® test strip category.

| Strip Category | SCC Testing Method | | SE | Color Chart[a] |
|---|---|---|---|---|
| | FC | TM | | |
| Healthy[b] | 5.2 [e] | 4.9[f] | .03 | < 5.3 |
| Healthy/ Inflammation | 5.5 [d] | 5.3[e] | .12 | 5.3 - 5.9 |
| Infection/ Subclinical Mastitis | 5.7 [d] | 5.7[d] | .10 | 5.9 - 6.3 |
| Chronic/ Clinical Mastitis[b] | 6.6[c] | 6.9[c] | .13 | > 6.3 |
| SE | .13 | .13 | | |

[a] Values represent estimated SCC ranges for each PortaSCC® test strip category.
[b] Row values with different superscripts differ (P < .03).
[c,d,e,f] Column values with different superscripts differ (P < .003).

Table 3. Effect of sampling day and PortaSCC® test strip category on protein, lactose and solids concentrations in ewe milk.[a]

| Strip Category | Protein % | | Lactose % | | Solids % | |
|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 1 | Day 2 | Day 1 | Day 2 |
| Healthy | 5.5 ± .07[c] | 6.0 ± .07[b,z] | 4.9 ± .05[z] | 4.9 ± .05[y] | 11.2 ± .06[c,z] | 12.0 ± .06[b,y] |
| Healthy/ Inflammation | 5.7 ± .30 | 5.7 ± .30[z] | 4.8 ± .20[z] | 4.8 ± .20[y] | 11.5 ± .23[z] | 11.5 ± .23[d,z] |
| Infection/Subclinical Mastitis | 5.2 ± .20[c] | 6.4 ± .34[b,z] | 5.0 ± .15[z] | 4.6 ± .23[y] | 11.0 ± .18[c,z] | 12.0 ± .26[b,y] |
| Chronic/Clinical Mastitis | 5.0 ± .45[c] | 7.2 ± .30[b,y] | 7.6 ± .30[b,y] | 3.1 ± .20[c,z] | 13.3 ± .40[b,y] | 11.2 ± .23[c,z] |

[a] Day 1 = weaning; Day 2 = 24 h post-weaning, mean ± SE.
[b,c] Row values within constituent with different superscripts differ (P < .0001).
[y,z] Column values within day and constituent with different superscripts differ (P < .0001).

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# LIMIT FEEDING NON-LACTATING, NON-PREGNANT BEEF COWS WITH BUNKERED WET DISTILLERS GRAINS PLUS SOLUBLES OR DISTILLERS SOLUBLES

**L. M. Kovarik*, M. K. Luebbe, R. J. Rasby, and G. E. Erickson**
University of Nebraska-Lincoln

**ABSTRACT:** Seventy non-lactating, non-pregnant beef cows (592 ± 63 kg) were used to evaluate the performance of limit-fed diets containing bunkered wet distillers grain plus solubles (WDGS; n = 24) and bunkered distillers solubles (DS; n = 22) compared to a control diet (CON; n = 24). The study was a completely randomized design with cows being stratified by age and BW and assigned to pens. Pens (3/treatment) were assigned randomly to treatment. Wet distillers grains plus solubles and DS were stored in a bunker with ground (17.78 cm screen) corn stalks 30 days prior to the start of the trial. Wet distillers' grains plus solubles were stored in combination with 30% cornstalks while DS were stored with 59% cornstalks (DM basis). The isocaloric, isonitrogenous diets were fed for 76 d and formulated to maintain BW. Wet distillers grains plus solubles diet contained 41% WDGS and 59% corn stalks with intake limited to 7.71 kg/d. Distillers solubles diet contained 41% DS and 59% corn stalks with intake limited to 7.71 kg/d. Control diet contained 43% bromegrass hay, 34% corn stalks and 23% alfalfa haylage and fed ad libitum (10.35 kg/d intake). Wet distillers grains plus solubles diet was 4.9% fat and 0.24% sulfur while the DS diet was 9.2% fat and 0.37% sulfur DM basis. Data were analyzed using Proc MIXED procedure of SAS. Initial BW and body condition score among treatments (1=emaciated; 9=obese) were similar. Final BW differed (P<0.01) between the WDGS (625.5 kg) treatment compared to the DS (611.8 kg) and CON treatments (610.9 kg). Average daily gain tended (P=0.09) to be greater for WDGS (0.374 kg/d) treatment compared to the CON treatment (0.20 kg/d). These data suggest that cows limit fed a diet of either WDGS or DS stored in a bunker with ground corn stalks has no negative impacts on performance.

**Key Words:** Beef Cows, By-products, Limit Feeding

## Introduction

Corn-based diets fed at a restricted intake can be used to meet nutrient needs for beef cows in gestation and early lactation without adverse affects on production (Loerch et al., 1998). Ethanol by-products are also a viable source of nutrients for cows and could be used with low quality forages to provide a limit-fed ration that meets maintenance requirements. The objective of this study was to evaluate the performance of non-lactating, non-pregnant beef cows limit-fed ethanol by-products compared with an ad libitum forage diet.

## Materials and Methods

Seventy non-lactating, non-pregnant beef cows (592 ± 62.7kg) were stratified by age and BW then randomly assigned to one of three treatments groups and fed to maintain BW. Cows were fed at the UNL ARDC feedlot near Mead, NE. Treatment diets were formulated to be isocaloric and isonitrogenous for the 76 d duration of the experiment. Cows (3 pens/treatment) were limit-fed a 41:59 ratio of bunkered wet distillers grains plus solubles and corn stalks limited to 7.71 kg/hd/d (1.3% of BW; WDGS; n = 24), bunkered distillers solubles (DS; n = 22) and corn stalks at a 41:59 ratio were offered at 7.71 kg/hd/d (1.3% of BW), or a control diet (CON; n = 24) containing 43% bromegrass, 34% corn stalks and 23% alfalfa haylage to provide ad libitum intake. The WDGS and DS were mixed and stored 30 d prior to the start of the trial. To prepare the material to be bunkered, corn stalks were ground through a 17.8 cm screen. Distillers solubles or WDGS and corn stalks were weighed into a Rotomix truck and mixed for five minutes then packed into a concrete bunker using a skid steer loader. The targeted ratio for storage in the bunker was a ratio of 65:35 by-product to corn stalks (DM basis). However, DS bunker would not pack at this ratio and so corn stalks were added until the bunker would pack and the optimal ratio was 41:59 distillers solubles to corn stalks ratio. The WDGS:corn stalks bunker was also adjusted to a storable ratio of 70:30 of wet distillers grains plus solubles and corn stalks, respectively. Wet distillers grains plus solubles and DS bunkered material were covered with plastic. Wet distillers grains plus solubles treatment was mixed at feed delivery with corn stalks to attain the 41%WDGS 59% corn stalks treatment ratio. DS was feed directly from the bunker. Prior to trial initiation cows were limit-fed a diet for five days to minimize error due to gut fill (1.9% BW) 40% brome hay, 10% alfalfa hay, and 50% wet corn gluten feed. Two d consecutive initial and final weights were recorded to determine performance characteristics. Limestone was added to limit-fed diets to achieve a minimum Ca: P ratio of 1.5:1. Salt and trace mineral blocks were offered free choice in the bunks. Data were analyzed using the Proc MIXED procedure of SAS with pen as the experimental unit.

## Results

Initial and final body condition scores were not different among treatments and averaged 5.9. Initial BW across treatments was similar (P=0.20). Final BW was greater (P<0.01) for the WDGS (626 kg) treatment compared to DS (612 kg) and CON treatments (610 kg). Dry matter intakes were 10.35 kg for the CON fed compared with 7.71kg for the limit-fed WDGS and DS fed treatments. ADG tended (P=0.09) to be greater for WDGS treatment (0.37 kg/d) compared to the CON treatment (0.20 kg/d).

## Discussion

Performance differences were not observed with cows limit-fed WDGS or DS treatments. Final BW was greater (P<0.01) for WDGS cows compared to DS and CON cows (Table 1). Although not significant, initial weight for WDGS cows was greater than DS cows. The difference in final BW may due to initial BW differences. ADG was not different between WDGS and DS (Table 1). Fat levels of the diets were 9.2% and 4.9% for DS and WDGS treatments, respectively. As fat level in the diet increased we hypothesized that ADG would be negatively impacted, thus anticipating a difference in ADG when comparing WDGS and DS treatments. Paven et al. (2007) stated that corn oil supplementation decreased NDF digestibility by 6 and 12% for corn oil supplementation of .75 g/kg of BW and 1.5 g/kg of BW respectively. A 3% improvement in NDF digestibility in steers fed a low fat distillers grain compared to steers fed a high fat distillers grain was observed by Corrigan et al. (2008) and was approaching significance (P=0.14). The CON treatment effects were likely due to lower DMI (1.8% of BW). Cows in the CON treatment visually sorted their diet. Cows on the WDGS and DS treatments did not sort their diet and consumed essentially 100% of their diets daily. Schoonmaker et al. (2003) concluded that limit fed corn, stockpiled orchardgrass, and ad libitum orchardgrass hay will all maintain cows in mid to late gestation, as well as in late gestation to early lactation. Therefore, selection of energy source for beef cows can be made based on individual economics of the feed resources available. With the increasing availability of ethanol by-products, producers may consider using WDGS and DS in limit-fed rations. However the dietary fat should be closely monitored because of its possible negative effect on forage digestion. Ethanol by-products follow cyclical prices in relationship to cattle on feed placements. By-products can be purchased and stored with low quality forages for later use.

## Implications

Spring and summer pasture is the most common for beef cows. However, if forage is limited, such as in drought conditions producers could consider limit-feeding ethanol by-products. Non-lactating, non-pregnant mature beef cows can be maintained on a limit-fed diet of WDGS or DS similarly to feeding forage diets ad libitum.

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Table 1. Effects of limit feeding non-lactating, non-pregnant beef cows

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| Performance Characteristics | WDGS | DS | CON | SEM | P-VALUE |
| Initial BW, kg | 597 | 588 | 595 | 10 | 0.20 |
| Final BW, kg | 626[a] | 612[b] | 611[b] | 7 | <0.01 |
| ADG, kg/d | 0.37 | 0.31 | 0.20 | 0.20 | 0.09 |
| DMI, kg/d | 7.71 | 7.71 | 10.35 | | |

[1]Dietery treatments: WDGS = Wet distillers grains plus solubles mixed with corn stalks; DS = Distillers solubles mixed with corn stalks; CON = Corn stalks , alfalfa haylage, and brome hay

[a,b] Within a row, means without a common superscript differ (P<0.01).

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# EFFECTS OF DISTILLER'S DRIED GRAINS WITH SOLUBLES AND CRUDE GLYCERIN ON PERFORMANCE AND CARCASS CHARACTERISTICS IN EARLY WEANED BEEF CALVES

**P. J. Gunn, R. P. Lemenager, M. C. Claeys, and S. L. Lake**
Department of Animal Sciences, Purdue University, West Lafayette, IN 47907

**ABSTRACT:** The objective of this study was to evaluate the effects of distiller's dried grains with solubles (DDGS) and crude glycerin (90% glycerin; GLY) on performance and carcass characteristics in early weaned (EW) beef calves. Fifty EW Angus-cross calves (123 ± 13 d of age, 170 ± 25 kg initial BW) were blocked by BW and randomly assigned (5 calves/pen, 2 pens/treatment) to one of five isocaloric, isonitrogenous dietary treatments: 1) 15% DDGS and haylage-based control (starchless; NEG), 2) corn and haylage-based control (POS), 3) 30% DDGS and corn (DDGS+C), 4) 30% DDGS with 15% GLY replacing corn (15GLY), and 5) 30% DDGS with 30% GLY replacing corn (starchless; 30GLY). Diets were fed ad libitum for 160 d and weights were recorded monthly to monitor performance. Cattle were commingled on d 161 and fed a corn-based finishing diet until harvest ($12^{th}$ rib fat depth of 1.0 ± 0.06 cm). Following a 24-h chill, carcass characteristics were measured. There were no differences among treatments during the EW phase for DMI or G:F ($P \geq 0.15$); however, final BW ($P = 0.04$) and ADG ($P = 0.004$) were greater for DDGS+C and 15GLY when compared to 30GLY. Calves fed DDGS+C required fewer days on feed ($P = 0.02$) than NEG, 15GLY or 30GLY; however, 15GLY and NEG resulted in greater final BW ($P = 0.004$) and HCW ($P = 0.01$) than POS and DDGS+C. Final carcass characteristics did not differ ($P \geq 0.08$) among treatments for dressing percent, KPH, yield grade, marbling score, or percent carcasses grading Choice or better; however, all NEG and POS calves graded choice. Calves fed 15GLY had the greatest LM area ($P = 0.04$) and higher quality grades ($P = 0.05$) than either DDGS+C or 30GLY, and a greater percent of carcasses grading Prime ($P = 0.03$) than NEG, POS, and DDGS+C. These data imply that early wean diets containing 15% GLY with 30% DDGS appear to improve feedlot performance and result in higher quality grades when calves are finished on a corn-based diet.

**Key Words**: distiller's grain, early wean, glycerin

## Introduction

When replacing a portion of corn in cattle diets, feeding distiller's dried grains with solubles (**DDGS**) have resulted in equal, and sometimes greater, performance of feedlot cattle (Gordon et al., 2002; Pingel and Trenkle, 2006). However, increasing DDGS levels in the diet significantly above that needed to meet the protein requirements not only reduces total dietary starch intake, but also results in decreased digestibility of starch derived from other dietary ingredients (i.e. corn; Pingel and Trenkle, 2006). The reduction of starch and starch digestibility could negatively impact marbling and carcass quality. Smith and Crouse (1984) demonstrated that glucose is the preferred substrate for lipogenesis in intramuscular fat depots, and therefore, dietary rations that alter starch intake or gluconeogenic precursors may ultimately affect carcass quality. Diets that contain elevated levels of starch or gluconeogenic precursors should increase rumen propionate and circulating glucose concentrations which, in turn, has the potential to increase marbling and carcass quality.

Glycerol, a by-product of the bio-diesel industry, could be used as a gluconeogenic precursor to replace starch in the diet since it can be rapidly converted to propionate in the rumen and act as a precursor for hepatic glucose synthesis (Johns, 1953). Therefore, reduced dietary starch intake created with inclusion of DDGS, as discussed above, conceptually could be over come with the addition of gluconeogenic precursors, such as glycerol, in the ration. Little research has been reported on glycerol feeding to beef cattle; however, Scröder and Südekum (1999) fed 10% glycerol at the expense of corn in dairy cow rations without negatively affecting DMI.

We hypothesized that supplementing young calves with a gluconeogenic precursor during adipocyte development will initiate greater intramuscular fat deposition without negatively affecting feedlot performance. Therefore, our objectives were to evaluate the supplementation of glycerol and DDGS on early wean feedlot performance and carcass characteristics.

## Materials and Methods

*General.* All procedures involving animals during this study were approved by the Purdue Animal Care and Use Committee. Fifty early weaned, Angus-cross calves (37 steers and 13 heifers) averaging 123 d of age (170 ± 25 kg initial BW) were used in a completely randomized design. Initial calf BW was the average of two weights taken on consecutive days at the beginning of the study. Calves were stratified by weight and randomly assigned to one of five treatments (5 steers/pen; 2 pens/treatment; Table 1): 1) haylage, 15% DDGS, and soybean hull negative control (no starch; **NEG**), 2) a corn based positive control (**POS**), 3) a corn based diet with 30% DDGS (**DDGS+C**), 4) 30% DDGS with 15% glycerol at the expense of corn (**15GLY**), and 5) a starchless diet containing 30% DDGS and 30% glycerol in total replacement of corn (**30GLY**). Diets were formulated to be isocaloric (with the exception of NEG) and isonitrogenous to meet or exceed the requirements (NRC, 2000) of 123 d old calves. Calves

were gradually adapted to their early wean dietary treatments over a 21 d period by increasing the amount of feed offered until calves reached ad libitum consumption. Feed was offered for ad libitum consumption once daily at 0700. Calves were housed in a 3-sided barn with concrete floors in 2.4-m x 9.1-m pens constructed of metal gates and given free access to an automated watering system. Cattle were commingled on d 161 and fed a common corn based finishing diet (Table 2). Three calves were removed from the study (1 each from the NEG, 15GLY, and 30GLY treatments) due to health concerns not related to treatment.

*Performance and Carcass Data Analyses.* Calf BW were recorded on 28 d intervals to monitor feedlot performance during the early wean phase (160 d) of the study. Final BW were determined using the average pre-feeding weight from two consecutive days. On d 158, calves were measured for LM area, 12th rib fat depth, rump fat depth and percent intramuscular fat by a Centralized Ultrasound Processing certified technician. Ultrasonic measurements for 12th rib fat depth were taken on 21 d intervals throughout the commingled phase to aid in harvest selection. To determine differences in marbling scores, cattle were individually selected for harvest when 12th rib subcutaneous fat depths reached approximately 1.0 cm. Upon exsanguination HCW was measured and following a 24-h chill, 12th rib fat thickness, LM area, KPH, preliminary yield grades and quality grades were determined by trained personnel. Final yield grade was calculated using the formula reported by Aberle et al. (2001).

*Sampling and Laboratory Analyses.* Feed refusals were weighed, recorded and discarded daily. Composite feed samples were collected on 21 d intervals, dried in a forced air oven at 60ºC for 48h, ground to pass a 1-mm screen, and analyzed for DM, ether extract, ash (AOAC, 1990), NDF, and ADF (ANKOM, Fairport, NY). Nitrogen composition was determined by combustion (Leco Instruments Inc., St. Joseph, MI; AOAC 976.06, 1990) and multiplied by 6.25 to obtain CP.

*Statistical Analyses*. Performance, ultrasound data and carcass characteristic data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) for a randomized complete block design. The effect of treatment and block were included in the model, and pen was the experimental unit. Block was considered a random effect in the model. For percent of carcasses grading USDA Choice or better and percent of carcasses grading USDA Prime, the CATMOD procedure in SAS was used with categorical animal data averaged per treatment.

## Results and Discussion

### *Early Wean Phase*

*Performance.* There were no differences in DMI ($P$ = 0.15) or G:F ($P$ = 0.83; Table 3) due to dietary treatment. To our knowledge there is no published data on the effects of feeding glycerol to early-weaned calves. However, similar results in dairy cows and finishing cattle reported no differences for DMI (Donkin et al., 2007) or DMI and G:F (Buckner et al., 2007; Ham et al., 1994) due to dietary inclusion of 15% glycerol or 30% DDGS, respectively. In contrast, Pyatt et al. (2007) reported decreased DMI and increased G:F with 10% crude glycerin in the finishing diet of feedlot steers. It should be noted that calves in the present study averaged 123 d of age at the initiation of the study, whereas traditionally weaned steers were utilized in the study of Pyatt et al. (2007).

Although there were no differences in G:F and DMI, DDGS+C fed calves had greater ($P$ = 0.004) ADG when compared with POS and 30GLY fed calves. Calves fed the NEG and 15GLY treatments obtained a greater ADG than calves fed the 30GLY treatment. Noting no differences in G:F across dietary treatments, reduced ADG in 30GLY fed calves was likely due to numerically lower DMI for those calves. By design, days on feed during the early wean phase were the same across treatments and any difference in final BW would be expected to follow treatment ADG. Calves fed the DDGS+C diet had greater ($P$ = 0.04) final BW at the conclusion of the early wean phase compared with the POS and 30GLY fed calves. The 15GLY fed calves had heavier final BW at the conclusion of the early wean feeding phase than that of the POS fed calves, but were intermediate to the NEG, POS, and DDGS+C fed calves.

*Ultrasonic Carcass Measurements.* Percent intramuscular fat measured by ultrasound on d 158 did not differ due to dietary treatments ($P$ = 0.58; Table 4).

Calves fed the DDGS+C diet displayed the greatest ($P$ <0.001) 12th rib fat depth when compared with all other treatments, while calves fed the NEG, POS, and 15GLY treatments had greater 12th rib fat depths than 30GLY fed calves. These measurements were consistent with results from rump fat measurements which were greater ($P$ = 0.04) for DDGS+C fed calves when compared with 30GLY and NEG fed calves, while POS and 15GLY were intermediate to all other treatments.

Calves fed the DDGS+C and 15GLY diets had greater ($P$ = 0.001) LM area than NEG, POS and 30GLY fed calves. The s.c. fat depth and LM area measurement differences observed in this study are likely due to differences in ADG resulting in greater BW at the time of ultrasound.

### *Final Carcass Characteristics*

No differences were detected in dressing percent ($P$ = 0.54), KPH ($P$ = 0.57), yield grade ($P$ = 0.48) or 12th rib fat depth ($P$ = 0.64) due to dietary treatment (Table 5). Because all cattle were harvested at an estimated 1 cm 12th rib fat depth, no differences in this measurement were expected.

Calves fed DDGS+C had fewer ($P$ = 0.01) total days on feed when compared with calves from the NEG, 15GLY and 30GLY treatments, while calves fed the POS diet were intermediate to all other treatments. Because the DDGS+C calves had a greater ADG and 12th rib fat accretion rate during the early weaned feeding phase, it was not surprising that these calves reached the 1.0 cm endpoint with a shorter finishing period.

Final BW ($P$ = 0.004) and HCW ($P$ = 0.01) were greater for 15GLY and NEG fed calves compared with POS and DDGS+C fed calves (Table 5), while calves fed 30GLY were intermediate to all treatments. A greater number of days on feed for NEG and 15GLY fed calves,

coupled with no differences in dressing percent, suggest both a greater final BW and HCW. Although calves fed DDGS+C had the fewest number of days on feed, they also obtained the lightest HCW, which is the traditional problem with early weaning of beef calves. In order to maximize profit, it is important to manage cattle to achieve not only higher quality grades, but also heavier HCW, considering HCW accounts for 70 to 90% of producer income, depending on the USDA Select-Choice spread (Tatum et al., 2006).

Longissimus muscle area was greater ($P$ = 0.04) for 15GLY when compared with DDGGS+C and POS fed calves. Calves fed 30GLY were intermediate to all diets while NEG fed calves had larger LM areas than DDGS+C. These differences are most likely attributed to the length in which calves were on feed and their consequent final BW and HCW. Because 15GLY fed calves had a greater number of days on feed, as well as greater final BW and HCW, it would be expected that these calves would measure a larger LM area.

The 15GLY fed calves tended ($P$ = 0.07) to have the greatest marbling score and had a higher ($P$ = 0.05) quality grade than DDGS+C and 30GLY calves, while calves on NEG and POS treatments were intermediate to all treatments. The decrease in quality grade for DDGS+C fed calves may be due to a decrease in starch content of the diet resulting in lower molar proportions of propionate in the rumen and reduced serum insulin concentrations as postulated by Schoonmaker et al. (2005). Decreased quality grades for 30GLY fed calves are most likely due to a lack of performance during the early wean diet phase. Conversely, an increase in quality grade for 15GLY fed calves could be due to glycerol acting in a similar manner to starch and being converted to propionate within the rumen. Of the VFAs absorbed for energy utilizations, propionate is the primary gluconeogenic precursor, and when converted to glucose, it becomes the predominant provider of acetyl units for lipogenesis in intramuscular adipose tissue depots (Smith and Crouse, 1984).

Although there were no differences in the percent of carcasses obtaining a USDA Choice grade or better ($P$ = 0.16), 15GLY fed calves had a greater percent of carcasses grading USDA Prime ($P$ = 0.03; 33% vs. 0%) when compared with NEG, POS, and DDGS+C treatments. Calves fed 30GLY were intermediate to all treatments with 11% of carcasses obtaining a USDA Prime quality grade (Table 5). It is possible that the addition of glycerol allowed for cattle to grow at a slow enough rate to develop not only heavier HCW, but also to achieve greater marbling scores through the priming of adipocytes for later growth.

## Implications

Early wean diets containing up to 15% crude glycerin with 30% DDGS appear to improve carcass characteristics upon harvest without hindering feedlot performance during the early wean phase. Further research should be directed at investigating the effects of increased levels of crude glycerin in the diet and its interactive effects with distiller's grains in order to develop improved feeding strategies of biofuel byproducts in feedlot systems.

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Table 1. Ingredients and chemical composition of diets fed to early weaned calves from day 0 through day 160 of trial

| | Treatments | | | | |
|---|---|---|---|---|---|
| Ingredient, % of diet DM | NEG | POS | DDGS+C | 15GLY | 30GLY |
| Dry-rolled Corn | --- | 67.4 | 49.3 | 15.0 | --- |
| Dried distillers grains[1] | 14.0 | --- | 30.4 | 29.2 | 29.8 |
| Haylage | 42.1 | 13.0 | 13.0 | 13.0 | 14.3 |
| Corn gluten meal | --- | 14.6 | 1.3 | 2.5 | 5.6 |
| Soybean hulls | 38.8 | --- | --- | 21.2 | 15.0 |
| Glycerol[2] | --- | --- | --- | 14.9 | 30.6 |
| | | | | | |
| Supplement | 5.1 | 5.0 | 6.0 | 4.1 | 4.7 |
| Limestone | 4.3 | 4.2 | 5.0 | 3.4 | 3.9 |
| Sodium Chloride | 0.48 | 0.48 | 0.57 | 0.39 | 0.45 |
| Akey beef premix No.[3] | 0.20 | 0.20 | 0.24 | 0.16 | 0.19 |
| Thiamine-10[4] | 0.16 | 0.16 | 0.19 | 0.13 | 0.15 |
| | | | | | |
| Nutrient composition[5] | | | | | |
| Crude Protein | 19.44 | 19.56 | 19.60 | 19.61 | 19.21 |
| Ether Extract | 4.33 | 3.65 | 6.64 | 6.84 | 7.61 |
| NDF | 45.71 | 20.15 | 25.90 | 29.57 | 27.45 |
| ADF | 36.26 | 14.69 | 16.61 | 18.43 | 12.35 |
| Ash | 9.77 | 9.19 | 9.08 | 9.02 | 10.33 |
| Dry Matter | 54.73 | 71.21 | 75.18 | 75.69 | 74.26 |

[1]Dried distiller's grains contained (DM basis): 29.5% CP, 13.9% Fat, 14.3% ADF, 0.85% P, 0.04% Ca, 1.03% K, 0.27% Mg, 0.71% S, 0.26% Na, and 0.54 NEg.
[2]Crude Glycerin (approx. 90% glycerol) was obtained from Integrity Biofuels, Morristown, IN.
[3]Akey beef premix No. 4 (Akey, Inc., Lewisburg, OH) contained: 9% Mg, 4% S, 0.02% Co, 1% Cu, 0.09% I, 2% Fe, 4% Mn, 0.03% Se, 4% Zn as well as 4,400,000 IU of vitamin A, 550,000 IU of vitamin D and 5500 IU of vitamin E/kg of premix
[4]Diet formulated to contain 30 mg/kg of thiamine (22g of thiamine/kg of premix)
[5]Based on values obtained from complete mixed feed samples in our laboratory.

Table 2. Ingredients and chemical composition of finishing diet fed from day 161of trial through harvest

| Ingredient | % of diet DM |
|---|---|
| Dry-rolled Corn | 69.8 |
| Silage | 24.2 |
| Soybean meal | 4.1 |
| | |
| Supplement | 1.9 |
| Soybean Meal | 1.27 |
| Limestone | 0.27 |
| Urea | 0.25 |
| Sodium Chloride | 0.07 |
| Akey beef premix No.4[1] | 0.03 |
| Rumensin-80 premix[2] | 0.004 |
| Tylan-40 premix[3] | 0.002 |
| | |
| Nutrient composition[4] | |
| Crude Protein | 11.06 |
| Ether Extract | 3.54 |
| NDF | 18.94 |
| ADF | 8.79 |
| Ash | 4.27 |
| Dry Matter | 77.14 |

[1]Akey beef premix No. 4 (Akey, Inc., Lewisburg, OH) contained: 9% Mg, 4% S, 0.02% Co, 1% Cu, 0.09% I, 2% Fe, 4% Mn, 0.03% Se, 4% Zn as well as 4,400,000 IU of vitamin A, 550,000 IU of vitamin D and 5500 IU of vitamin E/kg of premix
[2]Rumensin 80 (Elanco Animal Health, Indianapolis, IN) was added at 34 mg/kg of diet (176 g of monensin/kg of premix)
[3]Tylan 40 (Elanco Animal Health, Indianapolis, IN)was added at 11 mg/kg of diet (88 g monensin/kg of premix)
[4]Based on values for individual feed ingredients determined in our laboratory.

Table 3. Effects of distiller's dried grains with solubles and glycerol on performance in early weaned beef calves

| | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | NEG | POS | DDGS+C | 15GLY | 30GLY | SEM[1] | *P*-value[2] |
| Days on feed | 161 | 161 | 161 | 161 | 161 | | |
| Initial BW, kg | 168.8 | 170.2 | 170.4 | 170.3 | 170.5 | 5.7 | 0.99 |
| Final BW, kg | 345.6$^{abc}$ | 334.7$^{bc}$ | 367.0$^{a}$ | 359.0$^{ab}$ | 322.4$^{c}$ | 10.8 | 0.04 |
| DMI, kg/d | 6.71 | 5.64 | 6.97 | 6.02 | 5.02 | 0.51 | 0.15 |
| ADG, kg | 1.25$^{ab}$ | 1.16$^{bc}$ | 1.39$^{a}$ | 1.33$^{ab}$ | 1.07$^{c}$ | 0.06 | 0.004 |
| G:F, kg/kg | 0.086 | 0.094 | 0.089 | 0.097 | 0.088 | 0.004 | 0.50 |

[1]The greatest SEM was presented (n = 9 for NEG, 15GLY and 30GLY; n = 10 for POS and DDGS+C)
[2]Probabilites for overall treatment *F*-test
[a,b,c]Means within a row lacking a common superscript differ ($P \leq 0.05$).

Table 4. Effects of distiller's dried grains with solubles and glycerol on ultrasound carcass characteristics in early weaned beef calves[1]

| | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | NEG | POS | DDGS+C | 15GLY | 30GLY | SEM[2] | *P*-value[3] |
| 12$^{th}$ rib back fat, cm | 0.71$^{b}$ | 0.77$^{b}$ | 0.90$^{a}$ | 0.74$^{b}$ | 0.55$^{c}$ | 0.06 | <0.001 |
| Rump fat, cm | 0.71$^{b}$ | 0.75$^{ab}$ | 0.89$^{a}$ | 0.75$^{ab}$ | 0.59$^{b}$ | 0.07 | 0.04 |
| LM area, cm$^2$ | 23.64$^{b}$ | 23.70$^{b}$ | 26.05$^{a}$ | 26.38$^{a}$ | 21.75$^{b}$ | 0.85 | 0.001 |
| I.M. fat, % | 3.90 | 3.76 | 3.84 | 4.30 | 3.65 | 0.32 | 0.64 |

[1]Carcass characteristics based on Ultrasound data collected on d 158 by a Centralized Ultrasound Processing certified technician.
[2]The greatest SEM was presented (n = 9 for NEG, 15GLY and 30GLY; n = 10 for POS and DDGS+C)
[3]Probabilites for overall treatment *F*-test
[a,b,c]Means within a row lacking a common superscript differ ($P \leq 0.05$)

Table 5. Effects of distiller's dried grains and glycerol on finishing characteristics in early weaned beef calves

| | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | NEG | POS | DDGS+C | 15GLY | 30GLY | SEM[1] | *P*-value[2] |
| Days on feed | 310$^{a}$ | 270$^{ab}$ | 248$^{b}$ | 305$^{a}$ | 301$^{a}$ | 22 | 0.01 |
| Final BW, kg | 534.3$^{a}$ | 475.3$^{b}$ | 471.3$^{b}$ | 549.5$^{a}$ | 518.7$^{a}$ | 25.5 | 0.004 |
| Hot carcass weight, kg | 323.0$^{a}$ | 292.0$^{b}$ | 291.3$^{b}$ | 338.5$^{a}$ | 312.5$^{ab}$ | 10.5 | 0.01 |
| Dressing percent | 60.39 | 61.48 | 61.78 | 61.40 | 60.41 | 0.67 | 0.54 |
| 12$^{th}$ rib fat depth, cm | 0.99 | 1.02 | 1.08 | 0.96 | 0.97 | 0.06 | 0.64 |
| LM area , cm$^2$ | 88.15$^{ab}$ | 78.04$^{bc}$ | 74.56$^{c}$ | 99.72$^{a}$ | 81.91$^{abc}$ | 3.90 | 0.04 |
| KPH, % | 2.39 | 2.22 | 2.25 | 2.61 | 2.45 | 0.19 | 0.57 |
| Yield grade | 2.30 | 2.54 | 2.75 | 2.33 | 2.52 | 0.20 | 0.48 |
| Marbling score[3] | 630.5 | 601.6 | 546.0 | 671.7 | 568.3 | 38.0 | 0.07 |
| Quality grade[4] | 17.89$^{ab}$ | 17.55$^{ab}$ | 16.9$^{b}$ | 18.3$^{a}$ | 17.22$^{b}$ | 0.36 | 0.05 |
| USDA Ch or Prime, % | 100 | 100 | 70.0 | 88.9 | 66.7 | 12.2 | 0.16 |
| USDA Prime, % | 0$^{b}$ | 0$^{b}$ | 0$^{b}$ | 33.3$^{a}$ | 11.1$^{ab}$ | 8.8 | 0.04 |

[1]The greatest SEM was presented (n = 9 for NEG, 15GLY and 30GLY; n = 10 for POS and DDGS+C)
[2]Probabilites for overall treatment *F*-test
[3]Marbling score: 400 = Slight 0, 450 = Slight 50, 500 = Small 0, etc.
[4]Quality grade: 15 = Select$^{-}$, 16 = Select$^{+}$=, 17 = Choice$^{-}$, 18 =Choice$^{0}$, 19 = Choice$^{+}$, etc.
[a,b,c]Means within a row lacking a common superscript differ ($P \leq 0.05$)

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# DIVERSE DIETS: THE INCLUSION OF DISTILLERS DRIED GRAINS AND FIELD PEAS IN FEEDLOT RATIONS

**B. R. Ilse*, V. L. Anderson**
Carrington Research Extension Center, North Dakota State University, Carrington, ND

ABSTRACT: Field peas are grown in the same eco-region as barley, but there has been no research to investigate diets containing these two grains as the primary concentrates. Both of these grains contain highly rumen degradable protein. The objectives of this trial were to compare animal performance and carcass traits from feeding field peas with barley or corn, with and without distillers dried grain (DDGS) which was included as a rumen undegradable protein source. Treatment diets were formulated with field peas and barley (PB), field peas and corn (PC), field peas, barley and DDGS (PBD), and field peas, corn, and DDGS (PCD). Other ration ingredients were straw, condensed separator byproduct and an ionophore mineral supplement. One hundred twenty-five yearling steers (initial weight 473.46 ± 11.19 kg) were blocked by weight and allotted to 16 pens (four pens per treatment). Dry matter intake was highest (P = 0.04) for the PC (13.04 ± 0.28 kg/hd/d) followed by PCD, PBD, PB (12.77; 12.27; 11.82 ± 0.28 kg/hd/d respectively). Average daily gains and feed efficiency were not different due to treatment (P > 0.10). Hot carcass weight was affected (P = 0.06) by treatment. Field peas and barley HCW was less than the PC (433.83 vs. 450.86 ± 62.17 kg). The treatments that included DDGS were intermediate and similar (444.51; 445.82 ± 62.17 kg for PBD and PCD, respectively). Dressing percent was greater (P = 0.01) for PCD (62.99) than for PB (61.81) with PBD (62.47) and PC (62.22) intermediate. Ribeye area was greater (P = 0.01) for PB and PC (35.41; 35.00 ± 0.38 sq cm) compared to PBD (33.55 ± 0.38 sq cm) with PCD intermediate (34.51 ± 0.38 sq cm). Marbling score, yield grade, back fat and KPH were not affected by treatment (P > 0.10). The diverse diets did not affect feedlot performance but some differences were observed in carcass traits. More research is warranted to explore the complementarity of these feed ingredients.

**Key Words**: Field peas, Feedlot, DDGS

## Introduction

In 2007 North Dakota was the national leader in acres planted of both barley and field pea grain (USDA, NASS). Recent regional research has demonstrated that field pea grain is a valuable livestock feed (Reed et al., 2004; Stein et al., 2004; Soto-Navarro et al., 2004). Much of the preceding research has evaluated the compatibility of field peas and corn (Anderson et al., 2007). Field peas, corn and barley are highly rumen degradable (NRC, 2000). Optimum growth of beef cattle is based on the proportion of the rumen undegradable protein; amino acids that reach the duodenum (Cecava and Parker, 1993; Shain et al., 1998). There is increasing availability of distillers grains from the ethanol production throughout the region. Dry distillers grains contain high levels of rumen undegradable protein and a highly digestible fiber fraction (NRC, 2000) that may improve the animal performance if included in the barley-field pea diets.

The objective of this trial was to compare animal performance and carcass traits from feeding field peas with barley, and field peas with corn, and the effects of including dry distillers grains as a source of digestible fiber and rumen undegradable protein.

## Materials and Methods

One hundred twenty-five yearling steers (initial weight 473.46 ± 11.19 kg) were procured from commercial sources, fed step up rations for 35 days, then weighed individually and sorted into four weight blocks. Steers were allotted randomly within block to one of four dietary treatments (16 pens; four pens per treatment, 7 to 8 head per pen). Treatments were diets formulated primarily with field peas and barley (**PB**), field peas and corn (**PC**), field peas, barley, and dry distillers grains (**PBD**), and field peas, corn, and dry distillers grains (**PCD**). Animals were fed and cared for in accordance with the North Dakota State University Institutional Animal Care and Use Committee Guidelines.

The finishing rations (Table 1) were formulated with 85% concentrate (1.36 Mcal/kg) and met or exceeded nutrient requirements (NRC, 2000) with the exception of the PB diet which was low in by-pass protein

The supplement included an ionophore (Rumensin® Elanco Animal Health, Greenfield, IN) at 300mg/hd/day, added calcium to balance the high phosphorous in the ration and other minerals and vitamins. Steers were implanted with the commercial implant Synovex Choice® (Fort Dodge Animal Health, Fort Dodge, IA). Cattle were fed once daily to appetite in fenceline bunks and had free access to waterers.

Steers were weighed approximately every 28 days (3 weigh periods) to monitor progress and determine relative performance during the trial. Steer growth performance parameters measured during the trial included feed intake, gain, and feed efficiency.

Cattle were marketed after 82 days on feed to a commercial abattoir (Tyson Fresh Meats, Dakota City, NE) when it was determined 60% had reached USDA Choice grade by visual appraisal. Carcass traits measured and compared included hot carcass weight (HCW), marbling score, dressing

percent, ribeye area, fat thickness, and kidney pelvic and heart fat (KPH). Final Yield Grade was calculated from the above criteria.

Data were analyzed using SAS Mixed (SAS Inst., Inc., Cary, NC) procedures with pen as the experimental unit. Treatment effects were considered significant at $P = 0.10$.

## Results and Discussion

Treatment averages for steer weight, dry matter intake (DMI) and average daily gain (ADG) are found in Table 2. Body weights throughout the trial were not statistically different among treatments ($P > 0.10$). Dry matter intake during period 1 was similar for PB and PBD treatments (11.39, 11.29 kg/hd/d, respectively). However, PC steers consumed significantly more ($P = 0.04$; 12.66 kg/hd/d) with PCD intermediate (12.26 kg/hd/d). During period 2, DMI was similar between the two barley diets and between the two corn diets. However, calves fed PC and PCD consumed more than PB ($P = 0.04$; 13.35, 13.49 vs. 12.09 kg/hd/d, respectively). Intake for PBD was intermediate (13.01 kg/hd/d). During period 3, DMI did not differ due to treatment ($P > 0.10$). Over the entire study, DMI was highest ($P = 0.04$) for the PC (13.04 kg/hd/d) followed by PCD (12.77) and PBD (12.27) with PB consuming the least DM (11.82 kg/hd/d). Average daily gain for period 1 and 2 and for the entire study was not different due to treatment ($P > 0.10$). During period 3, however, ADG was greatest for the steers on PC treatment ($P = 0.09$; 1.72 kg) with PB, PCD, and PBD all gaining the same (AVG 1.32 kg/hd/day. Gain per unit feed (Table 3) was not different due to treatments ($P > 0.10$). Treatment averages for carcass quality traits are found in Table 4. Steer final live weights were similar between treatments ($P > 0.10$) however hot carcass weights (HCW) were significantly ($P = 0.06$) affected by treatment diet with PB HCW less than the PC treatment (433.83 vs. 450.86 kg). The two treatments that included DDGS were intermediate and similar (444.51, 445.52 kg for PBD and PCD respectively). Dressing percent was greater ($P = 0.01$) for PCD (62.99) than for PB (61.81) with PBD and PC intermediate. (62.47, 62.22, respectively). Ribeye area was greatest ($P = 0.01$) for PB and PC (35.41, 35.00 sq cm respectively) than PBD; (33.55 sq cm) with PCD intermediate (34.54 sq cm). Marbling score, Yield Grade, back fat and KPH were not different due to treatments ($P > 0.10$).

In this and other studies, it is apparent that corn and field peas complement each other very well for feed intake and gain. Starch from field peas ferments slowly but very thoroughly in the rumen while starch from corn digests in both the rumen and lower gut (Ørskov 1986; Robinson and McQueen, 1989). Protein from field peas also degrades very thoroughly in the rumen but corn protein contains a higher proportion of rumen undegradable protein (NRC, 2000). Barley and field peas tend to digest thoroughly in the rumen providing more nitrogen in the rumen than the microbes can use, and with less escape protein to support high levels of growth (Anderson et al., 2007; Reed et al., 2004; Wang et al., 2003). Barley is known for its rapid fermentation rate and potential for causing digestive upsets such as acidosis. There appears to be some mediation of this with the addition of DDGS. Given competitive prices for these commodities, feeding corn with peas appears to support excellent gains.

## Implications

Corn and field pea grain are very compatible as the primary concentrates in feedlot diets. However barley and field peas fed together had few negative effects on performance or carcass quality. The inclusion of DDGS in barley-field pea based diets increased the rumen undegradable protein but did not support improved performance compared to barley and field pea fed alone in the feedlot diet.

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Table 1. Rations feed to steers comparision of field peas with barley, corn and distillers grains.

| | | | Treatment | |
|---|---|---|---|---|
| | Peas Barley | Peas Barley DDGS | Peas Corn | Peas Corn DDGS |
| Ingredient | (PB) | (PBD) | (PC) | (PCD) |
| | ---------- | Percent - Dry Matter basis | | ---------- |
| Straw, chopped | 12 | 12 | 12 | 12 |
| Corn, dry rolled | - | - | 52 | 54 |
| Barley, dry rolled | 65 | 59 | - | - |
| Field Peas, dry rolled | 15 | 6 | 28 | 11 |
| Dried distillers grains | 0 | 15 | 0 | 15 |
| Cond separator by-product | 5 | 5 | 5 | 5 |
| Ionophore supplement | 2 | 2 | 2 | 2 |
| Calcium carbonate | 1 | 1 | 1 | 1 |
| Ration dry matter, % | 83.92 | 71.59 | 81.92 | 70.12 |
| Crude protein, % | 13.78 | 15.40 | 13.00 | 13.74 |
| Est. bypass protein, % CP | 20.00 | 32.00 | 32.00 | 47.00 |
| NEg | 60.02 | 60.05 | 62.49 | 62.36 |
| Ca, % | 0.67 | 0.66 | 0.64 | 0.63 |
| P, % | 0.41 | 0.40 | 0.37 | 0.36 |
| K, % | 0.66 | 0.66 | 0.65 | 0.61 |

Table 2. Feedlot performance of steers fed field peas with barley, corn, and distillers grains.

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| Weight, kg | PB | PBD | PC | PCD | St. Error | *P* Value |
| Initial Wt | 469.15 | 476.50 | 476.09 | 620.90 | 11.19 | 0.96 |
| Wt 2 | 505.45 | 514.89 | 514.83 | 511.70 | 12.19 | 0.94 |
| Wt 3 | 562.60 | 573.52 | 577.98 | 571.70 | 13.36 | 0.87 |
| Wt 4 | 595.34 | 606.17 | 620.90 | 604.58 | 13.27 | 0.60 |
| Dry Matter Intake, kg/hd/day | | | | | | |
| Period 1 | 11.39[a] | 11.29[a] | 12.66[b] | 27.02[ab] | 0.35 | 0.04 |
| Period 2 | 12.09[a] | 13.01[ab] | 13.35[b] | 29.75[b] | 0.32 | 0.04 |
| Period 3 | 12.03 | 12.66 | 13.16 | 27.80 | 0.36 | 0.23 |
| Overall | 11.82[a] | 12.27[ab] | 28.75[b] | 13.04[ab] | 0.28 | 0.04 |
| Average Daily Gain, kg/hd/day | | | | | | |
| Period 1 | 1.21 | 1.28 | 1.29 | 1.32 | 0.16 | 0.97 |
| Period 2 | 2.12 | 2.17 | 2.34 | 2.26 | 0.12 | 0.60 |
| Period 3 | 1.33[a] | 1.31[a] | 1.72[b] | 1.32[a] | 0.12 | 0.09 |
| Overall | 1.55 | 1.59 | 1.78 | 1.62 | 0.08 | 0.20 |
| [ab] Values with different superscripts are significantly different ($P < .10$) | | | | | | |

| Table 3. Feedlot efficiency of steers fed field peas with barley, corn, and distillers grains. | | | | | | |
|---|---|---|---|---|---|---|
| | Treatment | | | | | |
| | PB | PBD | PC | PCD | St. Error | *P* Value |
| Gain per Feed (Gain Efficiency) | | | | | | |
| Period 1 | 0.05 | 0.05 | 0.05 | 0.05 | 0.01 | 0.92 |
| Period 2 | 0.08 | 0.08 | 0.08 | 0.08 | 0.01 | 0.67 |
| Period 3 | 0.05 | 0.05 | 0.06 | 0.05 | 0.01 | 0.19 |
| Overall | 0.06 | 0.06 | 0.06 | 0.06 | 0.01 | 0.65 |
| [ab] Values with different superscipts are significantly different ($P < .10$) | | | | | | |

| Table 4. Carcass quality traits of steers fed field peas with barley, corn, and distillers grains. | | | | | | |
|---|---|---|---|---|---|---|
| | Treatment | | | | | |
| | PB | PBD | PC | PCD | St. Error | P Value |
| Item | | | | | | |
| Final Wt, kg | 595.34 | 606.17 | 620.90 | 604.58 | 13.27 | 0.60 |
| Hot Carcass Wt, kg | 433.83[a] | 444.51[ab] | 450.86[b] | 445.81[ab] | 62.17 | 0.06 |
| Marbling Score* | 485.16 | 446.00 | 482.07 | 484.19 | 18.42 | 0.38 |
| Dressing percent | 61.81[a] | 62.47[ab] | 62.22[ab] | 62.99[b] | 0.24 | 0.01 |
| Ribeye area, sq cm | 35.41[a] | 33.55[b] | 35.00[a] | 34.54[ab] | 0.38 | 0.01 |
| Yield Grade | 3.36 | 3.51 | 3.50 | 3.50 | 0.43 | 0.32 |
| Back Fat | 1.91 | 2.03 | 2.01 | 2.03 | 0.79 | 0.34 |
| KPH, % | 2.34 | 2.43 | 2.41 | 2.39 | 0.41 | 0.39 |
| * Marbling score is numeric value based on dispersion of fat inside ribeye muscle, | | | | | | |
| 300-399=select, 400-499=low choice. Higher scores = more marbling and higher carcass value. | | | | | | |
| [ab] Values with different superscripts are significantly different ($P < .10$) | | | | | | |

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# EFFECTS OF ADDED DIETARY PROTEIN AND FAT ON SUBCUTANEOUS ADIPOSE TISSUE OF FINSISHING LAMBS WHEN FED DIFFERING LEVELS OF DRIED DISTILLER'S GRAINS WITH SOLUBLES

**M.L. Van Emon[1], A.F. Musselman[1], P.J. Gunn[1], M.K. Neary[1], R.P. Lemenager[1], E.J. Scholljegerdes[2], and S.L. Lake[1]**
[1]Department of Animal Science, Purdue University, West Lafayette, IN 47907, [2]USDA-ARS, Northern Great Plains Research Laboratory, Mandan, ND 58554

**ABSTRACT:** The objectives of this study were to determine the effects of added dietary protein and fat in dried distiller's grains with solubles (DDGS) on s.c. adipose fatty acid (FA) profiles in finishing lambs. Sixty crossbred lambs (33.17 ± 4.67 kg; 30 ewes; 30 wethers) were allotted into pairs (ewe and wether) and fed one of five isocaloric dietary treatments: 1) a corn based diet with DDGS included to meet CP requirements (25% of DM; CON), 2) CON with DDGS included at twice the amount of CON (50% of DM; 50DDGS), 3) CON with added protein to equal the CP in the 50DDGS diet (CON+CP), 4) CON with added vegetable oil to equal the crude fat of the 50DDGS diet (CON+VO), and 5) CON with protein and crude fat added to equal that of the CP and fat in the 50DDGS diet (CON+CPVO). All data are expressed as concentrations (mg FA/g tissue) of fatty acids. Total fatty acid concentrations of s.c. adipose did not differ ($P$ = 0.77) between treatments. Lambs fed 50DDGS, CON+VO, and CON+CPVO had greater ($P$ = 0.03) concentrations of 18:1*trans*-11 in s.c. adipose than lambs fed CON+CP. The CON+CP treatment had greater ($P$ = 0.05) concentrations of 18:1*cis*-9 in s.c. adipose than lambs fed 50DDGS, CON+VO, and CON+CPVO diets. The lambs fed the CON+CPVO diet had greater concentrations of 18:2*trans*-9,*trans*-12 ($P$ = 0.04) and 18:2*cis*-9,*trans*-11 ($P$ = 0.04) in s.c. adipose than CON, 50DDGS, and CON+CP, and CON, CON+CP, and CON+VO treatments, respectively. Concentrations of 18:2*cis*-9, *cis*-12 in s.c. adipose were greater ($P$ = 0.04) in the CON+CP and 50DDGS diets compared with the CON+CPVO, and the CON+VO and CON+CPVO diets, respectively. Lambs fed the 50DDGS diet had greater concentrations of 18:2*trans*-10, *cis*-12 ($P$ = 0.02) compared with all other diets. Generally, the data would suggest that diets with elevated dietary fat appeared to have increased levels of linoleic acid biohydrogenation intermediates deposited in fat depots of finishing lambs.

**Keywords**: DDGS, fatty acids, lamb

## Introduction

As the price of traditional feedstuffs rise, producers are seeking for alternative feeds that reduce costs of the ration while maintaining both animal performance and carcass quality. Dried distiller's grains with solubles (DDGS) have become an alternative to corn in the diets of livestock. Ham et al. (1994) demonstrated a greater ADG, DMI, and G:F for finishing cattle can be fed DDGS at up to 40% of DM in the diet when compared with cattle fed a control diet containing dry rolled corn. However, increased availability has caused producers utilize DDGS as an energy source rather than a protein source. Little information is available on the effects of elevated levels of DDGS on performance and carcass quality in lamb rations. Additionally, how increased levels of dietary unsaturated fat that comes with feeding DDGS might an affect on carcass quality, fatty acid profile, and shelf life stability of lamb meat remains a question. In a review by Wood et al. (2003), shelf life of steaks was reduced and metabolites of lipid degradation were increased with increasing concentrations of PUFA concentrations.

An increase in CLA, an intermediate in biohydrogenation of linoleic acid, within sheep tissue may provide some human health benefits. As Belury (2002) stated, CLA may be an anticarcinogen, reduce adipose deposition, reduce atherosclerotic plaque formation, and decrease the onset of diabetes. Conjugated linoleic acid may effect bone formation and the immune system in a positive manner (Belury, 2002). Due to biohydrogenation of unsaturated fatty acids in the rumen, the fatty acid compositions of the LM tissue and s.c. adipose tissue are more difficult to predict (Wood and Enser, 1997). Dietary manipulations that alter these tissues could result in a healthier meat product for the consumer.

We hypothesized that increased dietary protein and fat would increase the amount of linoleic acid biohydrogenation intermediates deposited in the LM and s.c. adipose tissues. Our objectives were to determine if added dietary protein and fat from DDGS would have an effect on the fatty acid composition of LM tissue and s.c. adipose tissue in finishing lambs.

## Materials and Methods

### *General*

The Purdue University Animal Care and Use Committee approved all procedures involving animals for this study. Sixty crossbred lambs (33.17 ± 4.67 kg; 30 ewes and 30 wethers) were allotted into one of five isocaloric dietary treatments (Table 1): 1) a corn based diet with DDGS included to meet CP requirements of finishing lambs (25% of DM; CON), 2) CON with DDGS included at twice the amount of CON (50% of DM; 50DDGS), 3) CON with added protein to equal the CP level in the 50DDGS diet (CON+CP), 4) CON with added vegetable oil to equal the crude fat level of the 50DDGS diet (CON+VO), and 5) CON with protein and vegetable oil added to equal that of the CP and fat levels of the 50DDGS diet (CON+CPVO).

Initial lamb BW was the average of two weights taken on consecutive days at the beginning of the trial. Lambs were stratified by weight and blocked into pairs of one ewe and one wether, housed in a 1.83-m x 1.83-m pen. Lambs were fed once daily ad libitum and had free access to water. Three lambs were removed from the study due to non-treatment related illness and the remaining single lambs were kept in their original pens.

Lambs were fed to minimize sorting. Orts were collected and weighed twice weekly to calculate dietary intake. Dietary intake was calculated on a per pen basis. As expected, lambs fed fat had greater ($P$ = < 0.001) intakes of all fatty acids measured (Table 2).

***Sampling and Laboratory Analyses***

Dietary samples collected for analysis were dried in a forced air oven for 48 hr at 60°C for DM. Diets were analyzed for nitrogen content (Leco FP analyzer Model 602600, Leco Instruments Inc., St. Joseph, MI) and multiplied by 6.25 to obtain CP. Dietary samples were ground to pass a 1mm screen, and analyzed for NDF and ADF (ANKOM$^{200/220}$ Fiber Analyzer; ANKOM Technology, Fairport, NY).

Longissimus muscle and s.c. adipose samples were obtained 24 hr post-harvest from the 12$^{th}$-13$^{th}$ rib interface and were stored at -20°C for fatty acid analysis. Fatty acid analysis on feed samples, LM tissue, and s.c adipose tissue were analyzed at the USDA-ARS, Northern Great Plains Research Laboratory (Mandan, ND). Fatty acid analysis of the diets was accomplished using an acid catalyst in direct-trans esterification as described by Kucuk et al. (2001) and preparation of the s.c. adipose and LM tissues according to procedures outlined by Murrieta et al. (2003). Fatty acid concentrations were determined by gas chromatography (Model 3800, Varian Inc., Palo Alto, CA) using a 100-m capillary column (Supelco 2560, Supelco, Bellefonte, PA). Hydrogen was the carrier gas and was maintained at a column flow of 1.5 mL/min. The oven temperature was maintained at 120°C for 2 min, ramped up to 175°C at a 6°C/min interval, and finally to 250°C at a 10°C/min interval. Injector temperature was held at 260°C while detector temperature was held at 300°C. The split-ratio for the LM tissue was 30:1 and 100:1 for the s.c. adipose tissue. Purified fatty acid standards (Sigma-Aldrich, St. Louis, MO; Nu-Check Prep, Elysian, MN, Matreya, Pleasant Gap, PA) were used to identify the individual peaks. The s.c. adipose tissue (Table 3) and the LM tissue (Table 4).

***Statistical Analysis***

Data were analyzed by one-way analysis of variance for treatment using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Treatment was tested against the dependent variables, specific fatty acids. The model statement included the effect of treatment on specific fatty acid concentrations to calculate the variations from the grand mean. The LSMEANS statement of SAS was used to compute the adjusted treatment means. Two preplanned orthogonal contrasts were used to test treatment effects: 1) CON diet vs. the average of the diets containing elevated CP levels (50DDGS, CON+CP, and CON+CPVO), and 2) CON diet vs. the average of the diets containing elevated fat levels (50DDGS, CON+VO, and CON+CPVO).

## Results and Discussion

***Subcutaneous Adipose***

Total fatty acid concentrations in s.c. adipose tissue did not differ ($P$ = 0.77) due to dietary treatment (Table 3). In addition, fatty acids 14:0 ($P$ = 0.97), 14:1 ($P$ = 0.63), 16:0 ($P$ = 0.29), and 16:1 ($P$ = 0.79), which are made via de novo synthesis, were not different due to dietary treatment. Linolenic acid ($P$ = 0.18), 18:1*cis*-11 ($P$ = 0.82), 18:0 ($P$ = 0.24), saturated fatty acids ($P$ = 0.19), and MUFA ($P$ = 0.88) concentrations were not different between treatments. Lambs fed the CON+CPVO diet had greater concentrations of 18:2*trans*-9,*trans*-12 ($P$ = 0.04) and 18:2*cis*-9,*trans*-11 (CLA; $P$ = 0.04) in s.c. adipose than CON, 50DDGS, and CON+CP, and the CON, CON+CP, and CON+VO fed lambs, respectively. Concentrations of 18:3n-6 tended ($P$ = 0.06) to be greater for CON fed lambs compared with the CON+VO and CON+CPVO treatments. The 18:2*trans*-10, *cis*-12 CLA isomer concentrations were greater ($P$ = 0.02) in the 50DDGS fed lambs compared with all other diets. Linoleic acid concentrations were greater ($P$ = 0.04) in the 50DDGS fed compared with CON+VO and CON+CPVO; and the CON+CP fed lambs compared with CON+CPVO lambs. Concentrations of 18:1*cis*-9 were greater ($P$ = 0.05) in the CON+CP lambs compared with 50DDGS, CON+VO, and CON+CPVO diets. Lambs fed the 50DDGS, CON+VO, and CON+CPVO diets had greater ($P$ = 0.03) concentrations of 18:1*trans*-11 compared with the lambs fed the CON+CP diet. The PUFA concentrations tended ($P$ = 0.08) to be greater in 50DDGS fed lambs compared with both the CON+VO and CON+CPVO diets.

Prediction of the fatty acid composition of s.c. adipose and LM tissues based on dietary fatty acid composition is difficult, primarily due to the ability of rumen microbes to produce and biohydrogenate fatty acids (Wood and Enser, 1997). The increased amounts of linoleic acid intermediates in the elevated fat diets were likely the result of decreased biohydrogenation in the rumen. Greater concentrations of linoleic acid in the CON, 50DDGS, and CON+CP fed lambs observed in this study could be explained, in part, by the fact that fats associated with the DDGS granules being partially protected from biohydrogenation (Wood and Enser, 1997; Wood et al., 1999), whereas the added vegetable oil was readily accessible to rumen microbes for biohydrogenation. Similarly, Scholljegerdes, et al. (2001) noted an increased flow of both unsaturated and saturated fatty acids to the duodenum in heifers fed safflower seeds compared with their control diet. This could also indicate a natural protection of fatty acids from ruminal biohydrogenation when they are part of the feed particle. Increased concentrations of stearic acid in CON and CON+CP fed lambs may be the result of more complete linoleic acid biohydrogenation in the rumen. It is interesting to note the variation in fatty acid profile between the s.c. adipose tissue in the 50DDGS and CON+CPVO lambs, even though these diets were formulated to contain equal amounts of fat and CP.

***Longissimus Muscle***

Despite differences in fatty acid intake (Table 2), total fatty acid concentrations in LM tissue did not differ ($P$ = 0.24) between dietary treatments (Table 4). Fatty acid concentrations of 16:0 ($P$ = 0.28), 18:0 ($P$ = 0.44), 18:1*cis*-9 ($P$ = 0.23), 18:2*cis*-9, *cis*-12 ($P$ = 0.44), total SFA ($P$ = 0.28), MUFA ($P$ = 0.22), and PUFA ($P$ = 0.41) were not different between treatments. Fatty acid concentrations of 14:0 ($P$ = 0.08) and 16:1 ($P$ = 0.07) tended to be greater in the CON+VO fed lambs compared with the 50DDGS and CON+CP fed lambs. Lambs fed the CON and CON+VO diets tended ($P$ = 0.06) to have greater concentrations of 12:0 compared with the lambs fed the 50DDGS and CON+CP diets. Concentrations of 14:1 tended ($P$ = 0.11) to be greater in CON+VO fed lambs compared with the CON+CP diet. Lambs fed CON+VO tended ($P$ = 0.06) to have greater concentrations of 18:1*trans*-11 than the lambs fed CON+CP. Concentrations of 18:1*cis*-11 tended ($P$ = 0.08) to be greater in the CON+VO fed lambs compared with the 50DDGS, CON+CP, and CON+CPVO fed lambs. The 18:3n-3 fatty acid concentrations were greatest ($P$ = 0.01) in lambs fed the CON diet when compared with 50DDGS, and CON+CP fed lambs. Concentrations of CLA tended to be greater ($P$ = 0.14) in the CON+VO diet compared with the 50DDGS and CON+CP diets. The 18:2*trans*-10, *cis*-12 isomer of CLA tended to have greater ($P$ = 0.10) concentrations in the CON+VO diet compared with the CON and CON+CP diets.

Increased concentrations of CLA in LM tissue were observed in this study with the CON+VO and CON+CPVO treatments, this could prove to be more beneficial to human health (Belury, 2002). The low concentrations of 18:2*trans*-10, *cis*-12 in this study suggests that the majority of CLA is in the 18:2*cis*-9, *trans*-11 form and is in agreement with Belury (2002). Lambs fed the CON+VO diet contained the greatest concentrations of stearic acid and 18:1*trans*-11 in LM tissue, which could be due to the increased daily intake of stearic acid, 18:1*cis*-9, and 18:1*cis*-9, *cis*-12. Similar concentrations of stearic acid illustrates that there is a similar amount of deposition of biohydrogenation intermediates across treatments in the LM tissue, which might be explained by either a more complete biohydrogenation within the rumen or a greater daily intake of fatty acids.

## Implications

An increase in linoleic acid biohydrogenation intermediates in diets with added oil was observed, however the total fatty acid composition of the s.c. adipose and LM tissues was not affected. These results suggest that the fatty acids associated with DDGS are at least partially protected from ruminal biohydrogenation when fed to finishing lambs. Further research needs to be conducted to address the issue of how these differences in linoleic acid intermediates resulting from feeding differing levels of DDGS may affect meat quality of finishing lambs.

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Table 1. Dietary ingredients and chemical composition of diets fed to finishing lambs[1]

| | Dietary Treatments | | | | |
|---|---|---|---|---|---|
| Item | CON | 50DDGS | CON+CP | CON+VO | CON+CPVO |
| Ingredients, % of DM | | | | | |
| Dry-rolled corn | 59.8 | 34.9 | 48.9 | 48.9 | 38.3 |
| Distiller's dried grains | 25.0 | 50.4 | 25.1 | 25.1 | 25.2 |
| Ground hay | 10.7 | 10.6 | 10.7 | 10.7 | 10.8 |
| Corn gluten meal | — | — | 11.0 | — | 10.4 |
| Soybean hulls | — | — | — | 8.3 | 8.4 |
| Vegetable oil | — | — | — | 2.5 | 2.7 |
| Molasses | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 |
| Supplement | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Analyzed composition, % of DM | | | | | |
| CP | 14.57 | 18.45 | 20.26 | 15.49 | 19.76 |
| Ether Extract | 3.12 | 6.58 | 4.10 | 5.59 | 3.38 |
| ADF | 11.76 | 14.15 | 12.90 | 15.40 | 16.08 |
| NDF | 21.72 | 24.17 | 20.52 | 25.07 | 23.27 |
| DM | 10.31 | 9.52 | 9.50 | 9.41 | 9.43 |
| Fatty acid analysis, mg of fatty acid/g of feed | | | | | |
| 16:0 | 10.106 | 11.893 | 8.364 | 11.684 | 11.150 |
| 18:0 | 1.572 | 1.909 | 1.288 | 2.511 | 2.396 |
| 18:1*cis*-9 | 16.840 | 20.147 | 13.783 | 19.660 | 18.673 |
| 18:2*cis*-9, *cis*-12 | 40.339 | 47.815 | 34.029 | 50.293 | 48.510 |
| 18:3n-3 | 1.388 | 1.763 | 1.263 | 3.787 | 3.805 |
| 22:2 | 0.191 | 0.241 | 0.190 | 0.211 | 0.215 |
| Other | 1.635 | 1.959 | 1.426 | 2.566 | 2.388 |
| Total | 72.028 | 85.673 | 60.324 | 90.651 | 87.083 |

[1]CON: corn based diet with DDGS included to meet CP requirements; 50DDGS: CON with DDGS included at twice the amount of CON; CON+CP: CON with added protein to equal the CP in the 50DDGS diet; CON+VO: CON with added vegetable oil to equal the crude fat of the 50DDGS diet; CON+CPVO: CON with protein and crude fat added to equal that of the CP and fat in the 50DDGS diet.

Table 2. Effects of differing levels of CP and dietary fat from distiller's dried grains with solubles on fatty acid intake (g/d) of finishing lambs.

| | Dietary Treatment | | | | | | *P*-value[1] | | |
|---|---|---|---|---|---|---|---|---|---|
| Fatty Acid, g/d | CON | 50DDGS | CON+CP | CON+VO | CON+CPVO | SEM[2] | Treatment | CP | VO |
| 16:0 | 28.08$^{a}$ | 29.74$^{a}$ | 20.25$^{b}$ | 31.69$^{a}$ | 27.11$^{a}$ | 1.75 | 0.0005 | 0.21 | 0.45 |
| 18:0 | 4.37$^{c}$ | 4.77$^{c}$ | 3.12$^{d}$ | 6.81$^{a}$ | 5.83$^{b}$ | 0.32 | <0.0001 | 0.55 | 0.0003 |
| 18:1*cis*-9 | 46.79$^{ab}$ | 50.38$^{ab}$ | 33.37$^{c}$ | 53.33$^{a}$ | 45.41$^{b}$ | 2.93 | 0.0003 | 0.24 | 0.36 |
| 18:2*cis*-9, *cis*-12 | 112.07$^{b}$ | 119.56$^{ab}$ | 82.38$^{c}$ | 136.43$^{a}$ | 117.96$^{ab}$ | 7.25 | 0.0002 | 0.49 | 0.12 |
| 18:3n-3 | 3.86$^{bc}$ | 4.41$^{b}$ | 3.06$^{c}$ | 10.27$^{a}$ | 9.25$^{a}$ | 0.43 | <0.0001 | 0.0009 | <0.0001 |
| SFA | 32.59$^{b}$ | 34.66$^{ab}$ | 23.41$^{c}$ | 38.62$^{a}$ | 33.02$^{b}$ | 2.07 | 0.0002 | 0.32 | 0.21 |
| MUFA | 46.79$^{ab}$ | 50.38$^{ab}$ | 33.37$^{c}$ | 53.33$^{a}$ | 45.41$^{b}$ | 2.93 | 0.0003 | 0.24 | 0.36 |
| PUFA | 116.46$^{b}$ | 124.57$^{b}$ | 85.90$^{c}$ | 147.28$^{a}$ | 127.74$^{ab}$ | 7.68 | <0.0001 | 0.65 | 0.05 |
| Other | 5.07$^{c}$ | 5.50$^{bc}$ | 3.91$^{d}$ | 7.54$^{b}$ | 6.33$^{a}$ | 0.36 | <0.0001 | 0.66 | 0.002 |
| Total | 200.11$^{b}$ | 214.22$^{ab}$ | 146.04$^{c}$ | 245.92$^{a}$ | 211.77$^{ab}$ | 12.99 | 0.0001 | 0.50 | 0.10 |

[a-c]Means within a row that lack a common superscript differ (P≤ 0.05).

[1]Probabilites for overall treatment F-test and for orthogonal contrasts between CON vs. elevated CP diets and CON vs. elevated fat diets.

[2]Greatest SEM is presented (CON, n = 6; 50DDGS, n = 5; CON+CP, n = 6; CON+VO, n = 6; CON+CPVO, n = 6).

Table 3. Effects of differing levels of CP and dietary fat from distiller's dried grains with solubles on subcutaneous adipose tissue fatty acid profile in finishing lambs.

| | Dietary Treatment | | | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| Fatty Acid, mg of fatty acid/g of adipose tissue | CON | 50DDGS | CON+CP | CON+VO | CON+CPVO | SEM[1] | Treatment | CP | VO |
| 14:0 | 15.27 | 14.35 | 14.94 | 15.66 | 15.80 | 1.73 | 0.97 | 0.90 | 0.99 |
| 14:1 | 0.38 | 0.39 | 0.73 | 0.53 | 0.43 | 0.19 | 0.63 | 0.50 | 0.72 |
| 16:0 | 126.99 | 123.55 | 143.47 | 123.48 | 124.34 | 8.11 | 0.29 | 0.69 | 0.71 |
| 16:1 | 10.89 | 10.43 | 11.13 | 11.36 | 10.27 | 0.76 | 0.79 | 0.74 | 0.81 |
| 18:0 | 104.59 | 93.08 | 116.97 | 80.43 | 104.67 | 12.32 | 0.24 | 0.98 | 0.37 |
| 18:1*trans*-11 | 74.81$^{abc}$ | 95.22$^{ab}$ | 54.97$^{c}$ | 110.85$^{a}$ | 103.69$^{ab}$ | 13.69 | 0.03 | 0.51 | 0.06 |
| 18:1*cis*-9 | 200.72$^{ab}$ | 191.14$^{b}$ | 228.28$^{a}$ | 188.67$^{b}$ | 181.34$^{b}$ | 12.03 | 0.05 | 0.97 | 0.30 |
| 18:1*cis*-11 | 7.41 | 8.10 | 8.31 | 8.27 | 7.59 | 0.72 | 0.82 | 0.45 | 0.47 |
| 18:2*trans*-9, *trans*-12 | 0.46$^{b}$ | 0.43$^{b}$ | 0.54$^{b}$ | 1.01$^{ab}$ | 1.14$^{a}$ | 0.21 | 0.04 | 0.28 | 0.08 |
| 18:2*cis*-9, *cis*-12 | 42.47$^{abc}$ | 51.96$^{a}$ | 46.83$^{ab}$ | 35.26$^{bc}$ | 34.61$^{c}$ | 4.59 | 0.04 | 0.69 | 0.71 |
| 18:3n-6 | 0.14 | 0.07 | 0.08 | 0.04 | 0.01 | 0.03 | 0.06 | 0.02 | 0.01 |
| 18:3n-3 | 2.37 | 2.51 | 2.07 | 2.76 | 2.62 | 0.22 | 0.18 | 0.90 | 0.28 |
| CLA[3] | 3.19$^{b}$ | 3.68$^{ab}$ | 3.32$^{b}$ | 3.43$^{b}$ | 4.39$^{a}$ | 0.31 | 0.04 | 0.07 | 0.06 |
| *trans*-10,*cis*-12 | 0.53$^{b}$ | 0.96$^{a}$ | 0.55$^{b}$ | 0.61$^{b}$ | 0.68$^{b}$ | 0.09 | 0.02 | 0.06 | 0.04 |
| SFA | 267.41 | 250.01 | 294.59 | 237.88 | 263.33 | 18.01 | 0.19 | 0.92 | 0.38 |
| MUFA | 299.63 | 310.87 | 309.03 | 325.24 | 308.12 | 18.54 | 0.88 | 0.79 | 0.49 |
| PUFA | 49.68 | 60.06 | 54.60 | 43.62 | 43.91 | 4.80 | 0.08 | 0.79 | 0.49 |
| Other | 49.84 | 51.30 | 52.23 | 51.32 | 61.45 | 7.62 | 0.77 | 0.53 | 0.56 |
| Total | 639.58 | 647.19 | 684.42 | 633.68 | 653.04 | 9.00 | 0.77 | 0.52 | 0.88 |

[a-c]Means within a row that lack a common superscript differ (P≤ 0.05).

[1]Probabilites for overall treatment F-test and for orthogonal contrasts between CON vs. elevated CP diets and CON vs. elevated fat diets.

[2]Greatest SEM is presented (CON, n = 6; 50DDGS, n = 5; CON+CP, n = 6; CON+VO, n = 6; CON+CPVO, n = 6).

[3]cis-9,trans-11 CLA.

Table 4. Effects of differing levels of CP and dietary fat from distiller's dried grains with solubles on longissimus muscle tissue fatty acid profile of finishing lambs.

| | Dietary Treatment | | | | | | *P*-value[1] | | |
|---|---|---|---|---|---|---|---|---|---|
| Fatty Acid, mg of fatty acid/g of LM tissue | CON | 50DDGS | CON+CP | CON+VO | CON+CPVO | SEM[2] | Treatment | CP | VO |
| 12:0 | 0.12 | 0.07 | 0.06 | 0.13 | 0.08 | 0.02 | 0.06 | 0.03 | 0.22 |
| 14:0 | 2.28 | 1.47 | 1.46 | 2.68 | 1.83 | 0.37 | 0.08 | 0.09 | 0.48 |
| 14:1 | 0.02 | 0.02 | 0.00 | 0.07 | 0.02 | 0.02 | 0.11 | 0.76 | 0.44 |
| 16:0 | 23.52 | 16.46 | 19.20 | 26.19 | 19.68 | 3.50 | 0.28 | 0.19 | 0.47 |
| 16:1 | 1.88 | 1.11 | 1.25 | 2.15 | 1.36 | 0.30 | 0.07 | 0.06 | 0.30 |
| 18:0 | 14.42 | 10.20 | 13.14 | 15.30 | 13.00 | 2.04 | 0.44 | 0.30 | 0.47 |
| 18:1*trans*-11 | 6.98 | 6.85 | 3.68 | 12.49 | 9.25 | 2.19 | 0.06 | 0.87 | 0.28 |
| 18:1*cis*-9 | 41.17 | 24.98 | 31.91 | 42.36 | 30.82 | 6.35 | 0.23 | 0.09 | 0.22 |
| 18:1*cis*-11 | 1.84 | 1.30 | 1.34 | 2.05 | 1.43 | 0.23 | 0.08 | 0.06 | 0.32 |
| 18:2*cis*-9, *cis*-12 | 10.80 | 10.64 | 9.93 | 10.81 | 9.31 | 0.73 | 0.44 | 0.29 | 0.49 |
| 18:3n-3 | 0.44[ab] | 0.34[bc] | 0.29[c] | 0.57[a] | 0.43[abc] | 0.06 | 0.01 | 0.16 | 0.88 |
| CLA[3] | 0.46 | 0.35 | 0.35 | 0.58 | 0.54 | 0.08 | 0.14 | 0.59 | 0.75 |
| *trans*-10,*cis*-12 | 0.00 | 0.03 | 0.00 | 0.04 | 0.03 | 0.01 | 0.10 | 0.17 | 0.03 |
| Saturated FA | 42.84 | 29.94 | 35.65 | 46.82 | 36.37 | 6.01 | 0.28 | 0.18 | 0.43 |
| MUFA | 55.01 | 37.07 | 40.96 | 62.08 | 45.20 | 8.83 | 0.22 | 0.15 | 0.47 |
| PUFA | 12.26 | 11.87 | 11.03 | 12.57 | 10.82 | 0.82 | 0.41 | 0.25 | 0.56 |
| Other | 7.91 | 5.49 | 5.30 | 7.04 | 5.25 | 0.84 | 0.08 | 0.01 | 0.04 |
| Total | 111.85 | 79.30 | 87.92 | 122.46 | 93.03 | 15.60 | 0.24 | 0.14 | 0.42 |

[a-c]Means within a row that lack a common superscript differ (P≤ 0.05).

[1]Probabilites for overall treatment F-test and for orthogonal contrasts between CON vs. elevated CP diets and CON vs. elevated fat diets.

[2]Greatest SEM is presented (CON, n = 6; 50DDGS, n = 5; CON+CP, n = 6; CON+VO, n = 6; CON+CPVO, n = 6).

[3]cis-9,trans-11 CLA.

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# EVALUATION OF IN VITRO DEGRADATION OF PLANT CELL WALLS WITH RUMINAL MICROBES

**R. Reyna-Islas, S. L. Lodge-Ivey*, J. Browne-Silva, R. Creamer, and R. A. Khatib**
**New Mexico State University, Las Cruces, New Mexico**

**ABSTRACT:** For optimal ruminal digestion of plant cell walls, bacteria and fungi attach to the plant material. Attachment of bacteria to plant cell walls is controlled by a positive chemotatic attraction that may be reverse in response to secondary plant metabolites. To investigate this hypothesis locoweed (*Oxytropis spp.*) was used as a model. Toxic locoweeds are infected by a swainsonine-producing fungal endophyte (*Undifilum oxytropis)* which causes locoism in livestock. A study was conducted to evaluate cell wall degradation in endophyte-infected (E+) and endophyte-free (E-) locoweed using IVDMD and transmission electron microscopy (TEM). A single phase in vitro experiment was conducted for IVDMD and 24 h VFA production and TEM (12 or 24 h) analysis with E+ or E-. Locoweed E+ or E- IVDMD (78.3% ± 2.1 and 79.0% ± 1.2, respectively) did not differ (P = 0.45). Total VFA production was higher for E- than E+ (172.9 vs 159.9 ± 2.72 mmol; P < 0.01). Molar percentage of acetate was 7.3% lower (P < 0.01) and propionate was 15.6% higher for E- and than E+ (P < 0.02). The acetate:propionate ratio was 27% lower for E- than E+ (P < 0.01). These changes suggest an impact in bacterial metabolism or population dynamics. To compare the number of bacterial cells per micrometer of E+ and E- cell wall, transmission electron micrographs were made. Microbial cells were evaluated as attached or unattached (but within 3 µm of cell wall) from the length of cell wall visible in the micrograph. Attachment was not influenced by endophyte or time (P = 0.74 and 0.39, respectively). Time of incubation (P = 0.02) but not endophyte (P = 0.13) affected the number of unattached microbial cells. There were fewer cells unattached at 24 h vs 12 h (0.92 ± 0.18 vs 1.53 ± 0.16 cells/linear µm of plant cell wall). These research methods demonstrate the potential for secondary plant metabolites to impact bacterial function and may serve as a model to study the influence of plant secondary metabolites and may provide information about specialized microbial species.

**Keywords:** Locoweed, Endophyte, Cell wall degradation, Electron Microscopy

## Introduction

In the ruminant animal the microbial population in the rumen plays a key role in the survival of the animal by breaking down dietary substrates that would be otherwise indigestible without their intervention. Degradation of plant cell walls by the ruminal bacterial and fungal populations is related to adhesion of the microbe to the substrate. Varga and Kolver (1997) state that one of the major factors regulating ruminant fiber digestion is adhesion and hydrolysis by complexes of hydrolytic enzymes of the adherent microbial populations. Microscopic studies have revealed differences in rumen microbial adhesion and relative rates of degradability of plant cell walls depending on their composition (i.e. cellulose, hemicellulose, lignin; Hanna et al., 1973). Electron microscopy allows in situ visualization of forage cell wall degradation by ruminal microbes without physical or chemical pretreatment that can modify factors affecting the plant/microbe association during digestion. Inclusion of plants in ruminant diets with secondary metabolites can alter rumen fermentation patterns with effects observed on dry matter and protein digestion and VFA profile (Wallace, 1994). To investigate this hypothesis locoweed (*Oxytropis spp.*) was used as a model. Toxic locoweeds are infected by a swainsonine-producing fungal endophyte (*Undifilum oxytropis)* which causes locoism in livestock while some locoweeds are not infected by the endophyte and therefore not considered toxic. The availability of locoweeds with or without endophyte infection represents a good model to study the impact of secondary plant metabolites on ruminal microbe activities without altering plant material. The objective of this study was to evaluate digestibility of endophyte-infected and endophyte-free locoweed using IVDMD, VFA profiles, and transmission electron microscopy (TEM).

## Materials and Methods

*Locoweed samples*. Samples of locoweed (*Oxytropis spp.)* with (E+) or without (E-) endophyte infection were ground in a Wiley mill to pass a 2 mm screen, were weighed out (0.5 g) and placed in 30 mL in vitro digestion tubes in two quadruplicate sets. One set was used for IVDMD and VFA profile detection while the second set analyzed for microbial attachment to plant material using TEM. Swainsonine content of E+ was 0.135 % (DM basis).

*Evaluation of locoweed digestion.* To evaluate IVDMD locoweed substrates were mixed with ruminal fluid. Ruminal fluid was collected from a ruminally cannulated cow weighing approximately 750 kg allowed ab libitum access to medium quality forage (DM basis 12% CP, 56% NDF). Animal care and management practices were approved by the Institutional Animal Care and Use Committee at NMSU. A suction strainer was used to collect rumen fluid at the mat layer interface into collection thermoses that had been heated to 37°C. Rumen fluid was combined with equal parts of McDougall's buffer (Tilley and Terry, 1963). In vitro tubes with substrate were filled with 15mL of inoculum, sealed with a plastic cap and incubated (37◦C) in an anaerobic glove box with a 95%

$CO_2$: 5% $H_2$ atmosphere for 24 h. Tubes were manually agitated 10 times at the beginning of the experiment and were then agitated every two hours for twelve hours. Upon removal from the anaerobic glove box tubes were stored at -80◦C until IVDMD and VFA production analysis was performed. In vitro dry matter digestibility was calculated from the amount of substrate remaining after digestion with rumen fluid inoculum at 24 h. Gas chromatography was used to quantify VFA production (Goetsch and Galyean, 1983).

*Transmission electron microscopy*. For microbial association with fiber the second set of in vitro tubes with E+ and E- locoweed were incubated with ruminal fluid for 12 and 24 h. After incubation in vitro tubes were centrifuged and the supernant was discarded. The resulting solid portion was fixed with glutaraldehyde and $OsO_4$ and dehydrated with alcohol for TEM (Akin and Rigsby, 1985). To compare quantitatively the number of bacterial cells per micrometer of forage cell wall in the E+ vs E- transmission electron micrographs were made of plant cell walls undergoing degradation as shown by the removal or loss of wall material i.e. loss of electron density. Resulting images of plant cell wall and ruminal microbes were evaluated as attached or unattached (but within 3 μm of cell wall). Values were expressed as number of bacteria attached or unattached per linear micrometer of plant cell wall (Akin and Rigsby, 1985).

Data were analyzed using GLM procedure of SAS. Model included endophyte presence and time. Treatment means were separated using LSMEANS and PDIFF. A probability of 0.05 was considered significant.

## Results and Discussion

Locoweed E+ or E- IVDMD (78.3% $\pm$ 2.1 and 79.0% $\pm$ 1.2, respectively) did not differ (P = 0.45). There is limited data available in regards to locoweed IVDMD. Locoweeds are common legume plants found in the grazing areas of the southwest. Locoweed is comparable to alfalfa which also a legume in nutrient composition (DM basis 20% CP, 39% NDF; NRC, 1996). These data indicate that locoweed should be comparable in IVDMD to alfalfa. IVDMD values for alfalfa range from 51.4% to 88.7% (Collins and Taylor, 1984). Locoweed IVDMD obtained in this experiment were on the upper end of alfalfa range and indicate that locoweed is highly digestible. The high IVDMD could be related to plant cell wall structure of locoweeds used in this experiment having low NDF (average 30.4%) and high crude protein (average 18.9%). The presence of secondary plant metabolites, in this case swainsonine, did not seem to influence IVDMD. The fungal endophyte, *Undifilum oxytropis* in the E+ locoweed is correlated with swainsonine production in locoweeds (Ralphs et al., 2008).

Total VFA production was higher for E- than E+ (172.9 vs 159.9 $\pm$ 2.72 mmol; P < 0.01). Molar percentage of acetate was 7.3% lower (P < 0.01) and propionate was 15.6% higher for E- and than E+ (P < 0.02). The acetate:propionate ratio was 27% lower for E- than E+ (P < 0.01). These changes suggest an impact in bacterial metabolism or population dynamics resulting from swainsonine content and/or endophyte absence. VFA production is used to measure fermentation of feed by ruminal microbes. The ruminal microbes produce VFAs by digesting carbohydrates found in fiber or non fibrous sources feed sources. Depending on the species of ruminal bacteria different VFAs will be the end point of fermentation.

To compare the number of bacterial cells per micrometer of E+ and E- cell wall, transmission electron micrographs were made (Figure 1). Microbial cells were evaluated as attached or unattached (but within 3 μm of cell wall) from the length of cell wall visible in the micrograph. Attachment was not influenced by endophyte or time (P = 0.74 and 0.39, respectively). Time of incubation (P = 0.02) but not endophyte (P = 0.13) affected the number of unattached microbial cells. There were fewer cells unattached at 24 h vs 12 h (0.92 $\pm$ 0.18 vs 1.53 $\pm$ 0.16 cells/linear μm of plant cell wall). Constituents of plant cell walls are organized in a complex manner. Akin (1980) found that cool season grasses high is hemicellulose, pectins and xylans had less bacterial attachment to cell wall structures than warm season grasses with high levels of cellulose and lignin. Our lack of difference in microbial cell attachment between E+ and E- may be due to the fact that locoweed are low in cellulose and lignin and considered to be highly digestible. Digestion of plant material with low levels of cellulose and lignin can occur with minimal bacterial attachment and there have been reports of cell wall degradation by ruminal bacteria without adherence to the cell wall (Akin, 1980). According to the TEM micrographs digestion of the locoweed cell walls was apparent indicated by a loss of electron density (Figure 1).

Research into the interaction of plant toxicants and ruminal microbes are of interest in a number of areas. Currently, there is an interest on a national level for the production of biofuels from cellulose containing materials. An additional area of importance as an application of this research is the use of ruminants to control invasive or noxious plant species. Many cellulose containing plant materials also contain secondary plant metabolites that protect the plants from livestock and insect herbivory. An increase in knowledge of microorganisms that are capable of degrading cellulose in spite of secondary plant metabolites would be of great interest in the breakdown of cellulose to monosaccharides for the production of ethanol and for the control of noxious plants.

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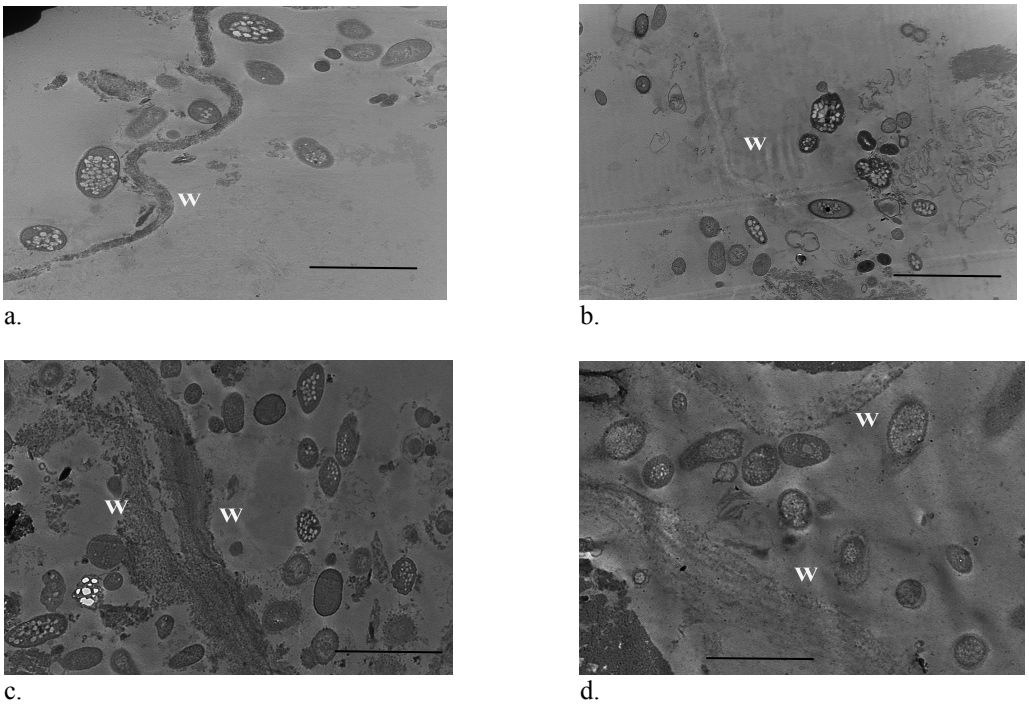


a. b.

c. d.

Figure 1. Transmission electron micrographs of attack by ruminal microbes from rumen fluid on endophyte-infected (E+; panel a and b) or endophyte negative (E-; panel c and d) *Oxytropis* spp. Cultures were incubated for 12 (panel a and c) or 24 h (panel b and d). Degradation is apparent at 24 h by loss of electron density of plant cell walls (W) near to the bacteria. Bar = 2 μm.

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# EFFECTS OF SHORT-TERM OILSEED SUPPLEMENTATION ON PLASMA FATTY ACID COMPOSITION IN LACTATING BEEF COWS

**E. J. Scholljegerdes*, L. A. Lekatz†, and K. A. Vonnahme†**

*USDA-ARS, Northern Great Plains Research Laboratory, Mandan, ND, USA, †North Dakota State University, Fargo, ND, USA

**ABSTRACT[1]:** Twenty-four three-year old Angus cows (512.2 ± 21.6 kg) and six ruminally cannulated beef heifers (523.1 ± 16.9 kg) were used to determine the impact of feeding oilseeds starting at the beginning of estrus synchronization until maternal recognition of pregnancy on plasma fatty acid composition. Starting approximately 60 d postpartum cows were synchronized with the Select Synch + CIDR® & timed AI protocol. The day CIDR® was inserted; cattle were randomly assigned to one of three treatments being grazing only (CON) or a supplement containing whole soybeans (WSB); or whole flaxseed (FLX). Supplements were formulated to provide similar quantities of N, TDN, and lipid. Cattle continued to receive these diets for 28 d. Blood was collected every three days until the end of the supplementation period and again on d 96 and 117. All cattle grazed a common pasture and supplemented cattle were individually fed their respective supplements once daily. Ruminally cannulated heifers were used to evaluate the impact our supplements had on forage intake, which was reduced ($P$ = 0.05) with oilseed supplementation. Feeding oilseeds increased total fatty acid intake ($P$ < 0.001) across treatments with WSB having greater ($P$ < 0.001) 18:2*n-6* intake than either CON or FLX. Likewise, cattle fed FLX had greater ($P$ < 0.001) 18:3*n-3* intake than either CON or WSB. There was a treatment × time interaction ($P \leq 0.05$) for all fatty acids identified except for 20:5*n-3* ($P$ = 0.99). Within 3 d after the start of supplementation, plasma concentrations of 18:2*n-6* increased ($P$ < 0.001) for cattle fed WSB compared to CON or FLX, whereas flax-fed cattle did not exhibit an increase ($P$ = 0.02) until d 18 over that of CON. Whereas, plasma concentrations for 18:3*n-3* was greater ($P$ < 0.013) for FLX than both CON and WSB by d 18. Feeding flaxseed tended to ($P$ = 0.065) increase and increased ($P$ = 0.007) plasma concentrations of 20:4*n-6* by d 18 over CON and WSB, respectively. Feeding oilseeds during the time of estrus synchronization will not only increase the energy density of the diet but will provide key fatty acids around the time of maternal recognition of pregnancy.

**Keywords** fatty acid, flaxseed, grazing, plasma, soybean

## Introduction

The results of short-term increases in energy, termed "flushing" have long been known to be beneficial to reproduction in sheep (Perkins, 1984; Nottle et al., 1997). However, the efficacy of flushing in cattle has been shown to be equivocal (Perkins, 1984). Specifically, flushing cattle with supplemental energy (more total feed or grains) has either slightly improved embryo quality (Nolan et al., 1998), follicular growth, or did not have any influence on any reproductive parameter measured (Dunne et al., 1999; Mackey et al., 1999).

Supplementation of fat can influence plasma fatty acids and subsequent energy status. This can be accomplished in a relatively short time frame because researchers (Filley et al., 2000; Scholljegerdes et al., 2006) have reported that plasma fatty acids differ within 7 days of initiation of dietary treatment. Of particular importance to reproduction is linoleic acid (18:2*n-6*), which is the precursor to the 2-series prostaglandins. An increase in dietary 18:2*n-6* will increase $PGF_2\alpha$ production (Lammoglia et al., 1997; Filley et al., 2000; Grant et al., 2005), which may negatively influence embryonic survival (Mattos et al., 2003; Hess et al., 2005). However, *n-3* fatty acids such as linolenic acid (18:3*n-3*) can inhibit 20:4*n-6* production and thereby decrease the formation of $PGF_2\alpha$. Ambrose et al. (2006) fed lactating dairy cows a barley silage-based diet that contained either rolled flaxseed (source of 18:3*n-3*) or rolled sunflower seed (source of 18:2*n-6*) for eight weeks starting 28 days prior to the breeding season and reported an early pregnancy loss (32 to 90 d) of 4.8% and 11.4% and an overall pregnancy loss of 9.8 and 27.3% for cows fed flaxseed versus sunflower seeds, respectively.

Timed AI is becoming increasingly attractive to livestock producers because of the reduction in heat detection; however, AI conception rates are generally lower than protocols using heat detection. Therefore development of a short-term feeding program that coincides with a timed AI protocol may help increase AI conception rates. Therefore our objectives were to evaluate the efficacy of a short-term increase in dietary energy and essential fatty acids around the time of AI until maternal recognition of pregnancy on plasma fatty acid concentrations over time in lactating beef cows grazing summer range.

## Materials and Methods

### *Animal Management*

Twenty-four Angus cows (avg. initial body weight = 512 kg and body condition score = 5.5 on a 1-9 scale) and six ruminally cannulated beef heifers (523.1 ± 16.9 kg)

[1] Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA 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.

grazing a 12.1 ha native range pasture were randomly allotted to one of three treatments being 1) grazing only and no supplement (**CON**); 2) supplemented whole soybeans (**WSB**); or 3) whole flaxseed (**FLX**). Supplements were formulated to provide equal amounts of protein, energy (TDN), and fat based on NRC (2000) predicted forage intake for a 544 kg cow producing 9.1 kg of milk. Approximately, 60 d postpartum, all animals were confirmed cyclic using ultrasound and serum progesterone as indicators of luteal activity. Two d prior to initiation of estrous synchronization, cattle were removed from feed and water for 12 hr prior to weighing for two consecutive days in order to account for any differences in gut fill and its bias on initial body weight and were weighed again on d 28 (shrunk) and again on d 117 and 118 (shrunk). Starting on d 0 a controlled intrauterine releasing device (CIDR®) was placed into the vagina and a blood sample was collected via coccygeal venipuncture. Cattle were also given 100 µg gonadorelin diacetate tetrahydrate (Fertagyl®). Animals that received supplement were locked into individual feeding stanchions that were placed in the pasture and offered their supplement. Cattle were given approximately 30 minutes to consume their supplement. Cows were sorted from the pasture and their calves each morning at 0730 and placed into individual stanchions for feeding. On d 7, the CIDR was removed from the vagina and cattle were given a shot of 25 mg of dinoprost tromethamine (Lutalyse®). Cows were observed for estrus for 72 h and bred upon appointment. Any cow that was not bred by 72 h was mass bred (d 10) and given another shot of 100 µg of Fertagyl®. The same AI sire was used for all cows.

It was important to know how supplements would influence forage intake because we know that fat supplements will reduce dietary intake (Schauff, and Clark. 1992) and in order to minimize stress and reduce any influence fecal sampling may have had on hormone production, 6 ruminally cannulated beef heifers to determine forage intake were utilized. These heifers were assigned to treatments (n = 2) and synchronized in the same manner as the cows. Starting on d 12 of the experiment, titanium dioxide was dosed to the cannulated heifers twice daily as an external marker fecal DM flow until d 21. Supplementation ceased on day 28 or 18 d post insemination, because Dunne et al. (2000) reported that the majority of embryo losses in beef heifers occurred prior to day 16 post insemination; therefore, supplement feeding was targeted to end just after maternal recognition of pregnancy. After supplementation ceased, all cattle were rotated to a similar pasture of similar quality until the final blood sample was collected.

***Sampling and Laboratory Analysis***

All cows were bled via coccygeal venipuncture every three days starting d 0 until d 18 after which blood samples were taken daily until d 12 then again every three days until day 20 when blood samples were taken daily until day 28 or the end of supplementation then again on d 96 and 117. Blood samples were immediately refrigerated for 4 h, after which serum or plasma was harvested by centrifugation at 2000 × *g* for 30 min. Cannulated heifers were used to evaluate the impact supplementation had on forage intake. Therefore, on d 11 heifers were completely evacuated of their rumen contents as described by Lesperance et al. (1960) and allowed to graze for 1 h. Heifers were then gathered and masticate was immediately collected, placed on ice and ruminal contents were returned to the rumen. Masticate was processed as outlined by Brokaw et al. (2001). On day 16, rumen fluid was collected from each heifer for masticate IVDMD. Each masticate sample was incubated in rumen fluid from the heifer from which it was collected. Fecal samples from cannulated heifers were collected twice daily starting on d 17 through d 21. Fecal samples were collected and composited for each heifer.

All supplements, masticate, and feces were analyzed for DM (AOAC, 1990), N (Carlo Erba Model NA 1500 Series 2 N/C/S analyzer, CE Elantech, Lakewood, NJ), NDF (ANKOM 200 fiber analyzer, ANKOM Technology, Fairport, NY) and IVDMD (ANKOM DaisyII Incubator, ANKOM Technology, Fairport, NY). Fecal samples were analyzed for $TiO_2$ according to the procedures of Myers et al. (2004).

Table 1. Supplement intake and ingredient and chemical composition of supplements fed to lactating beef cows supplemented with oilseeds and grazing native summer range

| | Supplements[1] | |
|---|---|---|
| Item | WSB | FLX |
| Supplement DM intake, kg·hd$^{-1}$·d$^{-1}$ | 2.95 | 2.67 |
| Ingredient composition, % DM | | |
| Corn | 19.6 | -- |
| Soybean meal | -- | 47.2 |
| Whole soybeans | 73.4 | -- |
| Whole flaxseed | -- | 45.1 |
| Molasses | 7.0 | 7.7 |
| Chemical composition, % DM | | |
| DM | 92.3 | 92.5 |
| CP | 32.1 | 38.0 |
| NDF | 20.7 | 22.0 |
| TDN[2] | 91.9 | 94.8 |
| IVDMD | 91.7 | 91.1 |
| Fatty acids | | |
| 16:0 | 1.38 | 0.77 |
| 18:0 | 0.49 | 0.44 |
| 18:1 | 2.51 | 2.12 |
| 18:2 | 7.02 | 2.56 |
| 18:3 | 1.01 | 5.63 |
| Total | 12.8 | 11.8 |

[1]WSB = Whole soybean supplement fed at 0.57% of BW (DM basis); FLX = Whole flaxseed supplement fed at 0.52% of BW (DM basis).
[2]Calculated based on published TDN values (NRC, 2000; Lardy and Anderson, 1999).

Feed and masticate was analyzed for fatty acids analysis via direct transesterification (Whitney et al., 1999) with methanolic-HCl (Kucuk et al., 2001) and plasma fatty acids were analyzed for fatty acid analysis using the procedures of Lake et al. (2006). Separation of fatty acid methyl esters was achieved by GLC (Model CP-3800, Varian Inc., Palo Alto, CA) with a 100 m capillary column (SP-2560, Supelco, Bellefonte, PA) and $H_2$ gas as a carrier gas at 1.5 mL/min. Initial oven temperature was maintained at 120°C for 2 min and then ramped to 210°C at 6°C/min and then ramped to 250° C at 5° C/min. Injector temperature was 260° C and flame ionization detector temperature was 300° C. Identification of peaks was accomplished using purified fatty acid standards (Sigma-

Aldrich, St. Louis, MO; Nu-Chek Prep, Elysian, MN; Matreya, Pleasant Gap, PA).

***Calculations and Statistical Analysis***

Fecal DM output was calculated using $TiO_2$ concentration. Intakes were estimated from fecal DM output and in vitro DM indigestibility of both the supplement and masticate. Therefore, cow forage intake was estimated using the average intake for each treatment, which was converted to g of forage DM intake/ kg of BW and multiplied by cow BW. Fatty acid intake and growth performance was analyzed as a completely randomized design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). All plasma fatty acid data was analyzed as a completely randomized design using the MIXED procedure of SAS. The model included diet, day, and any interaction between day and diet. Fixed effects included dietary treatment, day, and dietary treatment × day. Animal was used to specify variation between animals using the RANDOM statement and day was the repeated effect. Animal within dietary treatment was the nested effect using the SUBJECT statement. Single degree of freedom orthogonal contrasts were used to compare effects of Control vs. supplements (Corn and Flaxseed), as well as Corn vs. Flaxseed and sampling period effects were tested using orthogonal polynomial contrast (Steel and Torrie, 1980). Treatment differences within day were determined by using the PDIFF statement of SAS.

## Results

Estimated forage DM intake was lower ($P = 0.05$) for oilseed-fed cattle and no differences ($P = 0.76$) were observed between oilseeds (data not shown). Likewise, total DM intake was lower ($P = 0.01$) for heifers supplemented with oilseeds but did not differ ($P = 0.34$) between WSB and FLX (data not shown). Dietary intake of all fatty acids measured increased ($P < 0.001$) for fat-supplemented cattle (Table 2). Cattle fed whole soybeans had greater fatty acid intake for all fatty acids measured ($P \leq 0.02$) compared to flax-fed cattle with the exception of 18:3*n-3*, where FLX was greater ($P < 0.001$) than WSB.

Table 2 Effects of supplemental oilseeds on fatty acid intake of lactating beef cows grazing summer range

| | Treatments[1] | | | | Control | Flax |
|---|---|---|---|---|---|---|
| Item | CON | WSB | FLX | SE[2] | vs. Suppl | vs. WSB |
| 16:0 | 20.6 | 59.6 | 37.0 | 1.25 | <0.001 | <0.001 |
| 18:0 | 3.83 | 19.8 | 14.8 | 0.43 | <0.001 | <0.001 |
| 18:1*n-9* | 5.98 | 80.0 | 62.0 | 1.75 | <0.001 | <0.001 |
| 18:2*n-6* | 17.7 | 224.6 | 83.9 | 3.78 | <0.001 | <0.001 |
| 18:3*n-3* | 27.9 | 56.4 | 173.6 | 3.70 | <0.001 | <0.001 |
| Total | 98.5 | 471.2 | 395.4 | 10.9 | <0.001 | <0.001 |
| SFA | 24.4 | 79.4 | 51.8 | 1.68 | <0.001 | <0.001 |
| MUFA | 5.98 | 80.0 | 62.0 | 1.75 | <0.001 | <0.001 |
| PUFA | 45.6 | 281.0 | 257.5 | 6.80 | <0.001 | 0.02 |
| TUFA[3] | 51.5 | 361.0 | 319.5 | 8.54 | <0.001 | 0.002 |

[1]Treatments: CON = grazing only; WSB = Grazing plus Whole soybean supplement (73.4% whole soybeans, 19.6% cracked corn, and 7.0% dried molasses, DM basis) fed at 0.57% of BW (DM basis); FLX = Grazing plus flaxseed supplement (45.1% whole flaxseed, 47.2% soybean meal, 7.7% dried molasses, DM basis) fed at 0.52% of BW (DM basis).
[2]n=8.
[3]TUFA = total unsaturated fatty acids.

There was a treatment × time interaction for all plasma fatty acids measured ($P \leq 0.05$) with the exception of 20:5*n-3* ($P = 0.99$). Plasma concentrations of 18:2*n-6* increased ($P = 0.001$) above that of CON and FLX after 3 d of supplementation (Figure 1). Whereas, flax-fed cattle did not exhibit elevated plasma 18:2*n-6* concentrations above CON until d 18 ($P = 0.02$). Plasma 18:2*n-6* concentrations remained high ($P < 0.001$) 8 d past the end of supplementation and it was not until d 56 (29 d after supplementation ceased) that cattle fed whole soybeans tended ($P = 0.08$) to differ from CON and FLX.

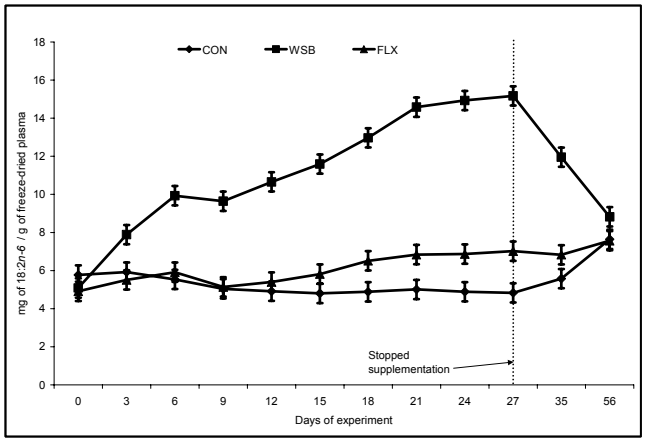


Figure 1. Effects of oilseed supplementation on plasma concentrations of 18:2*n-6* over time in lactating beef cows (SE = 0.51).

Plasma concentrations of 18:3*n-3* was greater ($P < 0.001$) for FLX compared to CON or WSB by d 15 (Figure 2). Despite higher 18:3*n-3* intake for WSB compared to CON, plasma concentrations for 18:3*n-3* tended to be lower by d 6 ($P = 0.07$) and were lower ($P = 0.04$) by d 9 for WSB than CON. This difference was short lived and by d 12 plasma concentrations of 18:3*n-3* only tended ($P = 0.09$) to differ from CON.

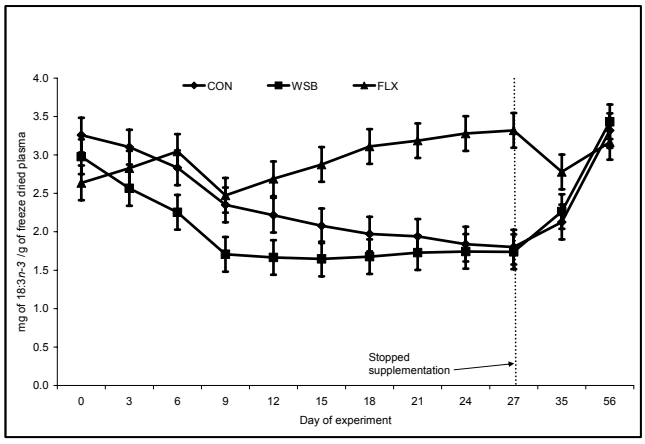


Figure 2. Effects of oilseed supplementation on plasma concentrations of 18:3*n-3* over time in lactating beef cows (SE = 0.23)

Plasma concentrations of 20:4*n-6* for cattle fed whole flaxseed tended to differ ($P = 0.07$) from CON and differed ($P = 0.04$) from WSB by d 18 (Figure 3). Cattle fed flaxseed continued to have elevated plasma concentrations of 20:4*n-6* above WSB ($P = 0.01$) 8 d past the end of supplementation.

Plasma concentrations of total unsaturated fatty acids (TUFA) increased by d 6 for WSB whereas additional

flaxseed did not increase ($P$ = 0.08) plasma TUFA concentrations until d 15 (Figure 4). Once supplementation ended, plasma concentrations of TUFA converged by d 56 ($P \geq 0.13$).

Total plasma fatty acid concentration was greater for WSB by d 6 ($P \leq 0.04$) when compared to either FLX or CON (Figure 5). Not until d 18 did plasma concentrations of total fatty acids differ ($P$ 0.04) between FLX and CON.

## Discussion

The estimated reduction in forage intake with fat supplementation was expected based on previous reports (Schauff and Clark. 1992; Scholljegerdes and Kronberg, 2007a,b) where forage intake was reduced with oilseed supplementation compared to unsupplemented controls. Nevertheless, this reduction in intake did not negatively affect animal performance because ADG in the current experiment was greater ($P$ = 0.01) for WSB and FLX than CON and no differences ($P$ = 0.12) being observed between supplemented groups (data not shown).

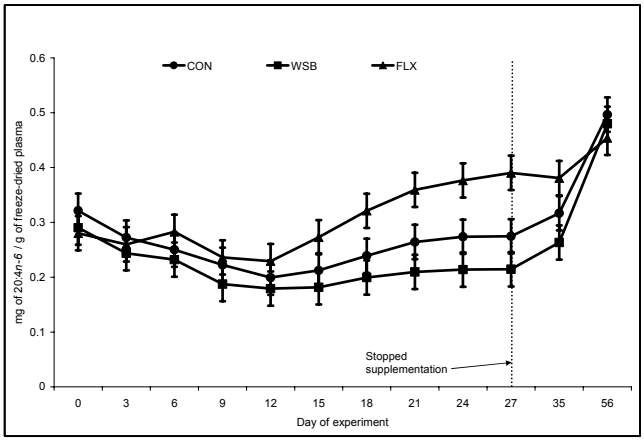


Figure 3. Effects of oilseed supplementation on plasma concentrations of 20:4*n-6* over time in lactating beef cows (SE = 0.01)

Fatty acid intake was expected to be higher for supplemented cattle compared to unsupplemented controls. However, the differences between supplemented treatments was not expected because we formulated the supplements based on NRC (2000) estimations for forage intake and fed each supplement at a level that would provide a diet that was approximately 3.3% crude fat. Nevertheless, cattle fed WSB consumed 75.8 g/d more total fatty acids than FLX based on estimated forage intake and actual supplement intake.

The observed increase in plasma concentrations of 18:2*n-6* in the current experiment has been previously reported by others where beef heifers were infused with soybean oil (Filley et al., 1999) or beef cows were fed cracked high-linoleate safflower seeds (Scholljegerdes et al., 2007). Despite the fact that cattle fed whole flaxseed had higher 18:2*n-6* intake compared to CON, it took 18 d for any differences in plasma concentration of 18:2*n-6* to develop. Cattle fed whole soybeans, saw an increase within 3 d over that of CON and FLX. It is not completely clear as to why there is a different lag phase between these two oilseeds. Supplementary data from the cannulated heifers would indicate that total tract DM digestibility did not differ ($P$ = 0.96) between WSB and FLX. Therefore, the difference was not likely due to differences in the ruminal digestibility between flaxseed and soybeans.

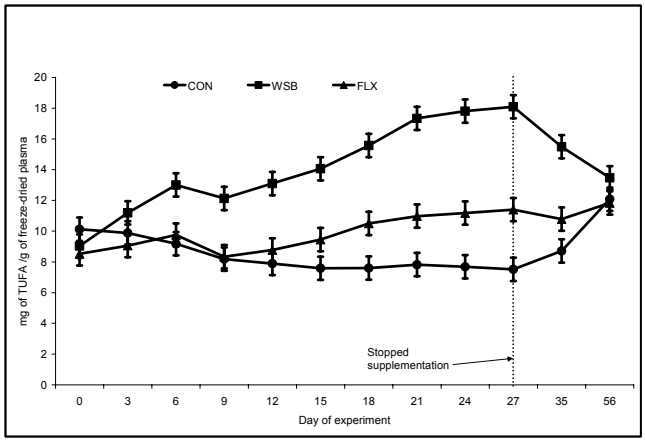


Figure 4. Effects of oilseed supplementation on plasma concentrations of total unsaturated fatty acids (TUFA) over time in lactating beef cows (SE = 0.76)

As expected, cattle fed flaxseed had higher plasma concentrations of 18:3*n-3* by d 15 compared to the other treatments. This difference remained high until d 35. Interestingly, there was a notable upswing in plasma concentration of 18:3*n-3* from d 35 to d 56. All cattle were rotated to a new pasture of similar quality and fatty acid composition after supplementation ceased on d 27, hence, it is unclear as to why plasma concentrations would increase at this time. Despite greater 18:3*n-3* intake for WSB compared to CON, plasma concentrations of 18:3*n-3* were lower for WSB compared to CON. We speculate that the reduction in plasma concentration is due to greater extent of ruminal biohydrogenation of 18:3*n-3* from whole soybean than forage alone.

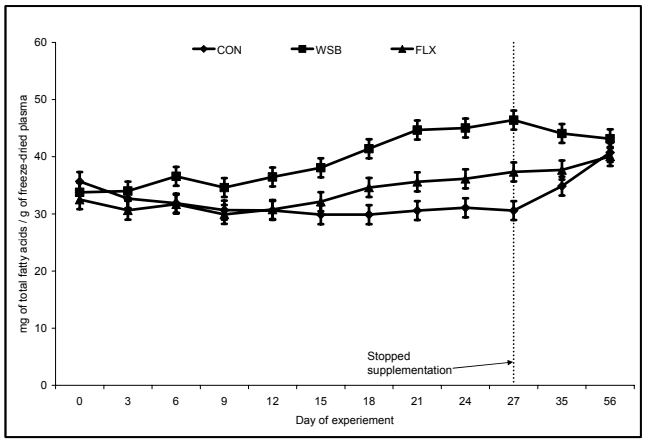


Figure 5. Effects of oilseed supplementation on plasma concentrations of total fatty acids over time in lactating beef cows (SE = 1.65)

Plasma concentrations of 20:4*n-6* was not expected to be lower for WSB than FLX because WSB had greater concentrations of 18:2*n-6,* which is a precursor for the production of 20:4*n-6*. Furthermore, 18:3*n-3* has been shown to be a potent inhibitor of 20:4*n-6* production (Mattos et al., 2000). Nevertheless, lower plasma concentrations of 20:4*n-6* in cattle fed whole soybeans may indicate that that 18:2*n-6* concentrations reported herein were sufficient to cause a reduction in 20:4*n-6* production

(Kaduce et al., 1982; Jenkins, 1988), while FLX stimulated 20:4*n-6* production. This is contrary to what we would have expected because 18:3*n-3* will compete with 18:2*n-6* for the Δ-6 desaturase enzyme thereby inhibiting 20:4*n-6* production. However, due to the extensive biohydrogenation of 18:3*n-3* (83%; Scholljegerdes and Kronberg, 2007b), the amount of 18:3*n-3* reaching circulation may not have been sufficient to significantly inhibit 20:4*n-6* production.

The greater plasma concentrations of TUFA for cattle fed the WSB supplement was due to a 41.5 g/d increase in TUFA intake over that of FLX. Nevertheless, both supplements increased plasma TUFA concentrations which have important implications to cow reproduction because increased intake of unsaturated fatty acids has been associated with improvements in reproduction (Staples et al., 1998).

This supplementation program was developed to coincide with a specific estrous synchronization protocol and provide certain nutrients at key time points during AI and pregnancy establishment. Specifically, it was our goal to provide a flush of energy in the form of fatty acids to improve the energy status of the cow which has been shown to improve cow fertility (Dunne et al., 1999). The improvement of ADG indicates that we were indeed able to improve energy status of the animal regardless of plasma fatty acid composition over that of CON. However, based on total fatty acid intake and plasma concentration, WSB would be expected to slightly out perform FLX, nevertheless, ADG was similar (0.83 and 1.10 kg/d, respectively) for both supplemented treatments averaged across the 28 d supplementation period. In addition to a flush of energy, oilseeds provided 18:2*n-6* or 18:3*n-3* which has been shown to improve follicular development (Thomas et al., 1997; Robinson et al. 2002) around the time of breeding (d 10). Although we realize that plasma fatty acid composition only gives a brief picture of what fatty acids are circulating in the blood at the time of sampling. It would appear that whole soybeans do a better job of supplying 18:2*n-6*, TUFA, and total fatty acids by d 10 or the day of timed AI than whole flaxseed. At this time it is not clear as to why there is this lag time between feeding and appearance of fat from flaxseed in the plasma.

A second major objective of this experiment was to provide increased levels of 18:2*n-6* or 18:3*n-3* around the time of maternal recognition of pregnancy (around d 15-16 post AI). At this time it is not clear whether an increased supply of 18:2n-6 is beneficial to reproduction. Because results of an increasing tissue supply of 18:2*n-6* are equivocal. Specifically, PG metabolite concentrations have been shown to increase (Filley et al., 1999; Robinson et al., 2002) or decrease (Burke et al., 1996; Cheng et al., 2001) with additional 18:2*n-6* supply. Elevated levels of PG after insemination have been linked to an increase in early embryonic mortality due to shortened estrous cycles (Burke et al., 1996). Whereas increasing the supply of *n-3* fatty acids has been shown to decrease PG metabolite production (Mattos et al., 2003; Petit and Twagiramungu, 2006). In the current experiment, both oilseeds increased the circulating levels of 18:2*n-6*, whereas only flaxseed increased the circulating levels of 18:3*n-3* around the time of maternal recognition of pregnancy. Unfortunately, flaxseed also increased the circulating levels of 20:4*n-6* around the time of maternal recognition of pregnancy, which is a precursor for PG synthesis. This increase in 20:4*n-6* may have a negative impact on embryonic survival.

In conclusion, using whole soybeans in a flushing protocol during estrous synchronization and breeding may improve reproductive success more so than whole flaxseed. However, it is important to note that plasma fatty acid concentration does not necessarily reflect the fatty acid composition of reproductive tissues (Scholljegerdes et al., 2007). Therefore, further investigation into the impact of these two oilseeds on reproductive hormone production is warranted.

## Implications

Feeding either whole soybeans or whole flaxseed starting at the beginning of estrous synchronization will increase the supply of key fatty acids known to influence reproduction and improve animal energy status.

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# SITE AND EXTENT OF NUTRIENT DIGESTION IN LAMBS FED WHOLE CANOLA, BROWN MUSTARD, OR CAMELINA SEEDS

**P. L. Price[1], V. Nayigihugu[1], D. C. Rule[1], J. M. Krall[2], and B. W. Hess[1]**
[1]Departments of Animal and [2]Plant Sciences, University of Wyoming, Laramie, 82071

**ABSTRACT:** The objective of this experiment was to compare site and extent of nutrient digestion in lambs fed diets containing whole canola, brown mustard, or *camelina sativa* seeds. Four black-face wether lambs (77.5 ± 4.2 kg BW) fitted with ruminal, duodenal, and ileal canulae were used in a 4 × 4 Latin square design experiment. Experimental diets consisted of 18% ground (2.54 cm) bromegrass hay, 65.2% cracked corn, 15% soybean meal, and 1.8% limestone (as-fed basis, Control) with oil seeds replacing enough of the soybean meal to provide 3% added fatty acid from each of the whole oil seeds. A 7-d adaptation period was followed by 3 d of duodenal, ileal, fecal, and ruminal sampling. Intake of OM was greater ($P < 0.0001$) for lambs fed camelina than Control with canola being intermediate and lambs fed brown mustard not differing from lambs fed camelina and canola. Site and extent of OM digestibility did not differ ($P \geq 0.217$) among treatments. Total ($P = 0.124$) and molar proportions ($P \geq 0.185$) of individual VFA did not differ among treatments. Intake of N was greater ($P < 0.0001$) for lambs fed camelina and brown mustard compared with Control; lambs fed canola were intermediate. Nonetheless, ruminal $NH_3$ concentrations were not affected ($P = 0.461$) by dietary treatment. Digestibility in the small intestine (% entering) of N was less ($P = 0.017$) for lambs fed Control than lambs fed any of the whole oil seeds. Expressed as a percentage of intake, total tract NDF digestibility was greater ($P = 0.038$) for lambs fed camelina and canola than the Control with brown mustard supplemented lambs being similar to all treatments. The feeding value of whole *camelina sativa* and brown mustard seeds are comparable to whole canola seeds when included in finishing lamb diets.

**Key words:** lambs, whole oil seed supplementation, digestion

## Introduction

The biodiesel industry in the U.S. has grown tremendously over the past decade. This growth has increased interest in the production of oilseeds to accommodate the demand of the industry. A variety of oilseeds are being grown across the high mountain plains region to find the most suitable crop for the area. Among the oilseeds being evaluated are brown mustard and *camelina sativa*. These particular oil seeds are easily grown and drought tolerant, making them suitable for cultivation in the intermountain and high plain region. Due to escalating costs of shipping and limited market opportunities in the high mountain plains region, growers of these oilseed crops are interested in alternative uses of the seed, because they are rich sources of fat and protein, one potential alternative is to incorporate the oilseeds in the diets fed to livestock.

Adding fat to the diets of livestock is often practiced to increase dietary energy. However, fat can have negative effects on digestion when supplemented at high levels. Nelson et al. (2001) reported a substantial decrease in total tract digestibility of fiber when tallow was added at 6% of finishing lamb diets. It has been recommended that dietary fat be limited to 16-20 percent of ME in ruminant diets (Palmquist 1994). We are unaware of any studies that have been conducted on site and extent of nutrient digestion of brown mustard and camelina in the diets of ruminant livestock. Our hypothesis was that the feeding value of brown mustard and camelina would be comparable to canola. Therefore, the objective was to determine site and extent of nutrient digestion in lambs fed oilseeds formulated to provide 3% added dietary fatty acids.

## Materials and Methods

### *General*

All procedures for the following experiment were approved by the University of Wyoming Animal Care and Use Committee. Four wether lambs (77.5 ± 4.2 kg BW) were fitted with ruminal, duodenal, and ileal canulae. After allowing time for recovery from the surgeries, lambs were maintained in individual metabolism crates (1.4 × 0.6 m) under continuous lighting in a climate controlled room where they had free access to water.

### *Diets and Sampling*

Following the design of a 4 x 4 Latin square experiment, lambs were assigned to 1 of 4 dietary treatments. The **Control** diet contained no supplemental fat and consisted of ground (2.54 cm) bromegrass hay, cracked corn, soybean meal, molasses, limestone, urea, and salt (Table 1). Oilseeds were added to replace enough of the soybean meal to provide 3% added fatty acids from canola, brown mustard, or camelina whole oilseeds. Due to a recent report indicating that oilseeds do not need to be processed to improve diet digestibility (Price et al., 2008), all seeds were fed whole for the present experiment. All dietary treatments were formulated to be isonitrogenous. Lambs were offered 25% their daily ration at 0530 and 75% at 1730. As an external marker of digesta flow, 2.5 g of $TiO_2$ was dosed intraruminally immediately before each feeding (Myers et al., 2006). Each 7-d adaptation period was followed by 2 d of duodenal and ileal sampling and 1 d of ruminal sampling. Duodenal and ileal samples were

collected beginning at 0500 on d 8 of each period with the collection of 150 mL of duodenal and ileal digesta repeated every 4 h. Collection times were advanced by 2 h on d 9 so that digesta was collected every other h of a theoretical 24-h clock. Beginning at 0500 on d 10, 500 mL of ruminal digesta was collected every 2 h for 12 h. Ruminal pH was immediately measured on whole rumen contents using a combination electrode (Orion Research Inc., Boston MA). A 10 mL aliquot was strained through 4 layers of cheesecloth, placed in a 12 mL conical vial with 0.10 mL of 7.2 *N* $H_2SO_4$, and stored in the freezer for subsequent VFA and $NH_3$ analysis.

**Table 1.** Ingredient composition of diets fed to lambs

| | Dietary treatment[1] | | | |
|---|---|---|---|---|
| | Control | Canola | B.M. | Camelina |
| Ingredients, % of DM | | | | |
| Bromegrass hay | 17.38 | 16.37 | 16.34 | 16.37 |
| Cracked corn | 61.12 | 61.12 | 61.12 | 61.12 |
| Soybean meal | 14.46 | 2.98 | 1.15 | 0.13 |
| Canola | - | 11.78 | - | - |
| Brown Mustard | - | - | 13.41 | - |
| Camelina | - | - | - | 14.46 |
| Salt | 1.05 | 1.05 | 1.05 | 1.05 |
| Urea | 0.00 | 1.01 | 1.08 | 1.05 |
| Limestone | 1.88 | 1.88 | 1.88 | 1.88 |
| Molasses | 4.10 | 4.10 | 4.10 | 4.10 |

[1]Supplemental whole oil seeds were added so that the diets contained 3% added fatty acids (as-fed) as canola, brown mustard (B.M.), or camelina.

### *Laboratory Analysis*

Beginning 2 d before and throughout the collection period, samples of all feedstuffs were taken on a daily basis for laboratory analysis. Duodenal and ileal samples were frozen at -20° C, lyophilized (Genesis 25 freeze dryer, The VirTis Co., Gardiner, NY), and composited within lamb for each collection period for analysis of $TiO_2$ (Myers et al., 2004). Feed and digesta samples were analyzed for DM and ash (AOAC, 1990), N (Leco Corp., Henderson, NV), and NDF (Ankom Technology, Fairport, NY). Acidified ruminal fluid samples were prepared (Goetsch and Galyean, 1983) and analyzed for VFA concentrations using a Hewlett-Packard 5890 GLC (Hewlett-Packard, Avondale, PA) equipped with a 15-m × 0.53-mm (i.d.) column (Nukol, Supelco, Bellafonte, PA). The initial oven temperature was 110°C and final temperature was 150°C with a ramp of 8°C/min. Ruminal $NH_3$ concentration was determined by the phenol-hypochlorite procedure (Broderick and Kang, 1980).

### *Calculations and Statistical Analysis*

Digesta flow was calculated by dividing the amount of $TiO_2$ dosed by the concentration of $TiO_2$ in duodenal and ileal samples. Nutrient flow was calculated by multiplying nutrient concentration by digesta flow. Digestion data were analyzed using the GLM procedures of SAS (Version 8.0, 1998, SAS Inst., Inc., Cary, NC) for a Latin square. After a significant preliminary F-test, Fisher's LSD was used to separate treatment means. Time series data were analyzed as a split-plot design. Treatment effects were tested using animal × period × treatment as the error term. Time and treatment × time interactions were tested using the residual error term. No interactions ($P$ = 0.088 to 0.999) were detected for time course data; therefore, only the main effects were reported.

## Results and Discussion

Intake of OM was greater ($P$ < 0.001) for lambs fed camelina than Control with canola being intermediate, and lambs fed brown mustard did not differ from lambs fed camelina and canola (Table 2). Nitrogen intake was greater ($P$ < 0.001) for lambs fed camelina and brown mustard compared with Control; lambs fed canola were intermediate. These differences in intake may be attributable to analysis of grab samples of soybean meal taken during the trial period differing from the initial analysis of soybean meal used to formulate diets. The differences in N intake; however, did not affect ($P$ = 0.461) ruminal $NH_3$ concentrations (Table 3), which have been shown to increase with an increase in dietary CP (Pritchard and Males, 1985). Additionally, site and extent of OM digestibility did not differ ($P \geq 0.217$) among treatments. This is consistent with Kucuk et al. (2004) who reported no differences in OM digestibility with increasing levels of soybean oil in a high-concentrate diet fed to lambs. Digestibility of N in the small intestine (% entering) was less ($P$ = 0.017) for lambs fed Control than lambs fed any of the whole oilseeds, suggesting that protein quality was improved by replacing soybean protein with protein from the oilseeds. In contrast, Aldrich et al. (1997) noted that small intestinal N digestibility was not affected by supplementing the diets of steers with canola seeds. The current study used a high-concentrate diet fed to lambs, whereas Aldrich et al. (1997) utilized a high-roughage diet for their control. Total tract digestibility of N was greater ($P$ = 0.002) for lambs fed camelina than Control with canola being intermediate, and lambs fed brown mustard did not differ from the other fat-supplemented treatments.

Intake of NDF was greatest ($P$ < 0.001) for canola followed by camelina, brown mustard, and Control. Supplementing fat did not affect ($P$ = 0.762) ruminal fiber digestibility, which was expected because diets were formulated to provide 3% added fat to avoid detrimental effects on ruminal fiber digestibility. Total tract NDF digestibility was greater ($P$ = 0.038) for lambs fed camelina and canola than the Control with brown mustard supplemented lambs being similar to all treatments. In contrast, total tract NDF digestibility of forage-based diets was not affected if steers were fed whole canola seeds (Leupp et al; 2006) or heifers were fed whole flaxseed (Scholljegerdes and Kronberg, 2007).

Ruminal pH was not affected ($P$ = 0.206) by dietary treatments (Table 3). In feeding dairy cows whole camelina seeds and camelina meal, Hurtaud and Peyraud (2007) detected a lower pH for camelina treatments before feeding and similar ruminal pH 3 h after feeding. Leupp et al. (2006) reported a lower ruminal pH in steers fed canola

seeds. Similar to other reports published by our laboratory (Kucuk et al., 2004; Atkinson et al., 2006); however, adding 3% dietary fat did not affect ($P = 0.124$) total ruminal VFA concentrations. Molar proportions of individual VFA did not differ ($P \geq 0.185$) among dietary treatments, which was also reported by Kucuk et al. (2004) who fed lambs high-concentrate diets containing 3.2% soybean oil.

In conclusion, although some variations in nutrient digestibility were noted, our overall hypothesis that nutrient digestibility of camelina and brown mustard would be comparable to canola held true. The feeding value of whole *camelina sativa* and brown mustard seeds were comparable to whole canola seeds when included in finishing lamb diets.

## Implications

Oilseed crop growers should be able to market brown mustard and camelina as a dietary supplement for lambs.

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**Table 2**. Intake and site and extent of OM, N, and NDF digestion in lambs fed whole canola, brown mustard, or camelina seeds

| | Dietary treatment | | | | | |
|---|---|---|---|---|---|---|
| Item | Control | Canola | Brown mustard | Camelina | SEM[1] | F-test *P*-value |
| OM | | | | | | |
| Intake, g/d | 1181.66[c] | 1235.11[b] | 1236.78[a,b] | 1245.60[a] | 2.7 | <0.001 |
| Digestibility | | | | | | |
| Apparent ruminal, % of intake | 55.13 | 50.59 | 51.00 | 57.27 | 4.0 | 0.604 |
| Small intestine, % entering | 49.55 | 54.35 | 54.84 | 53.65 | 3.2 | 0.653 |
| Large intestine, % entering | 24.10 | 19.21 | 21.51 | 18.78 | 6.5 | 0.931 |
| Lower tract, % entering | 61.81 | 64.10 | 64.46 | 63.59 | 2.4 | 0.871 |
| Total tract, % of intake | 83.13 | 82.42 | 82.96 | 84.43 | 0.6 | 0.217 |
| N | | | | | | |
| Intake, g/d | 17.23[c] | 24.55[b] | 26.61[a] | 26.18[a] | 0.3 | <0.001 |
| Digestibility | | | | | | |
| Small intestine, % entering | 67.06[b] | 71.08[a] | 72.51[a] | 71.62[a] | 0.9 | 0.017 |
| Large intestine, % entering | 9.17[b] | 10.21[b] | 15.05[b] | 28.43[a] | 3.8 | 0.038 |
| Lower tract, % entering | 70.34[c] | 74.01[b,c] | 76.61[a,b] | 79.80[a] | 1.2 | 0.009 |
| Total tract, % of intake | 54.23[c] | 69.57[b] | 73.82[a,b] | 78.96[a] | 2.6 | 0.002 |
| NDF | | | | | | |
| Intake, g/d | 298.23[d] | 339.68[a] | 323.75[c] | 333.13[b] | 1.0 | <0.001 |
| Digestibility | | | | | | |
| Ruminal, % of intake | 58.92 | 60.21 | 56.49 | 60.67 | 3.0 | 0.762 |
| Lower tract, % entering | 11.39 | 26.52 | 25.28 | 29.60 | 5.3 | 0.177 |
| Total tract, % of intake | 64.21[b] | 70.85[a] | 69.04[a,b] | 72.35[a] | 1.5 | 0.038 |

[1]n = 4

[a,b,c,d]Within the same row, means with unlike superscripts differ ($P < 0.05$).

**Table 3**. Ruminal pH, $NH_3$, and VFA in lambs fed whole canola, brown mustard, or camelina seeds

| | Dietary treatment | | | | | |
|---|---|---|---|---|---|---|
| Item | Control | Canola | Brown mustard | Camelina | SEM[1] | F-test *P*-value |
| pH | 5.87 | 6.09 | 6.04 | 5.87 | 0.08 | 0.206 |
| NH3, mM | 8.27 | 8.05 | 9.29 | 10.07 | 0.9 | 0.461 |
| Total VFA, mM | 133.00 | 127.40 | 132.80 | 146.40 | 4.8 | 0.124 |
| | ----------mol/100mol---------- | | | | | |
| Acetate | 54.9 | 57.9 | 55.5 | 52.8 | 1.4 | 0.191 |
| Propionate | 29.3 | 28.9 | 31.8 | 32.6 | 2.4 | 0.636 |
| Butyrate | 11.9 | 9.3 | 8.5 | 10.4 | 1.3 | 0.339 |
| Isobutyrate | 0.9 | 1.0 | 1.0 | 0.7 | 0.1 | 0.135 |
| Valerate | 1.4 | 0.9 | 1.0 | 1.2 | 0.2 | 0.292 |
| Isovalerate | 1.8 | 2.0 | 2.2 | 2.4 | 0.3 | 0.695 |

[1]n = 4

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# EFFECTS OF MATERNAL NUTRITION AND SELENIUM SUPPLY ON JEJUNAL CHARACTERISTICS AND mRNA EXPRESSION OF ANGIOGENIC FACTORS AND RECEPTORS IN OFFSPRING AT HARVEST

**T. L. Neville*, J. J. Reed*, K. A. Vonnahme*, P. P. Borowicz*, J. B. Taylor†, D. A. Redmer*, J. S. Luther*, C. J. Hammer*, L. P. Reynolds*, and J. S. Caton***

*Center for Nutrition and Pregnancy, Department of Animal Sciences, North Dakota State University, Fargo

†USDA-ARS, U. S. Sheep Experiment Station, Dubois, ID

**ABSTRACT:** To examine effects of maternal nutrition and Se supply on intestinal growth and vascularity in offspring, treatments were imposed during gestation to 82 pregnant Rambouillet ewe lambs (52.2 ± 0.8 kg) allotted randomly to 1 of 6 treatments in a 3 x 2 factorial. Factors were maternal nutrition (100% of ME requirement [**CON**], 60% of CON [**RES**], and 140% of CON [**EXC**]) and dietary Se (Se-enriched yeast) supply (9.5 µg Se•kg $BW^{-1}$•$d^{-1}$ [**ASe**] and 81.8 µg Se•kg $BW^{-1}$•$d^{-1}$ [**HSe**]). Selenium treatments were initiated at breeding and nutritional treatments at d 46 of gestation. At parturition, lambs were removed from ewes before nursing, placed in a common pen, and group-fed until necropsy at approximately 180 d of age. Jejunum weight, expressed as g or g/kg empty BW, was not different ($P \geq 0.31$). Small intestine weight (g) was greatest ($P \leq 0.06$) for CON-HSe, intermediate for RES-ASe, and least for CON-ASe, EXC-ASe, RES-HSe, and EXC-HSe. Small intestine weight (g/kg empty BW) was greatest ($P = 0.05$) in CON-HSe, intermediate in RES-ASe, CON-ASe, EXC-ASe, and EXC-HSe and least in RES-HSe. Concentration and total amounts of RNA, DNA, protein, RNA:DNA, and protein:DNA were not different ($P \geq 0.10$). Jejunal crypt depth and villi width and length were not different ($P \geq 0.19$). No differences ($P \geq 0.16$) were observed for percent proliferating nuclei and total number of cells, however total number of cells proliferating was greater ($P = 0.09$) in offspring from CON compared to RES or EXC ewes. No differences ($P \geq 0.13$) were measured in *TEK, GUCY1B3*, *NOS3*, *ANGPT1*, or *ANGPT2*. Maternal nutrition during gestation alters total proliferating jejunal crypt cells in offspring at harvest. This response is not explained by changes in angiogenic factors measured in this study.

Key words: maternal nutrition, selenium, jejunum

## Introduction

Selenium, an essential trace mineral, is important for normal growth and development (Sunde, 1997). Selenium is regulated by the FDA to an inclusion limit less than 0.3 ppm (FDA 21CFR573.920), however many rangelands in North and South Dakota contain much higher levels of Se due to the geographic formations in these areas (Rosenfeld and Beath, 1964). Nutrient restriction and overfeeding during adolescence in ewe lambs alters normal growth and development of the fetus and placenta (Wallace et al., 2000; Reed et al., 2007). The lifelong regulation of normal growth, development, and nutrient utilization are likely programmed *in utero* (Wu et al., 2006).

Lambs used in the current study were previously reported to be lighter and smaller when born to restricted or overfed ewes compared to maintenance ewes (Caton et al., 2007). Furthermore, total tract digestion of DM, OM, NDF and ADF were reduced by high maternal Se intake at 13 and 19 wk of age and offspring from overfed, adequate Se ewes had increased ADG and gain:feed (Caton et al., 2007). Maternal nutrition and high dietary Se altered IgG absorption after birth (Hammer et al., 2007). The underlying reasons for these differences are unknown, but may be related to intestinal function. Therefore, the objective was to determine the effects of maternal nutrition and selenium supplementation on offspring jejunal cellularity estimates, cellular proliferation, morphology, and expression of angiogenic factors and receptors.

## Materials and Methods

This experiment was approved by the Institutional Animal Care and Use Committee at North Dakota State University, Fargo, ND. Eighty-two pregnant Rambouillet ewe lambs (52.2 ± 0.8 kg; d 47 ± 5 d of gestation) were individually housed in 0.91 × 1.2 m pens. Ewes were randomly allotted to 1 of 6 treatments in a 3 x 2 factorial array. Main effects evaluated were dietary levels of Se (initiated at breeding; adequate [**ASe**; 9.5 µg Se•kg $BW^{-1}$•$d^{-1}$] vs. high [**HSe**; 81.8 µg Se•kg $BW^{-1}$•$d^{-1}$]), and plane of nutrition (initiated at d 46 of gestation; 60% [**RES**], 100% [**CON**], and 140% [**EXC**] of requirements for gestating ewe lambs).

All diets were fed once daily in a complete pelleted form (0.48 cm diameter; based on beet pulp, alfalfa meal, ground corn, and soybean hulls; 14.2% CP; 2.64 Mcal/kg ME DM basis). Pellets were similar in composition except in the HSe pellet, Se-enriched yeast (Diamond V Mills Inc., Cedar Rapids, IA) replaced soybean meal from the basal pellet. Nutrient requirements were based on NRC (1985) recommendations for 60 kg pregnant ewe lambs during mid to late gestation (weighted ADG of 140 g). Body weight was measured every 14 d. Selenium pellet was fed to meet the required Se level of HSe ewes (81.8 µg/kg BW) and remaining energy requirements were met with the basal pellet.

*Parturition to Slaughter*. Upon parturition, lambs were immediately separated from dams to assure that no ewe colostrum was consumed. Lambs were cared for in a separate, clean room. Lambs were offered artificial colostrum by bottle within 30 min of birth. Colostrum was fed in six feedings over the first 20 h post-parturition. At 24

h and until weaning, lambs were fed milk replacer (Super Lamb Instant Milk Replacer, Merrick's Inc., Middleton, WI). Lambs were weaned (avg 57 d age) to a totally pelleted diet and fed common diets. At harvest (avg 180 d age), lambs were stunned by captive bolt (Supercash Mark 2, Acceles and Shelvoke Ltd., England), exsanguinated, and tissues harvested as described by Reed et al. (2007).

Jejunal tissue samples (72 g) were collected for, RNA, DNA, protein, jejunal morphology, cellular proliferation analysis, and RT-PCR. Collection occurred at 15 cm down the mesenteric vein from the mesenteric-ileocecal vein junction then up the mesenteric arcade to the point of intestinal intersection. Tissue was preserved for analysis by snap freezing five 1 g samples in super-cooled isopentane (submerged in liquid nitrogen), then stored at -80°C (Neville, 2008) and fixing segments in Carnoy's solution (60% ethanol, 30% chloroform, 10% glacial acetic acid).

*Cellularity and Morphology*. Paraffin-embedded tissues were sectioned at 5 µm, and stained for a cellular proliferation marker using the mouse anti-proliferating nuclear cell antigen (PCNA) primary antibody (Chemicon International, Temecula, CA) and detected with a secondary biotinylated secondary antibody (horse anti-mouse IgG, Vectastain; Vector Laboratories, Burlingame, CA) and the Avidin-Biotin Complex system (Vectastain; Vector Laboratories, Burlingame, CA). Tissues were further stained with Periodic-Acid Schiff's reagent and counterstained with hematoxylin. Cellular proliferation was quantified using the Image-Pro Plus 5.0 analysis software (Media Cybernetics, Silver Spring, MD). Morphology was determined on the histological sections by computerized image analysis as previously described (Jin, 1994). A total of 10 villi and their associated length, width, and crypt depth were measured for each lamb.

*Cellularity Estimates*. Tissue homogenates were analyzed for concentrations of DNA and RNA by using the diphenylamine (Johnson et al., 1997) and orcinol procedures (Reynolds et al., 1990). Protein in tissue homogenates was determined with Coomassie brilliant blue G (Bradford, 1976), with bovine serum albumin (Fraction V; Sigma, St. Louis, MO) as the standard (Johnson et al., 1997).

*RT-PCR Procedures*. In this study, mucosal scrape mRNA was analyzed for angiogenic factors and their receptors (angiopoietin-1 [***ANGPT1***], angiopoietin-2 [***ANGPT2***], endothelial tyrosine kinase [***TEK***], endothelial nitric oxide synthase 3 [***NOS3***], and soluble guanylate cyclase [***GUCY1B3***]) were determined using quantitative real-time RT-PCR. Methods of extraction and quantification of mRNA and the analysis of major angiogenic factors followed Redmer et al. (2005) and Borowicz et al. (2007), with the following modifications. A multiplex reaction was performed. Into each well of the plate, 18S mRNA was added to serve as reference standard of total cellular RNA to minimize sample variation. Analyses were conducted using TaqMan reagents and procedures purchased from and recommended by Applied Biosystems (Foster City, CA).

Polymerization/amplification reactions were performed in a 96-well PCR plates sealed with optically clear adhesive covers using the Applied Biosystems ABI Prism 7000 sequence detector. Hybridization and polymerization were performed at 60ºC for 40 cycles for *ANGPT1* and *TEK* and 50 cycles for *NOS3*, *ANGPT2*, and *GUCY1B3*. All qRT-PCR data was normalized by dividing quantity of gene of interest by 18S.

*Statistics*. Data were analyzed as a completely randomized design with a 3 × 2 factorial arrangement of treatments using PROC GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Model contained effects for nutrition (RES, CON, and EXC), level of Se (ASe vs. HSe), and nutrition × Se interaction. Fetal number was included in the model and was retained when significant ($P \leq 0.10$) and dropped when not significant ($P > 0.10$). When interactions were present ($P < 0.10$), means were separated by least significant difference. Main effects were considered significant when $P < 0.10$.

## Results

Maternal nutrition and Se supplementation did not affect (Table 1; $P \geq 0.31$) total (g) or proportional (g/kg EBW) offspring jejunum weight. Small intestinal weight was greater (g; $P \leq 0.06$) for CON-HSe, and least for CON-ASe, EXC-ASe, RES-HSe, and EXC-HSe with RES-ASe intermediate. Interaction for small intestinal weight (g/kg EBW) resulted in CON-HSe being greater ($P = 0.05$) than RES-HSe with all other treatments intermediate. No differences (Table 2; $P \geq 0.16$) were observed due to maternal nutrition or selenium treatments for percent proliferating nuclei and total number of cells, however when multiplied to determine total number of cells proliferating, offspring from CON ewes were greater ($P \leq 0.09$) compared to RES or EXC. Morphology of the jejunal villi, crypt depth and villi width and length were not different (Table 2; $P \geq 0.19$) in offspring at 180 d due to treatments supplied to ewes during gestation.

No differences (Table 1; $P \geq 0.10$) in cellularity estimates DNA, RNA, protein, RNA:DNA, or protein:DNA were detected due to maternal nutrition or selenium supplementation. Neither nutrition nor selenium supplementation affected (Table 2; $P \geq 0.13$) the angiogenic factors and receptors measured (*TEK*, *GUCY1B3*, *NOS3*, *ANGPT1*, and *ANGPT2*).

## Discussion

Lambs, from this study, born to restricted or overfed ewes were lighter and smaller compared to maintenance ewes (Caton et al., 2007). Maternal nutrition and high dietary Se altered IgG absorption after birth (Hammer et al., 2007). Total tract digestion of DM, OM, NDF and ADF were reduced by high maternal Se intake (Caton et al., 2007) at 13 and 19 wk of age. Additionally offspring from overfed, adequate Se ewes had increased ADG and gain:feed (Caton et al., 2007) at 13 and 19 wk of age.

Other researchers have shown affects of maternal nutrition on fetal small intestine weight at d 135 of gestation was decreased due to 60% maternal nutrient restriction (Reed et al., 2007). Osgerby et al. (2002) found decreased gut weight (g and as % BW) at d 135 due to 70% maternal diet restriction initiated at d 22 of gestation,

though no differences were found in gut weight at d 45 or 90. Ewes at ad libitum intake produced fetuses at d 128 with decreased gut weight, but when expressed relative to fetal body weight gut weight was greater than moderate fed ewes (Wallace et al., 2000).

Nutrient restriction and Se have been shown to alter maternal proliferation in jejunum and jejunal mucosa of pregnant ewe lambs (Reed et al., 2007; Neville et al., 2008). Similar to the current data other research from our laboratory shows no differences in jejunal percent proliferating nuclei in fetuses (d 135) from selenium supplemented or nutrient restricted dams (not published). Conversely, when the percentage proliferating nuclei is multiplied by total number of cells CON offspring have a greater number of proliferating cells compared to RES or EXC offspring. The differences in total tract digestion, ADG, and gain:feed (Caton et al., 2007) are not explained by jejunal morphology in the current data set. Likewise, selenium supplementation in feedlot steers did not affect jejunal morphology (Soto-Navarro et al., 2004).

Form and level of maternal Se supplementation has been shown to affect RNA:DNA and protein:DNA in fetuses at d 134 of gestation (Neville et al., 2008). Fetal jejunum at d 135 of gestation had altered RNA:DNA due to maternal Se supplementation while maternal nutrient restriction decreased protein and protein:DNA (Reed et al., 2007). Maternal dietary restriction resulted in down-regulation of *GUCY1B3* in fetal jejunal tissue mRNA expression at d 135 of gestation (Neville et al., 2007). In the same study, maternal Se supplementation up-regulated *TEK* mRNA expression in fetal jejunum and nutrition × Se interaction was observed for *ANGPT2* where control-high Se and restricted-adequate Se were greatest, control-adequate Se intermediate and restricted-high Se lowest (Neville et al., 2007). Therefore, we have seen results due to Se and nutrient restriction on fetal hypertrophy, hyperplasia, and mRNA expression, but at 180 d of age these differences have been compensated for.

In summary, the observed differences in digestion and efficiency in offspring from nutritionally modulated mothers is not explained by jejunal morphology or those angiogenic factors measured. Small intestinal weight in offspring, however, was increased in ewes consuming high Se on moderate nutritional intakes. Furthermore, maternal nutrition during gestation alters total proliferating jejunal crypt cells in offspring at harvest.

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Table 1. Effect of maternal nutrition on DNA, RNA, and protein concentration and content of jejunum in offspring

| | Nutrition[1] | | | | Se[2] | | | *P*-Value[3] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | RES | CON | EXC | SEM | ASe | HSe | SEM | Nut | Se | Nut*Se |
| Jejunum, g | 395.3 | 419.7 | 411.6 | 17.8 | 408.0 | 409.7 | 13.5 | 0.49 | 0.93 | 0.31 |
| g/kg EBW[4, 5] | 8.71 | 8.85 | 9.15 | 0.39 | 8.99 | 8.82 | 0.29 | 0.67 | 0.67 | 0.48 |
| Small intestine, g[6] | 676.3 | 719.0 | 684.4 | 26.7 | 685.8 | 700.7 | 20.23 | 0.35 | 0.58 | 0.02 |
| g/kg EBW[4, 5, 7] | 14.9 | 15.1 | 15.1 | 0.5 | 15.0 | 15.0 | 0.4 | 0.89 | 0.94 | 0.09 |
| DNA, mg/g | 3.85 | 4.01 | 3.66 | 0.24 | 3.96 | 3.72 | 0.18 | 0.54 | 0.33 | 0.39 |
| DNA, g | 1.55 | 1.71 | 1.50 | 0.14 | 1.64 | 1.53 | 0.11 | 0.46 | 0.43 | 0.74 |
| RNA, mg/g | 7.05 | 6.21 | 6.21 | 0.98 | 6.44 | 6.54 | 0.74 | 0.70 | 0.91 | 0.10 |
| RNA, g | 2.78 | 2.66 | 2.50 | 0.44 | 2.60 | 2.70 | 0.33 | 0.88 | 0.82 | 0.32 |
| RNA:DNA | 2.00 | 1.60 | 1.78 | 0.31 | 1.69 | 1.90 | 0.23 | 0.54 | 0.50 | 0.23 |
| Protein, mg/g | 42.17 | 42.71 | 42.42 | 2.65 | 41.71 | 43.16 | 2.01 | 0.98 | 0.59 | 0.70 |
| Protein, g | 16.75 | 17.92 | 17.49 | 1.36 | 16.99 | 17.78 | 1.03 | 0.75 | 0.57 | 0.38 |
| Protein:DNA | 12.14 | 11.36 | 12.19 | 1.17 | 11.23 | 12.57 | 0.89 | 0.81 | 0.27 | 0.62 |

[1]Nutritional treatments were RES (60% of CON), CON (control; 100% requirements for gestating ewe lambs), and EXC (140% of CON).
[2]Selenium treatments were daily intake of organically bound Se; adequate Se (ASe; 9.5 µg/kg BW) vs. high Se (HSe; 81.8 µg/kg BW).
[3]Probability values for effects of nutrition (Nut), selenium (Se), and the interaction.
[4]Empty BW (EBW) = final BW – digesta weight.
[5]g/kg EBW = tissue mass (g)/Empty BW (EBW; kg).
[6]Interaction means for small intestine were 705.1, 662.9, 689.5, 647.6, 775.2, and 679.3 ± 39.7 g for RES-ASe, CON-ASe, EXC-ASe, RES-HSe, CON-HSe, EXC-HSe, respectively.
[7]Interaction means for small intestine, g/kg EBW were 15.5, 14.4, 15.2, 14.2, 15.8, and 14.9 ± 0.8 g/kg EBW for RES-ASe, CON-ASe, EXC-ASe, RES-HSe, CON-HSe, EXC-HSe, respectively.

Table 2. Effect of maternal nutrition on cellular proliferation and morphology of jejunal tissue in offspring

| | Nutrition[1] | | | | Se[2] | | | *P*-Value[3] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | RES | CON | EXC | SEM | ASe | HSe | SEM | Nut | Se | Nut*Se |
| Proliferating nuclei, % | 29.69 | 32.75 | 31.05 | 1.48 | 30.87 | 31.46 | 1.11 | 0.16 | 0.68 | 0.36 |
| Total cells, x $10^{11}$ | 2350.4 | 2585.8 | 2267.9 | 212.2 | 2487.1 | 2315.6 | 161.0 | 0.46 | 0.43 | 0.74 |
| Total cell prolif., x $10^{11}$ | 658.5[a] | 843.6[b] | 639.7[a] | 90.7 | 687.6 | 740.2 | 67.2 | 0.09 | 0.54 | 0.84 |
| Morphology | | | | | | | | | | |
| Crypt depth, µm | 296.83 | 289.88 | 285.08 | 16.88 | 292.86 | 288.34 | 12.37 | 0.82 | 0.77 | 0.39 |
| Villi width, µm | 273.21 | 277.98 | 304.25 | 15.03 | 286.58 | 283.71 | 11.01 | 0.19 | 0.83 | 0.29 |
| Villi length, µm | 451.95 | 445.16 | 429.09 | 27.40 | 436.79 | 447.35 | 18.85 | 0.80 | 0.69 | 0.88 |
| Angiogenic factors | | | | | | | | | | |
| *ANGPT1* | 0.0024 | 0.0023 | 0.0019 | 0.0005 | 0.0023 | 0.0021 | 0.0004 | 0.73 | 0.75 | 0.80 |
| *ANGPT2* | 0.0120 | 0.0135 | 0.0117 | 0.0028 | 0.0135 | 0.0113 | 0.0022 | 0.85 | 0.44 | 0.70 |
| *TEK* | 0.112 | 0.143 | 0.121 | 0.015 | 0.118 | 0.132 | 0.011 | 0.13 | 0.35 | 0.38 |
| *NOS3* | 0.207 | 0.235 | 0.216 | 0.039 | 0.220 | 0.219 | 0.030 | 0.81 | 0.99 | 0.20 |
| *GUCY1B3* | 0.219 | 0.226 | 0.219 | 0.050 | 0.232 | 0.211 | 0.038 | 0.99 | 0.67 | 0.83 |

[a,b]Within a row, means for nutritional treatment differ ($P \leq 0.08$).
[1]Nutritional treatments were RES (60% of CON), CON (control; 100% requirements for gestating ewe lambs), and EXC (140% of CON).
[2]Selenium treatments were daily intake of organically bound Se; adequate Se (ASe; 9.5 µg/kg BW) vs. high Se (HSe; 81.8 µg/kg BW).
[3]Probability values for effects of nutrition (Nut), selenium (Se), and the interaction.
[4]Total cells = total tissue DNA (mg) / (6.6 x $10^{-12}$ g DNA/cell) (Neville et al., 2008).
[5]Total cells proliferating = total cells x proliferating nuclei (Neville et al., 2008).

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# USE OF A SELF-FED, SMALL-PACKAGE PROTEIN SUPPLEMENT FOR BEEF COWS POST-WEANING[1]

**R. L. Endecott[2] and R. C. Waterman[3]**

[2]Montana State University, Miles City, MT
[3]USDA-ARS, Fort Keogh Livestock and Range Research Laboratory

**ABSTRACT:** A 60-d supplementation study conducted at Miles City, MT from mid-October to mid-December 2007 evaluated responses of beef cows (n = 141; avg BW = 535 kg) grazing dormant native range [8.8% CP (OM basis), 70.4% NDF (OM basis), 76.7% IVOMD] to 2 different supplementation strategies. Cows were stratified by age and weight at weaning and then assigned to one of two supplements: 1) self-fed loose mineral mix (**MIN**) or 2) self-fed mineral plus high-bypass protein sources (**MIN+PRO**; 50% mineral mix, 25% feather meal, 25% fish meal). Target intakes were 70 g/d for MIN and 140 g/d for MIN+PRO. Cows were weighed and hip height and girth measurements were taken at the beginning and end of the study. Weight-to-height and weight-to-girth ratio changes were calculated. Data were analyzed with cow age, supplement and their interaction in the model. Cows fed MIN consumed 28 g/d and MIN+PRO cows consumed 93 g/d, which was lower than the expected target amount for both supplements. Cow age × supplement interactions were not observed ($P \geq 0.22$). Weight change, weight-to-height ratio change, and weight-to-girth ratio change were similar regardless of cow age ($P \geq 0.79$). Cows lost similar ($P = 0.62$) amounts of weight during the study regardless of supplement treatment (-28 and -30 ± 4 kg for MIN and MIN+PRO, respectively). Likewise, weight-to-height ratio change (-0.31 and -0.32 ± 0.03) and weight-to-girth ratio change (-0.13 and -0.15 ± 0.02) were similar ($P \geq 0.63$) for MIN and MIN+PRO cows, respectively. Protein supplementation at this level did not impact cow performance. However, target intakes were not achieved, which may have contributed to the lack of response to supplementation with the mineral-protein mix.

Key Words: Beef Cows, Post-Weaning, Protein Supplementation

## Introduction

Low amounts of supplemental protein, particularly from sources high in ruminally undegradable protein (**RUP**), may enhance the efficiency of nitrogen utilization (Sawyer et al., 1998; Coomer et al., 1993). Further, nutrient restriction also increases the efficiency of nitrogen utilization in cows (Freetly and Nienaber, 1998). Supplement based on small quantities of high-RUP (> 70% of CP as RUP) ingredients combined with salt and minerals was demonstrated to maintain ruminal function with low quality forage diets (Sawyer et al., 2000) and was consumed in controlled and consistent patterns by cows grazing desert range (Stalker et al., 2002). In a 3-year field study in central New Mexico, gestating cows consuming a small-package, self-fed supplement (25% feather meal, 25% blood meal, and 50% mineral mix; < 250 g/d consumption) maintained BW and BCS during late fall and early winter, and had similar performance to cows hand-fed oilseed-based supplement at > 454 g/d (Sawyer et al., 2005). The objective of this study was to evaluate the effectiveness of a self-fed small-package supplement for maintaining BW of post-weaning beef cows grazing native range in the Northern Great Plains.

## Materials and Methods

A 60-d supplementation study was conducted at the Fort Keogh Livestock and Range Research Laboratory near Miles City, MT from mid-October to mid-December 2007. At this location, the potential natural vegetation is a grama-needlegrass-wheatgrass (*Bouteloua-Hesperostipa-Pascopyron*) mixed grass dominant. Average annual rainfall is 343 mm, with the majority occurring during the mid-April to mid-September growing season. Average precipitation compared to 2007 precipitation patterns by month is presented in Figure 1.

Hereford beef cows (n = 141; avg BW = 535 kg; ages 2 through 10 yr; ~135 d gestation) were stratified by age and BW at weaning and then randomly assigned to one of four pastures. Supplement treatments (n = 2) were then randomly assigned to each pasture resulting in 2 pastures per supplement treatment. Treatments consisted of: 1) self-fed loose mineral mix (**MIN**; Table 1) or 2) self-fed mineral plus high-bypass protein sources (**MIN+PRO**). The MIN+PRO supplement was formulated to contain 35% CP and was composed of 50% mineral mix, 25% feather meal, and 25% fish meal. The mineral portion of MIN+PRO was designed to provide the same level of mineral intake as cows receiving MIN. Target intakes were 70 g/d for MIN and 140 g/d for

[1]USDA-ARS, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Research was conducted under a cooperative agreement between USDA-ARS and the Montana Agric. Exp. Stn. Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, Montana Agric. Exp. Stn., or the authors and does not imply its approval to the exclusion of other products that also may be suitable. The authors gratefully acknowledge W. Kelly and S. Reil for their technical assistance.

MIN+PRO. Cows were weighed and hip height and girth measurements were taken at the beginning and end of the study. Weight-to-height and weight-to-girth ratio changes were calculated.

Diet quality was estimated from ruminal extrusa (Table 2). Extrusa samples were collected via ruminal evacuation techniques (Lesperance et al., 1960) at the beginning (mid-October) and end (mid-December) of the experiment. Four mature ruminally-cannulated cows that grazed in common with experimental cows were used for all diet sample collections (2 per pasture). Collected extrusa samples were lyophilized, ground to pass a 1-mm screen and stored until analysis for DM, OM (AOAC, 1990), and NDF (Goering and Van Soest, 1970). For CP analysis, sub-samples of ground extrusa were placed in glass square-bottom jars with metal rod inserts and dried in a 60°C oven. Upon removal from a drying oven, jars were capped with lids and subsequently placed on a roller grinder for 24 h (Mortenson, 2003). Nitrogen was determined by combustion techniques using a C-N analyzer (CE Elantech, Inc., Lakewood, NJ). Nitrogen values were multiplied by 6.25 to obtain CP.

At 0700 on the day of in vitro analyses, rumen extrusa (1/3 solids and 2/3 liquor) were collected at the interface of the forage mat and liquid fraction from 2 ruminally-cannulated cows on alfalfa hay diets and placed into a Dewar flask (Nalgene 4150-200, StevenJo & Steph, Rochester, NY) that had been incubated to 39°C for 24 h. Rumen extrusa in Dewar flask was immediately transported to the laboratory at Fort Keogh and forage (solids) were placed into a blender for 30 s. Once extrusa was blended, solids and remaining rumen liquor were strained through 4 layers of cheesecloth into a 6-L Erlenmeyer flask that had been pre-warmed in a 39°C water bath under continuous $CO_2$ flushing. Next, 500 mL of rumen liquor was measured out into a graduated cylinder and was then combined with 500 mL of pre-made phosphate buffer [70.8% $Na_2HPO_4$ and 29.2% $KH_2PO_4$; Menke et al., (1979)] and McDougal's buffer (Tilley and Terry, 1963) already in vessels of a DAISY$^{II}$ apparatus (ANKOM Technology Corp., Fairport, NY) maintained at 39°C. Vessels also contained samples [250 mg of sample/bag (F57; 5 × 5.55 $cm^2$, ANKOM Technology Corp, Fairport, NY)]. Vessels were purged with $CO_2$ for 30 s and a lid was secured onto the jar and immediately placed back into the DAISY$^{II}$ apparatus (process was repeated for each of two vessels). Samples were then subjected to in vitro incubation for 48 h at 39°C. At the end of 48 h, incubation bags containing samples were removed and rinsed under reverse-osmosis water until effluent was clear. In vitro organic matter disappearance (IVOMD) was calculated as the OM which disappeared from the initial OM weight inserted into the bag.

Data were analyzed as a completely randomized design by analysis of variance using the MIXED procedure of SAS (SAS Institute, Cary, NC) with pasture as the experimental unit. The model included cow age (2-yr-old, 3-yr-old, or 4-yr-old and older), supplement and their interaction as fixed effects and supplement(pasture) as a random effect.

## Results and Discussion

Forage quality and quantity are important factors that influence domestic rangeland livestock production. Forage CP concentrations were similar ($P = 0.26$) in mid-October and mid-December, which is not uncommon in the Northern Great Plains. However, CP concentrations were closer to meeting animal requirements than expected. Additionally, no differences ($P \geq 0.16$)) were observed between extrusa NDF and IVOMD concentrations in October and December. These results may have been influenced by low stocking rates used in the present study which allowed animals to select diets of higher quality throughout the 60-d study.

Cow age × supplement interactions were not observed ($P \geq 0.22$). Cows fed MIN consumed 28 g/d and MIN+PRO cows consumed 93 g/d, which was lower than the expected target amount for both supplements (70 and 140 g/d, respectively). Sawyer et al. (2005) fed a supplement similar to MIN+PRO (containing blood meal instead of fish meal) to prepartum cows, who consumed an average 230 g/d over a three-year study. Stalker et al. (2002) also reported higher intake (128 g/d) of a self-fed supplement similar to MIN+PRO than observed in the current study.

Weight change, weight-to-height ratio change, and weight-to-girth ratio change were similar regardless of cow age ($P \geq 0.79$; Table 3). Cows lost similar ($P = 0.62$) amounts of weight during the study regardless of supplement treatment (Table 4). Likewise, weight-to-height ratio change and weight-to-girth ratio change were similar ($P \geq 0.63$) for MIN and MIN+PRO cows, respectively. Sawyer et al. (2005) reported that cows fed a small-package (< 250 g/d) self-fed protein supplement maintained weight and body condition during late winter, while cows that were fed self-fed loose mineral supplement lost weight. These researchers reported that cows on the mineral-only treatment were also fed 454 g/d oilseed-based supplement during adverse weather. Lack of a response to protein supplementation in the current study might be due to less-than-target intake or to effects of low stocking rate on diet selection.

## Implications

Strategic protein supplementation with a small-package, self-fed supplement did not impact cow performance. However, target intakes of supplement were not achieved, which may have contributed to the lack of response to supplementation with the mineral-protein mix. In addition, cows were able to select a higher quality diet due to low stocking rates, which may have influenced their voluntary supplement intake. Further research identifying liming nutrients in range forages and the use of strategic small-package supplementation may be beneficial to optimize range livestock production.

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Table 1. Composition of self-fed loose mineral supplement

| Item | % |
|---|---|
| Calcium | 12.0 |
| Phosphorus | 12.0 |
| Salt | 27.0 |
| Sodium | 10.0 |
| Magnesium | 1.5 |
| Potassium | 0.1 |
| | ppm |
| Copper | 1,200 |
| Manganese | 4,000 |
| Iodine | 100 |
| Selenium | 25 |
| Zinc | 2,500 |
| | IU/kg |
| Vitamin A | 330,000 |
| Vitamin D | 39,600 |
| Vitamin E | 220 |

Table 2. Crude protein and neutral detergent fiber concentration and in vitro organic matter disappearance of rumen extrusa samples at the start (mid-October) and end (mid-December) of supplementation from experimental pastures

| | Extrusa Collection | | |
|---|---|---|---|
| Item | Start | End | SE |
| CP %, OM basis | 9.2 | 8.4 | 0.47 |
| NDF %, OM basis | 70.0 | 70.8 | 1.34 |
| IVOMD, % | 76.7 | 79.4 | 4.83 |

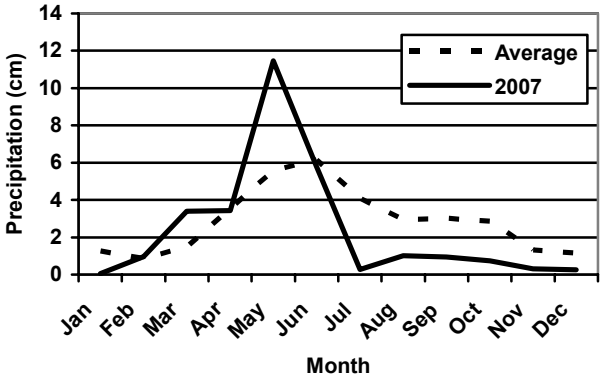


Figure 1. Average annual precipitation (30-yr) and 2007 precipitation by month for Miles City, MT (NOAA, 2007).

Table 3. Weight change, weight-to-height ratio change, and weight-to-girth ratio change by cow age

| | Cow Age | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | 2 | SE | 3 | SE | ≥ 4 | SE | *P*-value |
| | n = 27 | -- | n = 18 | -- | n = 96 | -- | -- |
| Weight change, kg | -28 | 5 | -31 | 6 | -28 | 3 | 0.85 |
| Weight-to-height ratio change | -0.31 | 0.04 | -0.32 | 0.05 | -0.32 | 0.02 | 0.93 |
| Weight-to-girth ratio change | -0.12 | 0.03 | -0.15 | 0.04 | -0.14 | 0.02 | 0.79 |

Table 4. Supplement impacts on weight change, weight-to-height ratio change, and weight-to-girth ratio change

| | Supplement | | | |
|---|---|---|---|---|
| Item | MIN | MIN+PRO | SE | *P*-value |
| Weight change, kg | -28 | -30 | 4 | 0.62 |
| Weight-to-height ratio change | -0.31 | -0.32 | 0.03 | 0.80 |
| Weight-to-girth ratio change | -0.13 | -0.15 | 0.02 | 0.63 |

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# ENERGY VALUE OF CORN AND BARLEY IN BEEF FINISHING DIETS

**A. V. Grove, J. A. Cookson, T.K. Blake, and J.G.P. Bowman**
Montana State University, Bozeman 59717

**ABSTRACT:** Eighteen feedlot studies were conducted at 2 locations (Bozeman and Havre, MT) from 1997 to 2006 in order to evaluate the performance, nutrient digestibility, and grain energy content of finishing diets based on corn or barley. For each study, 80 Angus crossbred steers were allotted by weight to 16 pens. Grains were dry rolled prior to being fed and diets were formulated to be isonitrogenous (2.3% N). Diets were formulated (DM basis) to contain 80 to 83% grain, 6% straw, 3% soybean oil, and 8 to 11% supplement. Steers were weighed on 2 consecutive days at the beginning and end of the 84 to 159-d studies. Diet, ort, and fecal samples were collected from individual steers and composited by pen every 28 d. Acid insoluble ash was used as an internal marker to estimate fecal output and calculate apparent nutrient digestibility. Steers were slaughtered when 70% were visually estimated to grade Choice and carcass measurements were collected after a 24-h chill. Data from all trials were combined and analyzed using the GLM procedure of SAS with pen as the experimental unit. Treatment (corn or barley), year, and location were included in the model. Dry matter intake did not differ ($P$ = 0.77) between cattle fed corn and barley finishing diets, averaging 10.4 kg/d. Corn-fed cattle had higher ($P < 0.10$) ADG and G:F than cattle fed barley (1.71 vs. 1.66 kg/d ADG and 16.5 vs. 16.1 kg gain/100 kg feed). There was no difference ($P > 0.34$) in grain $NE_m$ or $NE_g$ between corn and barley, averaging 2.29 and 1.61 Mcal/kg, respectively. Digestible intake of DM, starch, N and ADF was greater ($P < 0.08$) by cattle fed corn- than barley-based finishing diets. Corn-fed cattle had greater ($P < 0.08$) HCW, KPH, $12^{th}$-rib fat thickness, and yield grade than barley-fed cattle; however, LM area, marbling score, quality grade, and carcass value did not differ ($P > 0.83$) between treatments. Percent retail cuts was greater ($P = 0.04$) from barley- than corn-fed carcasses (49.8 vs. 49.5%). Greater intakes of digestible nutrients by corn-fed cattle appeared to be directed toward external fat deposition. Net energy values of corn and barley grain were similar.

Key Words: barley, beef, carcass traits, corn, energy value

## Introduction

Barley is commonly fed in finishing rations in Canada and throughout the Pacific Northwest; however, corn accounts for 80% of the energy grains fed to cattle (USITC, 2000) and it is widely believed that corn has superior feeding value compared to barley. Historically, Morrison (1956) reviewed 21 research trials conducted between 1887 and 1955 and stated that ground barley was worth 88% the value of shelled corn for fattening cattle, in spite of the fact that gains were similar between cattle fed corn or barley. NRC (1984, 1996) has also assigned higher energy values to corn than barley; however, Owens et al. (1997) reviewed finishing studies after 1974 and reported similar ME for barley and corn (3.42 Mcal/kg). These values were higher than NRC (1996) estimates for barley, but closer to NRC estimates for corn. The objective of the current study was to evaluate the performance, nutrient digestibility, and grain energy content of finishing diets based on corn or barley.

## Materials and Methods

Eighty Angus crossbred steers were allotted by weight to 16 pens with 4 pens per treatment at 2 locations: Bozeman and Havre, MT from 1997 to 2006. Steers were fed finishing rations based on corn or various barley varieties. Grains were dry rolled prior to being fed and diets were formulated to be isonitrogenous (2.3% N). Diets were formulated (DM basis) to contain 80 to 83% grain, 6% straw, 3% soybean oil, and 8 to 11% vitamin/mineral supplement. Animals were cared for under protocols approved by the Montana State University Animal Care and Use Committee.

Steers were weighed on 2 consecutive days at the beginning and end of the 84 to 159-d studies. Steers were fed once daily at 0800 and were given ad libitum access to water. Steers were gradually brought up to ad libitum intake of their respective treatment diets over 28 days. Steers were implanted in 9 trials. Diet, ort, and fecal samples were collected from individual steers and composited by pen every 28 d. Diet and fecal samples were dried in a 60° C forced-air oven, ground through a Wiley mill (1-mm screen), and analyzed for DM, (AOAC, 1999), N (Leco Corporation, St. Joseph, MI), ADF (Van Soest et al., 1991), starch (Megazyme, Sidney, Australia), and AIA (4N HCl method; Van Keulen and Young, 1977). Acid insoluble ash was used as an internal marker to estimate fecal output and calculate apparent nutrient digestibility. Grain energy content ($NE_m$ and $NE_g$) was calculated based on steer average weight, DMI, and ADG using NRC (1984) equations. Carcass value was calculated using the quality grade based grid with a $10 Choice-Select spread and other pricing used by Tatum et al. (2006).

Steers were slaughtered when 70% were visually estimated to grade Choice, and hot carcass weights were collected. All other carcass measurements were taken after a 24-h chill. A USDA grader assigned quality grades.

Data from all trials were combined and analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. Grain (corn or barley), year, and location were included in the model.

Least square means were separated using the Least Significant Difference method when $P < 0.10$.

## Results

Dry matter intake was similar ($P = 0.77$) between cattle fed corn and barley-based finishing diets averaging 10.4 kg/d (Table 1); however, corn-fed cattle had higher ($P = 0.08$) ADG than cattle fed barley (1.71 vs. 1.66 kg/d ADG). This relationship resulted in greater ($P = 0.10$) G:F by cattle fed corn compared to cattle fed barley-based finishing diets (16.5 vs. 16.1 kg gain/100 kg feed).

There was no difference ($P > 0.34$) in grain $NE_m$ or $NE_g$ between corn and barley, averaging 2.29 and 1.61 Mcal/kg, respectively. The range in $NE_m$ was 1.90 to 2.62 Mcal/kg for corn and 1.95 to 2.93 Mcal/kg for barley. The range in $NE_g$ was 1.25 to 1.89 Mcal/kg for corn and 1.29 to 2.17 Mcal/kg for barley.

Corn-fed cattle had greater ($P < 0.001$) starch and N intake but lower ($P = 0.002$) ADF intake compared to barley-fed cattle. Corn-based finishing diets had higher ($P < 0.01$) DM digestibility (78.0 vs. 75.3%) and ADF digestibility (53.1 vs. 21.8%) compared to barley-based finishing diets, while N digestibility was similar ($P = 0.92$) between diets averaging 75.7%. Total tract starch digestibility was greater ($P < 0.001$) for barley compared to corn-based finishing diets. Digestible intake of DM, starch, N, and ADF was greater ($P < 0.08$) by steers fed corn vs. barley-based finishing diets.

Corn-fed cattle had greater ($P < 0.08$) HCW, KPH, $12^{th}$-rib fat thickness, and yield grade compared to cattle consuming barley-based finishing diets; however, LM area, marbling score, and quality grade did not differ ($P > 0.87$) between treatments. Percent retail cuts was greater ($P = 0.04$) from carcasses of steers fed barley compared to corn. Carcass value did not differ ($P = 0.83$) between treatments, averaging $941.70/carcass.

## Discussion

The increase in total tract starch digestibility by steers consuming barley did not appear to impart any energetic advantages to these cattle. Extra digestible nutrients consumed by corn-fed cattle appeared to be directed toward external fat deposition ($12^{th}$-rib fat thickness, KPH) which may be responsible for higher HCW and yield grades compared to barley-fed cattle. Boss and Bowman (1996) reported that there was a linear relationship between ADG and digestible starch intake ($R^2 = 0.37$), but not between ADG and digestible DM intake.

NRC (1996) reported that $NE_m$ and $NE_g$ for corn (cracked, grain) was 2.24 and 1.55 Mcal/kg, respectively, while $NE_m$ and $NE_g$ for barley (heavy) was 2.06 and 1.40 Mcal/kg, respectively. Our NE values for barley grain were 11 and 14% higher than that reported by NRC (1996) while our net energy values for cracked corn were only 3 and 4.5% higher than NRC estimates. Similar to NRC (1996), early reports suggested that barley contained slightly less TDN than corn (Morrison, 1956). However, in agreement with our data, Owens et al. (1997) reported similar ME for barley and corn grains which converts to 2.37 Mcal/kg $NE_m$ and 1.66 Mcal/kg $NE_g$. These values are higher than ours and therefore also higher than NRC (1996) estimates. Energy values for feedstuffs reported by NRC (1996) are based on lab analysis of TDN which does not take into consideration energy losses due to urine, combustible gases, and heat (Jurgens, 1988). Differences in the type of energy estimated could explain differences in energy values that we and others obtained.

## Implications

Corn-fed cattle had higher digestible intake of all nutrients which appeared to be partitioned toward external fat deposition contributing to slightly higher ADG, G:F, HCW, KPH, $12^{th}$-rib fat thickness, and yield grade. Barley-fed cattle had higher total tract starch digestibility and percentage of retail cuts; however, there was no difference in LM area, marbling, quality grade, or carcass value between finishing diets. The National Research Council reports lower energy values for barley than corn; however, our data suggests that there were no differences in net energy value between corn and barley grain.

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Table 1. Nutrient intake and digestibility, animal performance, and carcass characteristics of steers fed finishing diets based on corn or barley at 2 locations over 10 years

| | Corn | Barley | SE | *P*-value |
|---|---|---|---|---|
| Feedlot performance | | | | |
| DMI, kg/d | 10.3 | 10.4 | 0.09 | 0.77 |
| ADG, kg/d | 1.71 | 1.66 | 0.016 | 0.08 |
| G:F, kg gain/100 kg feed | 16.5 | 16.1 | 0.15 | 0.10 |
| Calculated energy content | | | | |
| Diet $NE_m$, Mcal/kg | 2.19 | 2.16 | 0.013 | 0.22 |
| Diet $NE_g$, Mcal/kg | 1.51 | 1.49 | 0.012 | 0.22 |
| Grain $NE_m$, Mcal/kg | 2.30 | 2.28 | 0.016 | 0.52 |
| Grain $NE_g$, Mcal/kg | 1.62 | 1.60 | 0.014 | 0.35 |
| Intake, kg/d | | | | |
| Starch | 6.1 | 4.8 | 0.08 | <0.001 |
| N | 0.25 | 0.23 | 0.003 | <0.001 |
| ADF | 0.89 | 0.97 | 0.016 | 0.002 |
| Digestibility, % | | | | |
| DM | 78.0 | 75.3 | 0.57 | 0.002 |
| Starch | 91.5 | 95.0 | 0.36 | <0.001 |
| N | 75.7 | 75.6 | 0.70 | 0.92 |
| ADF | 53.1 | 21.8 | 1.72 | <0.001 |
| Digestible Intake, kg/d | | | | |
| DM | 8.0 | 7.8 | 0.09 | 0.07 |
| Starch | 5.6 | 4.6 | 0.08 | <0.001 |
| N | 0.19 | 0.18 | 0.003 | <0.001 |
| ADF | 0.54 | 0.21 | 0.021 | <0.001 |
| Carcass characteristics | | | | |
| HCW, kg | 326 | 322 | 1.2 | 0.07 |
| KPH, % | 2.1 | 2.0 | 0.02 | 0.01 |
| $12^{th}$-rib fat thickness, cm | 1.24 | 1.16 | 0.023 | 0.02 |
| LM area, $cm^2$ | 75.2 | 75.1 | 0.46 | 0.92 |
| Marbling score | 454 | 453 | 6.0 | 0.95 |
| Quality Grade[1] | 12.1 | 12.1 | 0.06 | 0.88 |
| Yield Grade | 3.2 | 3.0 | 0.04 | 0.04 |
| Retail cuts, % | 49.5 | 49.8 | 0.09 | 0.04 |
| Carcass value, $ | 942.69 | 940.71 | 5.804 | 0.83 |

[1] 11= Select, 12 = Choice$^-$, 13 = Choice, 14 = Choice$^+$

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# SORGHUM SILAGE DIGESTIBILITY IN SHEEP[1]

G. M. Abdelrahim [*2], C. Melton[†], V. Allen[†], and K. Pond[‡];

[*]Department of Food and Animal Sciences, Alabama A & M University, Normal, AL, 35762;
[†]Department of Animal and Food sciences, Lubbock, TX 79409

**ABSTRACT**: A metabolism trial was conducted to investigate the impact of feeding four types of protein supplemented silages on lambs′ performance. Diets used in the trial were silages of corn, Brown midrib-100 (bmr-100), Fame, and Cow Vittles II (CVII). The three types of sorghum and corn were grown at Texas Tech university farm. Supplements used were cottonseed with cottonseed meal, coated cottonseed, or uncoated cottonseed. During the trial twenty four lambs were used. Lambs were housed in individual false-bottom metabolism stalls for total collection of urine and feces. Data were analyzed as a randomized complete block design with a 3 x 4 factorial arrangements of treatments. Body weight was used as the blocking criterion. Our results shows that DMI, nitrogen intake, dry matter apparent digestibility (DMD), fecal excretion (FE), Nitrogen (N) apparent absorption, hemicellulose apparent digestibility were greater ($P$<0.05) for bmr-100 than for the mean of Fame, and CVII. DMD, N FE, N apparent absorption, NDF, ADF, and the apparent digestibility for lignin, hemicellulose, and ash were greater ($P$<0.05) for Fame than for CVII. Interactions of silage and supplement were detected ($P$<0.05) when we measured dry DMD, NDF, ADF, lignin digestibility, and ash apparent digestibility. Magnesium (Mg) consumption, FE, and apparent absorption were greater ($P$<0.01) for bmr-100 than for the mean of Fame and CVII, and were greater ($P$<0.01) for CVII than for Fame. Phosphorus (P) intake and retention were greater ($P$<0.01) for bmr-100 than for the mean of Fame and CVII. Calcium (C) intake, apparent absorption, apparent retention, and FE were higher ($P$<0.01) for bmr-100 than for the mean of Fame and CVII. We concluded that bmr-100 was advantageous when it comes to the amount of DM, N, Mg, P, and C it provided compared to the mean of Fame and CVII. Also we conclude that hemicellulose apparent digestibility was greater ($P$<0.05) for Fame than for CVII, and it was higher for corn silage ($P$<0.01) than for the mean of sorghum silages.

**Keywords**: Digestibility, Silage, Sorghum

[1]Supported by the Brunette Center at Texas Tech University
[2]Corresponding author: gamal.abdrahim@aamu.edu, phone: 256- 372-4164; fax: 256 372-5432

## Introduction

Sorghum, the fifth most important cereal grain in the world after wheat, rice, maize, and barley, is a hardy drought- resistant crop adapted to environmental conditions too harsh for the production of corn. It requires less water than corn and can survive dry conditions and then resume growth when moisture becomes available (Dahlberg, 1993).

Sorghum is produced in the United States predominantly on the southern Great Plains, although it is grown in over 30 states. In the United States and in other countries, sorghum is valued because of its ability to produce in areas with marginal rainfalls and high temperatures where other cereals often fails, and because of its relatively short growing season requirement- thus its suitability for double-cropping and rotation systems (Wayne Smith and Frederickson, 2000).

The United States exports from a third to a half of its grain sorghum production. Five states, Kansas, Texas, Oklahoma, Nebraska, and Missouri- produce about 90% of the grains in the United States (Wayne Smith and Frederickson, 2000) Digestibility of NDF is an important parameter of forage quality. Fibrous fractions of feed ferment slowly and are retained in the rumen longer than nonfibrous fractions of feeds. Because physical fill in the rumen often limits maximum DMI, faster disappearance of the NDF fraction from the rumen because of increased rate of digestion or passage may reduce physical fill in the rumen over time and allow greater voluntary feed intake (6). In addition, more digestible fiber might increase the energy density of diets and microbial N production (Oba and Allen, 2000).

Brown midrib sorghum silage (brmSS) was compared with alfalfa, corn, and normal sorghum silages for its effect on performance, ruminal metabolism, and digestive kinetics of Holstein dairy cows in midlactation (Grant et al., 1995).

Brown midrib (bmr) forage genotypes typically contain less lignin and may have altered lignin composition and cross-linking with cell wall carbohydrates, resulting in improved NDF digestibility (Vogel and Jung, 2001). In vitro and in situ digestion studies have shown that bmr forage sorghum and bmr sorghum-sudangrass hybrids have a greater extent of NDF digestion than their conventional counterparts (Fritz et al., 1990; Grant et al., 1995; Dann et al., 2008).

## Materials and Methods

*Animals, Facilities, and Diets*. The study was conducted at the Burnett Center in New Deal, Texas. The procedures were approved by Texas Tech University Animal Care and Use Committee. A total of twenty-four cross breeds wether lambs weighing 32.27±2.08 kg were housed in individual false-bottom metabolism stalls for total collection of urine and feces. Lambs had free access to freshwater and were fed silages based diet at 1.75 % of BW (DM basis). Water-soluble carbohydrates were determined on postensiled forage. The diet was divided into two equal portions and fed twice daily at 0800 and 1700.

*Design and Treatments.* The experiment was a randomized block design and lasted 28 d, which allowed 7 d for adjustment period in the stalls, and 7 d for adaptation to diets and dietary treatments, followed by 7 d as preliminary period, and 7 d for collections. Treatments, in a 3 × 4 factorial arrangements, were four types of silages (corn and three types of sorghums including bmr-100, Fame, and Cow Vittles II (CVII), and three protein supplements including cottonseed with cottonseed meal, coated cottonseed, or uncoated cottonseed.

*Collections*. Feed refusals were collected every morning. At the end of the collection period ruminal fluids samples were collected via stomach tube four hours after feeding and were acidified with $H_3PO_4$. Blood samples were collected via jugular vein puncture six hours after feeding at the conclusion of the trial. Samples of feed, feces, and refusals were dried and ground.

*Samples Analysis.* Samples were analyzed for neutral detergent fiber, acid detergent fiber, hemicelluloses, cellulose, lignin and ash. Total nitrogen was determined in feeds, refusals, feces, and urine samples by micro-Kjeldahl method.

*Statistical Analysis:* All data were analyzed using SAS (SAS Inst. Inc., Cary, NC). Initial body weight was used as the blocking criterion. The model statement tested for effects of treatment, block and silages×supplement interaction.

Treatment comparisons were made using the following orthogonal contrasts: 1) corn silage versus the mean of sorghum silages, 2) bmr-100 versus the mean of Fame and CVII, 3) CVII versus fame, and 4) Interaction.

## Results and Discussion

In agreement with previous research (Aydin et al., 1999; Oliver et al., 2004) our results shows that DMI, DMD, FE were greater ($P$<0.05) for bmr-100 than for the mean of Fame, and CVII (Figure 1).

Results of the current study shows DMD, NDF, ADF, and the apparent digestibility for lignin, hemicellulose, and ash were greater ($P$<0.05) for Fame than for CVII (Figure 2). These results are in agreement with previous data by Dann et al. (2008) who reported that the 30- and 48-h in vitro NDF digestibility for the bmrSS was approximately 10 percentage units greater than for the CS hybrid, and by Aydin et al., (1999) who observed no significant difference in total tract NDF digestibility between bmr

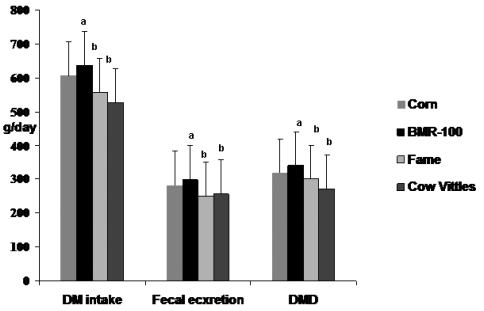


Figure 1. Dry matter intake, dry matter digestibility, and fecal excretion by wethers fed corn and sorghum silages. [a,b]Means without common superscript letters differ ($P$ < 0.05).

forage sorghum and CS. Furthermore, the current study shows hemicellulose apparent digestibility were greater ($P$<0.05) for bmr-100 than for the mean of Fame, and CVII (Figure 2).

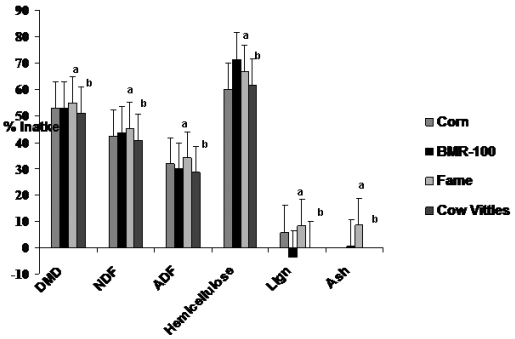


Figure 2. Apparent digestibility of dry matter, crude protein and fiber components of corn and three sorghum silages fed to wethers. [a,b]Means without common superscript letters differ ($P$ < 0.05).

In the current study, N intake and apparent absorption were found to be greater ($P$<0.05) for bmr-100 than for the mean of Fame, and CVII (Figure 3). The results also shows that N FE, and apparent absorption were greater ($P$<0.05) for Fame than for CVII (Figure 3). There is no available data to which we can compare our results; however, previous data (Oba and Allen, 2000) shows that apparent total tract digestibility of N was greater for high NDF diets contained brown midrib hybrid corn silage.

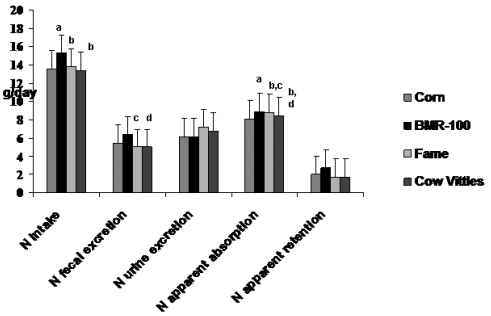


Figure 3. Nitrogen balance in wethers fed corn and sorghum silages. [a,b,c,d]Means without common superscript letters differ ($P$ < 0.05).

In addition to intake and absorption of Mg, and Ca, and intake of P, our results shows that fecal excretion for Mg and C; and retention for P and C, were greater ($P$<0.01) for bmr-100 than for the mean of Fame and CVII, and

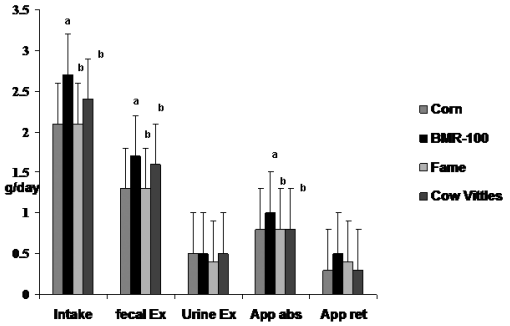


Figure 4. Magnesium balance in wethers fed corn and three sorghum silages. [a,b]Means without common superscript letters differ ($P < 0.05$).

were greater ($P<0.01$) for CVII than for Fame (Figures 4, 5, 6). These results agrees with previous data on P (Oliver et al., 2004) where total tract apparent phosphorus digestibility was improved significantly for bmr-6 forage sorghum compared with the CS hybrid evaluated.

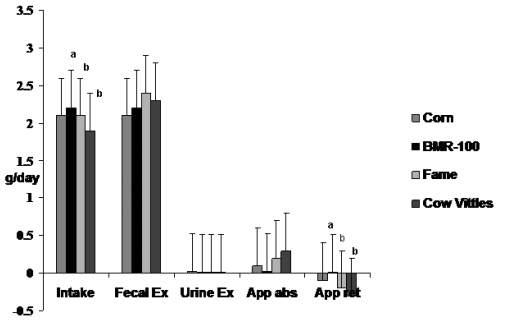


Figure 5. Phosphorous balance in wethers fed corn and three sorghum silages. [a,b]Means without common superscript letters differ ($P < 0.05$).

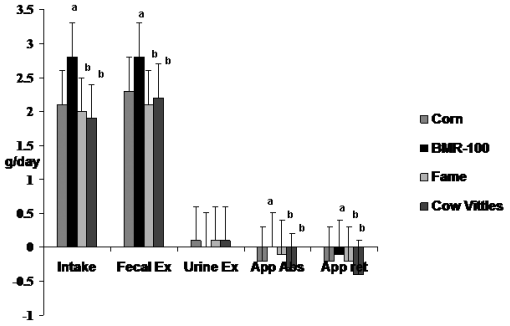


Figure 6. Calcium balance in wethers fed corn and three sorghum silages. [a,b]Means without common superscript letters differ ($P < 0.05$).

## Implications

We concluded that bmr-100 was advantageous when it comes to the amount of DM, N, Mg, P, and C it provided compared to the mean of Fame and CVII. Also we conclude that hemicellulose apparent digestibility was greater ($P<0.05$) for Fame than for CVII, and it was higher for corn silage ($P<0.01$) than for the mean of sorghum silages. However, research is needed to compare bmrSS hybrids with other common forages and to develop alternatives to corn silage in regions or situations where corn is less agronomically suitable.

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# EFFECTS OF ELEVATED DIETARY NITRATE ON PRODUCTION AND REPRODUCTION PARAMETERS IN SUFFOLK EWES

**R. Cockrum[1], K. Austin, P. Ludden, K. Cammack**
Department of Animal Sciences, University of Wyoming, Laramie, 82072

**ABSTRACT:** High dietary nitrate has a significant economic impact on livestock production worldwide due to both chronic and acute effects. Nitrate toxicity is often observed in cattle and sheep populations during periods of drought when forage crops become toxic. In ruminants, nitrate is converted to nitrite in the rumen and reduced to ammonia. The conversion of nitrate to nitrite exceeds the reduction process when ruminants consume high nitrate forages. Nitrite ($NO_3^-$) is then absorbed into the blood and binds with hemoglobin, reducing the ability of blood to carry oxygen to peripheral tissues. Symptoms include decreased feed efficiency, reproductive complications, weight loss, and even death. The purpose of this study was to confirm individual variation in response to subacute levels of dietary nitrate and identify those individuals more and less tolerant respectively to elevated dietary nitrate. Purebred Suffolk ewes were administered a $KNO_3$ supplement (300 mg $NO_3^-$/kg BW daily; n = 47) or control supplement (n = 8) for an 8 d period. Six nitrate tolerant and six nitrate intolerant ewes were identified based on performance and behavior traits. Supplement intake was lower ($P < 0.0001$) in nitrate-treated ewes than in control ewes, indicating elevated dietary nitrate influences feed intake. Supplement intake was not different in control and tolerant ewes, but was lower ($P < 0.0001$) in intolerant ewes. The average supplement intake of tolerant and intolerant ewes was 84% and 24%, respectively. Weight change and plasma nitrite were not different ($P > 0.05$) between control, tolerant, and intolerant ewes. Plasma urea nitrogen values were not different between control, nitrate tolerant, or nitrate intolerant ewes, but control ewes were numerically higher than tolerant and intolerant ewes ($P > 0.05$). Eight nitrate-treated ewes gave birth to either open or dead fetuses. These results show that individual animal performance differs in response to elevated dietary nitrate and that reproduction may be affected by short-term exposure to subacute levels of nitrate prior to breeding.

Key Words: Nitrate, performance, reproduction, sheep

## Introduction

More than $340,000,000 are lost annually by western United States producers due to livestock consumption of toxic plants (Nielsen and James, 1992). Death rates from rapid consumption of high nitrate forage (224 - 547 mg $NO_3^-$/kg BW in sheep) range from 7 - 44% death loss in affected cattle and up to 42% death loss in affected sheep (Harris and Rhodes, 1969). However, sheep have a higher tolerance to nitrate due to their ability to increase the concentration of red blood cells in the blood. While nitrate itself is not toxic to livestock, ingested nitrate is converted to nitrite by rumen bacteria. Once absorbed into the blood, nitrite combines with hemoglobin forming methemoglobin, thereby reducing the ability of red blood cells to transport oxygen, resulting in suffocation (Koch and Paisley, 2002). The transfer of rumen nitrite into the bloodstream is influenced by nitrate intake, rate of feed digestion and subsequent nitrate release, rate of nitrite reduction to ammonia, and absorption of nitrite from the rumen. Symptoms of acute toxicity include brown or black mucous membrane discoloration, respiratory distress, coma, cyanosis, and possibly death (Rogers, 1995). Chronic nitrate toxicity can result in lethargy, head pressing, and impaired animal production as evidenced by depressed appetite, reduced or no weight gain, lowered milk production, and increased susceptibility to infection (Yaremcio, 1991). Nitrate toxicity can also result in reproductive failure due to hormonal imbalances, reduced implantation rate, and impaired sperm quality (Yaremcio, 1991; Zraly et al., 1997). Pregnant females often abort their fetuses when exposed to elevated nitrate levels while not displaying outward symptoms of nitrate poisoning. The long-term and short-term effects of chronic nitrate toxicity have received little research attention, resulting in frequent misdiagnosis in livestock operations and incorrect or lack of appropriate treatment. Variation in susceptibility to toxicity can be partially attributed to the rate and duration of exposure as well as individual tolerance and metabolism levels. In this study, animals that are more or less tolerant to subacute levels of nitrate intake are identified based on supplement intake, weight gain, and demonstration of chronic nitrate toxicity symptoms.

## Materials and Methods

Purebred Suffolk ewes (n = 60; initial average BW = 85.7 ± 46.4 kg) were randomly allocated to one of two project start days (contemporary groups) due to time and labor limitations. Within each contemporary group, ewes were randomly allocated to a control (n = 5) or elevated nitrate (n = 25) diet. The basal diet consisted of bromegrass hay fed at 2.5% of initial BW. A supplement (11.5% total dietary CP) consisting of (92% DM basis) 53.9% soybean meal, 28.7% beet pulp, 10.0% molasses, and 7.4% of vitamins/minerals was fed three times per day (125 g/feeding, as fed basis). Weights were taken on d 1, d 3, d 8 of the trial, as well as 4 d after completion of the study.

Liver biopsies were performed on d 3 or 4 and again at the end of the study using modified procedures of Ferreira (1996) to accommodate larger sample extraction. A surgical area of approximately 16 $cm^2$ was sheared and

sterilized with Lugol's iodine solution (2% iodine, 4% K+ iodide). A local anesthetic of 8cc of 2% injectable Lidocaine (VEDCO, St. Joseph, MO) was administered topically and subcutaneously around the incision area. A 1 cm incision was made with a #22 scalpel blade between the intercostal space of the $10^{th}$ and $11^{th}$ ribs approximately 9 cm below the processus spinosus. A bone marrow biopsy punch (Jorgensen Vet Supply, Loveland, CO) modified with a syringe and tubing was inserted vertically through the intercostal space. The liver was penetrated approximately two to four times with suction to obtain a 1 g sample. Tissue was rinsed with PBS, snap frozen in dry ice, and stored at -80°C. Incisions were sutured and cleaned with Lugol's iodine solution, and ewes were given 3cc of penicillin for precaution against infection. An 11% death rate typically occurs in sheep when performing liver biopsies as opposed to < 0.5% in cattle (Anderson et al., 1962). A total of 121 biopsies were performed in this study, with a 7% death loss due to complications. The RNA obtained from the liver biopsies will be used to conduct microarray analyses to determine differentially expressed genes between control, nitrate tolerant, and nitrate intolerant ewes. Expression levels of differentially expressed genes will be confirmed using real-time RT-PCR.

Following the initial biopsies, ewes were randomly assigned to one of two nitrate treatments consisting of 0 (control) or 300 mg supplemental $NO_3^-$/kg BW/d. As a precautionary measure, a mixture of 1 – 4% aqueous solution in saline of methylene blue was prepared which was to be injected intravenously based on 5 – 20mg/kg LW basis. Blood was drawn for analyses at the time of biopsy, 12 h after nitrate exposure, every 24 h for the remaining 8 d of the trial, and 4 d after the cessation of treatment. Blood was drawn through the jugular vein and mixed with heparin to prevent clotting. All samples were immediately mixed and put on ice for 1 hour. Samples were then centrifuged for 20 minutes at 1520 × g after which plasma was obtained and stored at -20°C for future analyses.

Nitrate levels in the bromegrass hay and supplement were analyzed using a Standard Range Lab Nitrate Test Kit (L-NTK; NECi, Lake Linden, MI). Plasma samples were also tested for nitrite levels using the same kit with the omission of the nitrate reductase and NADH reagents from the assay. Plasma urea nitrogen (PUN) levels were confirmed using a QuantiChrom™ Urea Assay Kit (DIUR-500; BioAssay Systems, Hayward, CA).

Data were analyzed using the GLM procedures of SAS (SAS Inst., Inc., Cary, NC). Mean separation of least-squares means was performed using an LSD assuming an alpha level of 0.05.

## Results and Discussion

Under normal conditions, soil nitrate is absorbed by plant roots and then reduced to amino acids. During periods of drought, nitrate accumulates in the plant, as high temperatures inhibit the conversion of nitrate to amino acids and during severe drought conditions, plants do not absorb nitrate due to the lack of moisture. However, eventual moisture leads to rapid absorption of nitrate by plants. Ingested nitrate is converted to nitrite by rumen bacteria and reduced to ammonia. The excess nitrite in the blood forms methemoglobin resulting in a conversion of the ferrous ion of hemoglobin to the ferric form; thereby, reducing the ability of blood to carry oxygen to the body (Yaremcio, 1991). Chronic and acute symptoms of nitrate toxicity may appear when > 20% of hemoglobin is converted to methemoglobin.

Nitrate levels in bromegrass hay and basal supplement (without added nitrate) were < 4 ppm. There was no effect ($P > 0.10$) of ewe age on supplement intake. Based on production and reproduction results treated groups were selected for nitrate tolerant and intolerant ewes (n = 6) and were further analyzed for weight, supplement intake, nitrite plasma levels, and PUN's. These results were compared to control ewes (n = 6) that did not have nitrate in their diet. All samples will be analyzed for differential expression of genes by microarray in the future. Supplement intake ($P < 0.0001$; Figure 1) differed between control and $NO_3^-$ treated ewes and also between the two contemporary groups ($P < 0.001$; Figure 2). There was no difference in supplement intake ($P > 0.10$) between control ewes and tolerant ewes (Figure 3); however, intake was lower ($P < 0.0001$) in intolerant ewes compared to control and tolerant ewes (Figure 3). Intolerant ewes had a decrease in feed intake, weight, and were noticeably more lethargic than tolerant and control ewes. There was no effect ($P > 0.10$) of nitrate on weight change (Figure 4). Plasma nitrite levels were not significantly different between nitrate treated and control ewes, and did not differ throughout the trial (Figure 5). Control ewes had numerically greater PUN than tolerant ewes and intolerant ewes, but no differences in PUN's between the selected groups were detected ($P > 0.05$; Figure 6). Reproductive performance of ewes was recorded during the breeding and lambing season. Eight nitrate-treated ewes were either open or gave birth to dead fetuses at parturition.

Cattle and sheep are two ruminant livestock species that are particularly affected by nitrate toxicity. Both species exhibit similar symptoms in response to high levels of nitrate in forage. However, sheep have a higher tolerance to nitrate due to the ability to increase the concentration of red blood cells in the blood. The similar symptoms exhibited by cattle and sheep, combined with the higher tolerance of sheep to nitrate toxicity, make sheep an ideal model for cattle. The higher tolerance of sheep to nitrate allows for the identification of intolerant sheep at the chronic, not acute, level.

## Conclusions and Future Aims

Individual response in ewes to nitrate treatment varied as evidenced by differences in performance and behavior. **S**upplement intake was significantly decreased by increasing dietary nitrate. Contemporary groups 1 and 2 differed in average supplement intake, which may be attributable to weather patterns or temperature. Further investigation will be required to determine reasons for variation between the two contemporary groups. Lack of differences in plasma nitrite levels between control and nitrate treated ewes may be due to the nitrite being bound in the blood or may be a result of individual threshold levels

of nitrate intake, which may be further explained with gene chip analyses. These results show that individual animal performances differ in response to elevated dietary nitrate and that reproduction may be affected by short-term exposure to subacute levels of nitrate prior to breeding.

Microarray analyses will be performed at the University of Missouri during the summer of 2008 to identify genes differentially expressed between control, tolerant, and intolerant ewes. Plasma samples will be analyzed to determine cortisol, glucose, and Vitamin E levels in selected ewes. Finally, elevated nitrate levels have been associated with female reproductive performance such as hormonal imbalances and reduced implantation rate, potentially resulting in abortion (Yaremcio, 1991; Zraly et al., 1997). Therefore, future studies will examine the effects of nitrate toxicity on female reproduction.

**Acknowledgements**

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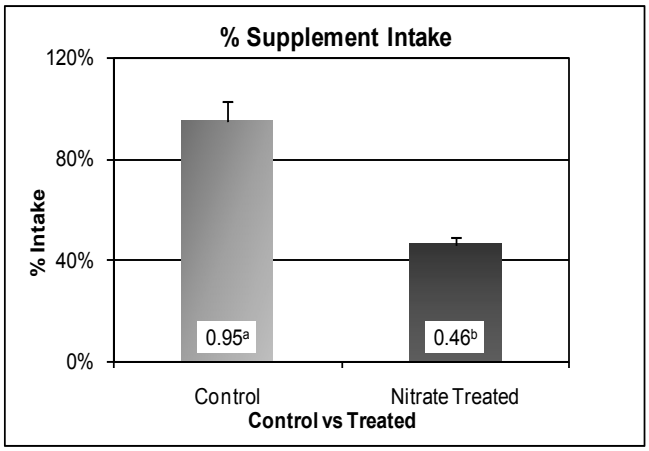


**Figure 1.** Percent supplement intake for controls and $NO_3^-$ treated ewes.

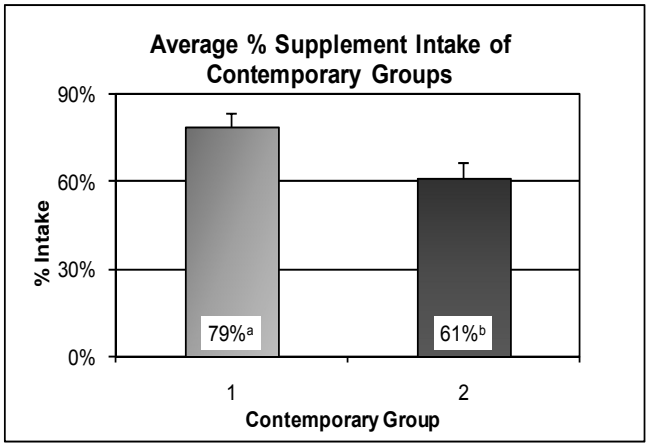


**Figure 2.** Average% supplement intake for contemporary groups 1 and 2.

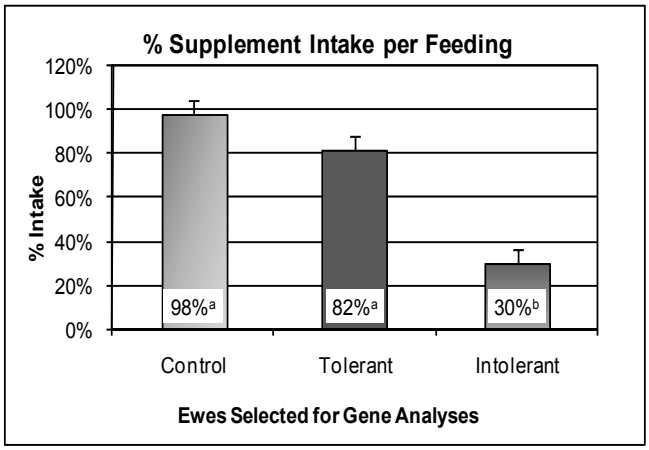


**Figure 3.** Percent supplement intake of control, tolerant, and intolerant ewes.

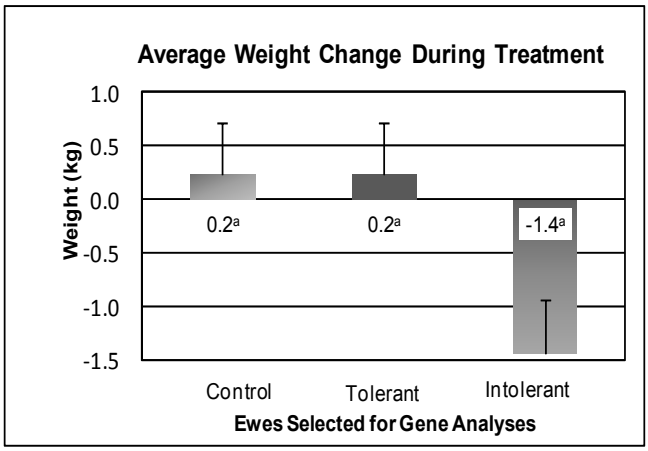


**Figure 4.** Average weight change of control, tolerant, and intolerant ewes.

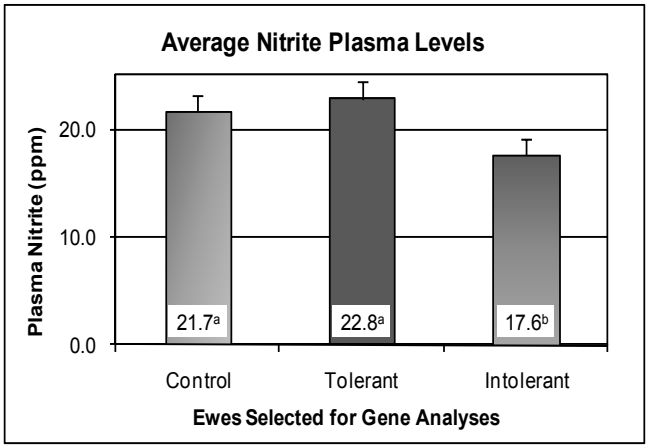


**Figure 5.** Average nitrite plasma levels of control, tolerant, and intolerant ewes

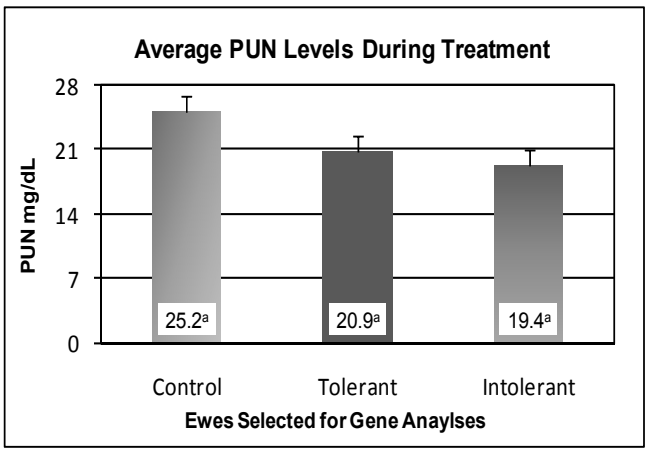


**Figure 6.** Average PUN during treatment period of study.

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# EFFECTS OF VITAMIN E, ZINC, BY-PASS PROTEIN, AND CHLORTETRACYCLINE SUPPLEMENTED TO EWES OF DIFFERENT AGE AND BODY CONDITION ON LAMB PRODUCTION AND INDICES OF IMMUNE FUNCTION.

**R. R. Redden[1], R. W. Kott[1], J. A. Boles[1], A. W. Layton[2], and P. G. Hatfield[1]**
[1]Department of Animal and Range Sciences,
Montana State University, Bozeman, MT 59717
[2]Montana Department of Livestock, Bozeman, MT 59717

**ABSTRACT:** Western White face ewes were used in two experiments to compare production responses of lambs to **HIGH** (12.5% rumen by-pass protein, 400 IU of supplemental Vitamin E, 176 ppm chelated Zn, and 72.7 mg/kg chlortetracycline) or **LOW** (7.56% rumen by-pass protein, with no supplemental Vitamin E, chelated Zn, or chlortetracycline) iso-caloric, iso-nitrogenous supplement fed to ewes of different age (Exp. 1; **3** vs **6** yr old; n = 52) and different body condition score (Exp. 2; **GOOD** vs **POOR**; 3.0 and 1.7 $\pm$ 0.5, respectively; n = 40). Supplement was individually fed 0.227 kg·ewe$^{-1}$·d$^{-1}$ for 29 d prior to expected lambing. Thereafter, each ewe was mass fed the appropriate supplement until lambing. Exp. 3 group fed HIGH or LOW supplements to the Red Bluff Research Ranch ewe flock. Total weight of lambs per ewe was measured at birth, turnout, and weaning. Colostrum was collected 4 h postpartum and analyzed for IgG. Two parainfluenza type 3 ($PI_3$) intranasal vaccinations were administered to ewes 31 and 17 d prior to expected lambing. Day 3 postpartum lamb sera was collected and analyzed for anti-$PI_3$ antibody titers. **Exp. 1:** Lambs born to 3 yr old ewes on HIGH supplement had greater ($P < 0.05$) anti-$PI_3$ antibody titers than lambs born to 3 yr old ewes on LOW supplement. **Exp. 2:** Lambs born to GOOD ewes on HIGH supplement had greater ($P < 0.10$) anti-$PI_3$ antibody titers than other treatments. **Exp. 3:** Kilograms of lamb per ewe at weaning were greater ($P < 0.10$) for HIGH than LOW supplemented ewes. We detected improvements in $PI_3$ immune transfer dependent of age and condition of ewe and production of weaned lamb was improved when the HIGH supplement is fed to ewes during late gestation.

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**Keywords:** Vitamin E, Zinc, By-pass Protein, Chlortetracycline, Parainfluenza 3, and Sheep

## Introduction

Montana range ewe operations commonly provide supplementary feed during late gestation and early lactation to improve the ewes' plane of nutrition, to spare body reserves, and ultimately improve lamb production. Supplementation with high rumen undegradable protein (UDP; Roeder et al., 2000), zinc-methionine (Hatfield et al. 1995), vitamin E (Kott et al., 1998), and chlortetracycline (SID, 2002) has shown to improve factors associated with lamb production.

Al-Sabbagh et al. (1995) found that ewe production was greater for 2.5 than 3.5 body condition score (BCS) ewes. Dickerson and Glimp (1975) reported low production in 2 and 3 yr old ewes, maximum production from 4 to 7 yr old ewes, and depressed production past 7 years of age.

Our objective was to incorporate UDP, vit E, zinc, and chlortetracycline into one supplement and compare it against an iso-caloric, iso-nitrogenous supplement fed during late gestation to ewes of two different age categories and body condition scores on indices of immune function (Exp 1 and 2). Supplement impact on lamb production was investigated in a large production setting (Exp 3).

## Materials and Methods

*Treatments.* Iso-caloric and iso-nitrogenous pelleted supplements were fed to ewes at 227 g·ewe$^{-1}$·d$^{-1}$. The **HIGH** supplement treatment contained 12.5% UIP, 400 IU of supplemental Vit E, 176 ppm chelated Zn (Availa-Zn 100; Zinpro, Eden Prairie, MN), and 72.7 mg/kg chlortetracycline (Aureomycin; Alpharma, Bridgewater, NJ). The **LOW** supplement treatment contained 7.56% UIP, no supplemental Vit E, no chelated Zn, and no chlortetracycline. Supplements were fed March 10, 2007 until April 24, 2007 or until each ewe lambed, which ever occurred first.

*Exp. 1 & 2.* Fifty two Targhee ewes were moved on March 8, 2007 from the range flock at Montana State University's Red Bluff Research Ranch near Norris, Montana to the Montana State University Fort Ellis facilities near Bozeman, Montana. Ewes had ad libitum access to long stemmed grass hay and water. Ewes were drenched with an anthelmintic (Valbazen; Phizer Animal Health, Exton, PA) prior to initiating treatment.

In Exp 1 & 2, ewes were assigned randomly to a 2 X 2 factorial arrangement of treatments. All ewes were assigned to either the HIGH or LOW supplemental treatments. In Exp. 1 (n = 52) half of the ewes were from the 6 yr old and half from the 3 yr old Targhee ewe population. In Exp. 2 (n = 40) half of the ewes were in POOR (average BCS = 1.7; range of 1.5 to 2) body condition and half the ewes were in GOOD (average BCS = 3; range of 2.5 to 3.5) body condition

For 29 days, ewes were individually supplemented (March 10 to April 7, 2007) in pens (1.5 m$^2$) every other day with 454 g·ewe$^{-1}$ of the appropriate treatment supplement. On April 7 ewes were drenched

with an anthelmintic, vaccinated for overeating and tetanus (Bar-Vac CDT**;** Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO), and treated for external parasites (Permectrin; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO). On April 8 ewes were moved back to Red Bluff. Ewes were then mass fed their respective supplement until lambing.

*Parainfluenza Type 3 (Exp. 1 & 2).* Ewes were treated with an intranasal injection of bovine rhinotracheitis-parainfluenza$_3$ vaccine ($PI_3$; Pfizer Animal Health, NY, NY) two days prior to initiation of supplement treatment. Two weeks later an additional intranasal treatment of $PI_3$ was administered. Three days post lambing, lambs were bled via jugular puncture using a non-hepranized vacutainer. Blood samples were centrifuged for 20 min at 1000 x g. Serum was decanted into plastic tubes and stored at -20ºC. Lamb serum was analyzed for anti-$PI_3$ titers at the Montana State University Diagnostic Laboratory by the hema-absorption method using an end point titer assay as described by Daniels et al. (2000). A greater dilution giving positive hema-absorption equates to a greater amount of anti-$PI_3$ antibody in the sample.

*Colostrum (Exp. 1 & 2).* Within two hours of birth, colostrum samples were taken, placed in 100 mL containers, and frozen for later analysis. Colostral immunoglobulin G (IgG) concentrations were measured by RIA at New Mexico State University's Endocrinology Lab in Las Cruces, NM.

*Lamb Production Data. Exp. 3:* Ewe not used in Exp. 1 and 2 (n = 499) were separated into two groups and mass fed one of the two treatment supplements. Supplements were fed to ewes at Red Bluff from March 10 to April 24. Ewes were fed equal portions of alfalfa and barley hay at a rate of approximately 2 $kg \cdot ewe^{-1} \cdot day^{-1}$. Lambs were born between April 12 and May 6, 2007. Lamb birth weights were recorded within 12 h of birth, at turn out (May 23, 2007), and at weaning (August 23, 2007). Kilograms of lambs/ewe were calculated as ewe production. Lambs that died were included in the analysis as 0 kg BW. Ewes were removed from production data if they did not lamb.

*Statistical analysis.* Data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Exp. 1 & 2 model included effects of supplement, age (Exp. 1) or condition (Exp. 2), number of lambs born per ewe, and treatment x age/condition. In Exp. 3 the model included supplement, number of lambs born per ewe, breed, and birthday. Kilograms lamb/s per ewe was analyzed at birth, turnout, and weaning. Data are presented as least squares means with differences considered significant at $P < 0.10$.

## Results and Discussion

*Exp. 1:* Colostral IgG concentrations did not differ between supplemental treatments ($P > 0.43$; Table 1). However, 3 yr old ewes tended ($P = 0.15$) to have higher colostral IgG concentrations than 6 yr old ewes. Lambs born to 3 yr old ewes on HIGH supplement had greater ($P < 0.05$) anti-$PI_3$ antibody titers than lambs born to 3 and 6 yr old ewes on LOW and HIGH supplements, respectively. Lambs born to 6 yr old ewes on LOW supplement had greater ($P < 0.05$) anti-$PI_3$ antibody titers than lambs born to 3 yr old ewes on LOW supplement and 6 yr old ewes on the HIGH supplement.

*Exp 2*: Colostral IgG concentrations were not different among treatments ($P > 0.41$; Table 2). However, lambs born to GOOD ewes on HIGH supplement had greater ($P < 0.10$; Table 4) anti-$PI_3$ antibody titers than other treatments.

*Exp. 3:* Kilograms of lamb per ewe at birth and turnout were not different ($P > 0.50$) between treatments. However, kilograms of lamb per ewe at weaning were greater ($P < 0.05$) for HIGH than LOW supplemented ewes (Table 3).

*Discussion:* Roeder et al. (2000) found that supplementation with by-pass protein to late gestational ewes increased colostral IgG concentrations. However we found no difference between supplements in ewe colostral IgG concentrations. The HIGH supplementation did altered $PI_3$ immune transfer from ewe to lamb differently depending on age and condition of ewe. Three yr old HIGH supplemented ewes showed an improvement in immune transfer, however, 6 yr old ewes had their $PI_3$ immune transfer to lambs depressed when fed the HIGH supplement. In Exp. 2, ewes in GOOD condition had increased $PI_3$ immune transfer due to the HIGH supplementation but supplement had no impact on POOR ewe $PI_3$ immune transfer. Daniels et al. (2000) supplemented vitamin E to gestating ewes and saw no effect on $PI_3$ transfer from ewe to lamb.

Supplementation in Exp. 3 did not affect birth weights or turnout weights but improved weaning weights. Ewes fed the HIGH supplement had a 6% increase in kg of lamb weaned per ewe. Similarly, Kott et al. (1998) who found a 9% increase in ewe weaning weights when vitamin E is supplemented during late gestation.

*Conclusion:* We measured differences in indices of ewe/lamb immune function dependent of age and condition of ewe when supplemented during late gestation with a supplement high in vitamin E, zinc, by-pass protein, and chlortetracycline. In addition, the supplement improved ewe production of weaned lamb.

## Acknowledgments

We would like to thank the Montana Sheep Institute at Montana State University for funding this project.

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Table 1. Least square means of colostral immunoglobulin G (IgG) concentrations (mg/mL) and serum parainfluenza type 3 ($PI_3$) titer dilutions taken from lambs at three days of age born from 3 and 6 year old ewes fed 227 g·d$^{-1}$ of either a HIGH[1] or LOW[2] supplement the last 30 d gestation in Exp. 1.

| n = 35 | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 yrs | | 6 yrs | | | *P* value | | |
| | HIGH | LOW | HIGH | LOW | SEM | S x A[3] | 3 vs 6 yr old | High vs Low |
| $PI_3$ | 29[a] | 11[b] | 13[b] | 26[a] | 5.51 | <0.01 | | |
| IgG | 54.0 | 56.6 | 45.9 | 50.6 | 5.57 | 0.88 | 0.15 | 0.46 |

[1] HIGH = 12.5% UIP, 400 IU supplemental Vit E, 176 ppm chelated Zn, and 72.7 mg/kg chlortetracycline.
[2] LOW = 7.56% UIP, no supplemental Vit E, no chelated Zn, and no chlortetracycline.
[3] S x A = Interaction between type of protein supplement and age of ewe.

Table 2. Experiment 2 least square means of colostral immunoglobulin G (IgG) concentrations (mg/mL) and serum parainfluenza type 3 ($PI_3$) titer dilutions taken from lambs at three days of age born from GOOD[1] and POOR[2] conditioned ewes fed 227 g·d$^{-1}$ of either HIGH[3] or LOW[4] supplemental treatments the last 30 d gestation.

| n = 25 | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GOOD | | POOR | | | *P* value | | |
| | HIGH | LOW | HIGH | LOW | SEM | S x C[5] | Good vs Poor | High vs Low |
| $PI_3$ | 44[a] | 10[b] | 15[b] | 15[b] | 10.4 | 0.10 | | |
| IgG | 53.7 | 57.3 | 59.6 | 53.7 | 5.63 | 0.40 | 0.85 | 0.85 |

[1] GOOD = average body condition score = 3; range of 2.5 to 3.5
[2] POOR = average body condition score = 1.7; range of 1.5 to 2
[3] HIGH = 12.5% UIP, 400 IU supplemental Vit E, 176 ppm chelated Zn, and 72.7 mg/kg chlortetracycline.
[4] LOW = 7.56% UIP, no supplemental Vit E, no chelated Zn, and no chlortetracycline.
[5] S X C = Interaction between type of protein supplement and condition of ewe.

Table 3. Least square means of lamb BW born to ewes fed 227 g·d$^{-1}$ of either HIGH[1] or LOW[2] supplemental treatments the last 30 d gestation in Exp. 3.

| n = 499 | Treatments | | |
|---|---|---|---|
| | HIGH | LOW | SEM |
| Birth Wt, kg | 7.0 | 7.0 | 0.17 |
| Turnout Wt, kg | 16.3 | 16.0 | 0.81 |
| Weaning Wt, kg | 36.6[a] | 34.4[b] | 2.06 |

[a,b] Within row, means without a common superscript letter differ, $P < 0.10$
[1] HIGH = 12.5% UIP, 400 IU supplemental Vit E, 176 ppm chelated Zn, and 72.7 mg/kg chlortetracycline.
[2] LOW = 7.56% UIP, no supplemental Vit E, no chelated Zn, and no chlortetracycline.
[3] Lamb were born between April 12 and May 6, 2007. Birth weights were taken within 12 hours of birth. Turnout and weaning weights were taken on June 23rd and August 23rd 2007, respectively. Multiple births were summed for a total lamb weight.

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# NATURAL SOURCE VITAMIN E SUPPLEMENTATION AND REPRODUCTIVE EFFICIENCY IN BEEF COWS

**M. J. Richardson*[1], R. P. Lemenager[1], N. Pyatt[2], and S. L. Lake[1]**
[1]Department of Animal Sciences, Purdue University, West Lafayette, IN, [2]ADM Animal Nutrition Research, Decatur, IN

**ABSTRACT**: The objective of this study was to determine the effect of supplemental natural-source vitamin E (NSVE) on reproductive efficiency in beef cows. In a two year study, one hundred twenty-seven Angus-cross beef cows (n = 77 in year one, n = 50 in year two; initial BW = 607 kg; initial BCS = 5.2) were randomly assigned to one of two isocaloric dietary supplements: 1) corn-based supplement (CON) or 2) corn-based supplement formulated to contain 1000 IU/d NSVE (NAT). Supplementation began 6 wk prepartum and continued until the breeding season. Cow BW and BCS were measured throughout the study to evaluate changes in energy balance. Cow blood samples were collected at calving to determine α-tocopherol concentration and weekly beginning four weeks postpartum to determine progesterone concentrations and return to estrus. Cows were synchronized using CO-Synch + CIDR® and bred by AI based on heat detection. Non-responding cows were time bred (AI) 66 h after prostaglandin injection. Cows returning to estrus following AI were bred by natural service. Dietary supplement did not affect ($P > 0.10$) change in BCS or BW. Cows supplemented with NSVE had greater ($P < 0.001$) concentrations of α-tocopherol at calving than CON cows. Dietary supplement did not affect ($P = 0.79$) the percentage of cows cycling before the breeding season; however, supplementation of NSVE tended to increase first service ($P = 0.09$; NAT 55%, CON 41%) and overall ($P = 0.09$; NAT 90.6%, CON 80%) pregnancy rates. Numerically, NSVE supplementation increased second service conception rates ($P = 0.23$) by 15% and first plus second services combined ($P = 0.15$) by 12% when compared to CON cows. These data suggest that while supplementing NSVE does not improve resumption of cyclicity, it may improve first service and overall conception rates. Further investigation is needed to elucidate the mechanisms associated with improved conception rates in cows supplemented with NSVE in absence of improved cyclicity.

**Key Words:** Beef cows, Reproduction, Vitamin E

## Introduction

The overall goal of cow-calf producers is to optimize pounds of calf weaned per cow exposed. Calves born earlier in the season will wean heavier and are thus more profitable than those born later in the season. Cows must be bred to calve within a condensed time period to produce a uniform group of calves at weaning. The average gestation length for beef cows is 281 d with an estrus cycle length of 21 d (Aiello, 1998), leaving only 84 d for the cow to undergo uterine involution, resume cyclicity, and become pregnant. Therefore, beef producers need to utilize production and management practices that will improve reproductive efficiency through enhanced postpartum rebreeding.

Supplementation of vitamin E has been shown to improve reproductive parameters in beef and dairy cows; however, there is a paucity of research involving the effects of vitamin E in reproducing beef cows. Supplementation of vitamin E has improved conception rates in primiparous beef heifers by as much as 50% compared to heifers receiving no vitamin E supplementation (Laflamme and Hidiroglou, 1991). Campbell and Miller (1998) reported a reduction in number of days to resumption of estrus from 70 to 50 in dairy cows receiving 1000 IU vitamin E per day. Similarly, Baldi et al. (2000) reported a decrease in the number of days to conception from 111 to 84 and total number of inseminations required for conception from 2.2 to 1.3 in dairy cows due to vitamin E supplementation at 2000 IU/d compared with 1000 IU/d.

As an antioxidant, vitamin E can prevent oxidation of lipids within cell membranes and increase cellular integrity (Machlin, 1991). Vierk et al. (1999) suggested that luteolysis of the corpus luteum (**CL**) is due to accumulation of toxic oxidative species, and vitamin E may suppress these oxidants, thus enhancing the maintenance of the CL, allowing for adequate progesterone secretion to maintain pregnancy.

The vitamin E requirement for beef cows has not been well established due its interrelationships with other dietary components, although Machlin (1991) stated that levels of 15 to 60 IU per kg DM were sufficient for young calves. Dairy cows are supplemented with 500 to 2,000 IU per day, leading some researchers to suggest that supplementation of 1000 IU of vitamin E per day to beef cows may improve reproductive performance (Franklin, 1998).

We hypothesized that 1000 IU vitamin E per day will improve reproductive efficiency in beef cows through a decrease in postpartum interval; therefore, our objectives are to evaluate the effects of natural source vitamin E (**NSVE**) on reproductive efficiency by investigating possible decreases in postpartum interval and improved overall conception rates.

## Materials and Methods

### *Experimental Design*

All protocols for this study were approved by the Purdue Animal Care and Use Committee. In a two-year study, 127 two- and three-year-old Angus-cross beef cows (n = 77 in year one, n = 50 in year two; initial BW = 607 ± 7 kg; initial BCS = 5.2 ± 0.14; 1 = emaciated, 9 = obese; Wagner et al., 1988) were blocked by age, BW, and BCS into one of two supplemental dietary treatments. Beginning an average of 6 wk prepartum, cows were given *ad libitum*

access to hay and water. Cows were fed corn silage once daily and given a corn-based supplement containing either no added vitamin E (**CON**) or 1000 IU NSVE · $cow^{-1}$ · $d^{-1}$ (**NAT**; Vitamin E 405 Natural Source, d-α-tocopheryl acetate, ADM Alliance and Nutrition, Inc., Quincy, IL) as a top dress. Supplementation was provided until the beginning of the breeding season. At an average of 75 d postpartum, cows were synchronized using the CO-Synch + CIDR® protocol. An intravaginal controlled internal drug release device (**CIDR**, Pfizer Animal Health, New York, NY) and GnRH (100 µg, i.m.; Cystorelin, Merial, Iselin, NJ) were administered to cows. Seven days later, the CIDR was removed and prostaglandin $F_2\alpha$ (**$PGF_2\alpha$**, 25 mg, i.m.; Lutalyse®, Pfizer Animal Health, New York, NY) was administered. Cows were monitored for signs of estrus behavior twice daily and those detected in estrus were bred by AI using the am/pm rule where cows detected in estrus in the morning are bred that evening and those detected in estrus in the evening are bred the following morning. Cows not exhibiting estrus by 66 h post-CIDR removal were bred (AI) and given GnRH (100 µg, i.m.). Cows were placed with a bull 14 d after AI. Pregnancy and fetal age were determined by ultrasonography 90 d after AI. First, second, first plus second combined, and overall conception rates were determined in relation to the AI date.

***Sample Collection***

Blood samples were collected via the coccygeal vein into 5-mL Vacutainer tubes (Becton, Dickson and Co., Franklin Lakes, NJ) 24 h after parturition for analysis of α-tocopherol concentration and weekly beginning 4 wk postpartum until breeding for analysis of progesterone concentration to determine days to resumption of estrus. Blood samples were immediately refrigerated for 8 h, centrifuged at 939 × *g* for 20 min, and serum was collected and stored at -20°C.

***Sample Analysis***

Serum samples were analyzed for vitamin E as α-tocopherol by HPLC. Briefly, in a 13 × 100 mm glass tube, 250 µL of serum was dissolved in 250 µL ethanol containing butylated hydroxytoluene (0.1 mg/mL) and 20 µL of δ-tocopherol (100 µM) as the internal standard and vortexed. Hexane (1 mL) was added and samples were centrifuged (3 min, 1200 × *g*). The hexane layer was removed, the extraction was repeated, and the hexane layers were combined and dried under nitrogen flow at 37°C. The residue was dissolved in 400 µL ethanol, filtered, transferred to a 300 µL auto sampler vial, and injected into the HPLC for analysis of vitamin E. Tocopherols were separated by isocratic HPLC at 0.8 mL/min using a reverse phase MD-150 column (150 cm × 3.2 mm, 3 µm particle size; ESA, Inc., Chelmsford, MA). The column was equilibrated in ammonium acetate (0.2 M) in a mixture of methanol:ammonium acetate (90:10 V/V, pH 4.36). The tocopherol isoforms were eluted over a 15-min period. Monitoring was performed with an electrochemical ESA CoulArray® detector (ESA, Inc., Chelmsford, MA) with potentials set at 200, 400, 600, and 800 mV. Identification and quantification of vitamin E were accomplished by comparison of retention time and peak areas with the internal standard.

Progesterone concentrations were measured using RIA (Coat-A-Count In-vitro Diagnostic Test Kit, Siemens Corp., Tarrytown, NY). Briefly, 100 µL of serum and 1 mL iodinated ($^{125}I$) progesterone were added to progesterone antibody-coated tubes. Tubes were incubated at room temperature for 3 h, decanted, and counted for one minute in a gamma counter (Cobra® II Auto-gamma® Counting Systems, Packard Instrument Co., Meriden, CT). Circulating progesterone concentrations were determined from the logit-log representation of the standard curve. Cows exhibiting circulating progesterone concentrations greater than 1.0 ng/mL were considered cycling. The intra- and inter-assay coefficients of variation were 5% and 2.1%, respectively.

***Statistical analyses***

Initial and final BW and BCS and changes were measured using the GLM procedures (SAS Institute, Cary, NC). Percentage of cows cycling prior to the breeding season and conception rates were determined using the CATMOD procedures (SAS Inst. Inc., Cary, NC). The model included the effects of maternal dietary supplement, year, age, and all possible interactions. No interactions ($P \geq 0.12$) were detected; therefore, only main effects of supplement are presented. All means presented are least squares means of each group and greatest SEM are reported. Significance was declared at $P < 0.05$.

## Results

The effects of NSVE on cow BW and BCS measurements and circulating α-tocopherol concentrations are presented in Table 1. By design, there were no differences in initial BW ($P = 0.62$) or BCS ($P = 0.60$). Natural source vitamin E supplementation did not affect changes in cow BW ($P = 0.69$) or BCS ($P = 0.88$) throughout the study.

Table 1. Effects of natural source vitamin E supplementation on cow BW, BCS, and serum α-tocopherol concentration

| | Supplements[1] | | | |
|---|---|---|---|---|
| Item | CON | NAT | SEM[2] | *P* value |
| Initial BW, kg | 604 | 609 | 6.86 | 0.62 |
| Final BW, kg | 596 | 598 | 8.32 | 0.84 |
| BW Change, kg | -9.64 | -11.60 | 3.84 | 0.69 |
| Initial BCS | 5.29 | 5.19 | 0.14 | 0.60 |
| Final BCS | 5.13 | 5.09 | 0.13 | 0.81 |
| BCS Change | -0.11 | -0.13 | 0.10 | 0.88 |
| α-tocopherol, µg/mL[3] | 2.15 | 3.34 | 0.15 | < 0.001 |

[1] Dietary supplement NAT formulated to contain 1000 IU natural source vitamin E · $cow^{-1}$ · $d^{-1}$; CON contained no additional natural source vitamin E.

[2] Greatest SEM presented.

[3] Serum α-tocopherol concentration measured 24 h after parturition.

Circulating concentrations of α-tocopherol were greater ($P < 0.001$) in NAT compared with CON cows 24 h postpartum.

The effects of NSVE supplementation on resumption of estrus prior to the breeding season, as well as conception rates, are presented in Table 2. There was no difference in days to resumption of estrus ($P = 0.52$) or the percentage of cows returning to estrus prior to the breeding season ($P = 0.79$) due to vitamin E supplementation. There was a tendency for higher first service ($P = 0.09$), first plus second service combined ($P = 0.15$), and overall ($P = 0.09$) conception rates in NSVE cows compared with cows supplemented the CON treatment. Age of cow affected ($P = 0.01$) resumption of estrus, with fewer two-year-old (first parity) heifers (37%) resuming cyclicity before the breeding season than three-year-old (second parity) cows (62%). First service conception rates were greater ($P = 0.01$) in year two (63%) compared with year one (33%).

Table 2. Effects of natural source vitamin E supplementation on resumption of estrus and conception rate

| | Supplements[1] | | | |
|---|---|---|---|---|
| Item | CON | NAT | SEM[2] | *P* value |
| Days to estrus[3] | 60.3 | 58.4 | 2.29 | 0.52 |
| Cycling, %[4] | 48.4 | 50.9 | 7.09 | 0.79 |
| Conception Rate, % | | | | |
| First Service | 40.6 | 55.7 | 6.79 | 0.09 |
| Second Service | 36.7 | 51.4 | 9.12 | 0.23 |
| First + Second | 64.0 | 76.0 | 6.40 | 0.15 |
| Overall | 80.0 | 90.6 | 4.83 | 0.09 |

[1] Dietary supplement NAT formulated to contain 1000 IU natural source vitamin E · $cow^{-1}$ · $d^{-1}$; CON contained no additional natural source vitamin E.

[2] Greatest SEM presented.

[3] Average number of days for cows to resume estrus following parturition.

[4] The percentage of cows resuming estrus prior to the breeding season.

## Discussion

Circulating serum α-tocopherol concentrations were 2.15 and 3.34 μg/mL in CON and NAT cows, respectively. Wichtel et al. (1996) and Hidiroglou et al. (1992) suggested that serum concentrations less than 2 μg/mL are deficient. Vitamin E is stored in all body tissues, but depletion rates vary between tissues and small amounts can be maintained in the body for long periods of time (McDowell, 1989). Therefore, the lack of response in resumption of estrus and postpartum interval in this study may be attributed to the CON cows having sufficient circulating α-tocopherol concentrations. Alternatively, supplementation of NSVE above 1000 IU/d may improve these reproductive parameters by increasing circulating α-tocopherol concentrations above 4 μg/mL, which Hidiroglou et al. (1992) considered adequate.

Although dietary supplement did not affect postpartum interval prior to the breeding season, more three-year-old (second parity) cows resumed cyclicity before the breeding season compared with two-year-old (first parity) heifers. This difference in postpartum interval between two- and three-year-old cows is in agreement with previously reported data (Strauch et al., 2001; Renquist et al., 2006). Postpartum interval is increased in two-year-old (first parity) heifers due to greater nutrient demands, a greater incidence of dystocia, and longer uterine involution compared with multiparous beef cows (Bellows et al., 1982; Renquist et al., 2006).

Previous research involving vitamin E supplementation on postpartum interval has been inconclusive. Campbell and Miller (1998) reported a decrease in the number of days to resumption of estrus in dairy cows due to vitamin E supplementation. Likewise, Harrison et al. (1984) also reported that postpartum interval was decreased in dairy cows when vitamin E was supplemented; however, the authors also reported the incidence of metritis, as well as cystic ovarian disease, were not affected by vitamin E supplementation, further complicating the insight into potential mechanisms of vitamin E on uterine health and postpartum interval. In the present study, adequate concentrations of vitamin E in CON cows likely explains the lack of significant differences in postpartum interval prior to the breeding season.

Laflamme and Hidiroglou (1991) reported similar increases to the present study with regards to overall conception rate with vitamin E supplemented heifers having greater overall conception rates than non-supplemented heifers by 50%. While the results of the present study are not as drastic, NAT cows had a tendency to increase first service and overall conception rate when compared with CON cows. The effect of vitamin E on conception rates could be due to the role of vitamin E in preventing early embryonic death and fetal resorption as demonstrated in rats by Evans and Bishop (1922). Rats reared on vitamin E deficient diets showed normal ovarian behavior but had increased fetal resorption by the second day of gestation. Early embryonic death has been associated with premature luteolysis of the CL. Prostaglandin $F_2\alpha$ is responsible for lysis of the CL which in turn induces ovulation, and is derived from arachidonic acid through the cyclooxygenase pathway (Murdoch et al., 1993; Mattos et al., 2000). A vitamin E deficiency may result in increased $PGF_2\alpha$ concentrations through enhanced phospholipase A or cyclooxygenase activity within the cyclooxygenase pathway (Panganamala and Cornwell, 1982) which may lead to early embryonic death. Vierk et al. (1998) demonstrated that as an antioxidant, α-tocopherol in ewes may salvage the CL from apoptosis by suppressing oxidative stress, thus possibly explaining the tendency for first service and overall conception rates to be improved with NSVE supplementation in the present study.

The original hypothesis investigated decreases in postpartum interval and post-breeding effects of NSVE; however, postpartum interval before the breeding season were not affected by supplementation of NSVE, leading the authors to believe that NSVE affects reproductive efficiency through post-breeding mechanisms. Further research is needed to elucidate the mechanisms that affect

conception rates when resumption of cyclicity is not affected.

## Implications

These data suggest that supplementation of NSVE at 1000 IU/d could improve first service and overall conception rates in beef cows without affecting energy balance. It is possible that a more beneficial response in reproductive efficiency might be seen when levels greater than 1000 IU/d of NSVE are supplemented.

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# EFFECT OF LEVEL OF DRY DISTILLERS GRAINS PLUS SOLUBLES ON SERUM HORMONE CONCENTRATIONS IN FEEDLOT LAMBS

**Y. Diaz, A. Islas, D. M. Hallford, L. E. Camacho, J. M. Benavidez, and S. A. Soto-Navarro.**

**New Mexico State Univ., Las Cruces 88003**

**ABSTRACT**: Effect of level of corn dry distillers grains plus solubles (DDGS) on serum prolactin (PRL), triiodithyronine (T3), insulin, and insulin-like growth factor-I (IGF-I) were evaluated in 24 Rambouillet lambs (27.8 ± 1.47 kg initial BW) in a 63-d finishing experiment. Finishing diets were 80% concentrate based on rolled corn with 15% sudangrass hay and 5% alfalfa hay. Treatments consisted of 4 levels (8, 16, 24, and 32% of diet DM) of DDGS replacing dry-rolled corn. Lambs were blocked by BW and assigned to 1 of the 4 DDGS levels. Lambs were housed individually (2 x 4 m pens) and fed once daily at 0800. Fresh water was always available. Blood samples were collected at d 0, 21, 42, and 63. Increasing level of DDGS had no effect on PRL ($P$ = 0.47; 370.8, 362.2, 321.4, and 268.2 ± 49.7 ng/mL for 8, 16, 24, and 32% DDGS, respectively). Serum T3 was not affected ($P$ = 0.38) by increasing DDGS level (1.30, 1.35, 1.47, and 1.43 ± 0.07 ng/mL for 8, 16, 24, and 32% DDGS, respectively). Likewise, insulin was similar ($P$ = 0.80) among DDGS replacement levels (1.06, 0.92, 1.00, and 1.13 ± 0.16 ng/mL for 8, 16, 24 and 32% DDGS, respectively). Serum IGF-I was also not affected ($P$=0.49) by increasing DDGS level (254, 240, 239, and 221, ± 14.7 ng/mL for 8, 16, 24, and 32% DDGS, respectively). We conclude that lamb serum hormones of feedlot lambs are not altered by level of DDGS replacement. Therefore, DDGS can be used in finishing diets for lambs up to 32% without negatively affecting metabolic hormone status.

Key Words: DDGS, hormones, lambs

## INTRODUCTION

The ethanol industry is expanding rapidly and by-products such as corn dried distillers grains with solubles (**DDGS**) are becoming widely available (Renewable Fuels Association, 2005). Corn dried distillers grains with solubles are relatively high in CP (10%), fat (12%), NDF (36%), and P (0.9% of DM). Also, DDGS are competitively priced compared with other protein and energy sources. The use of DDGS as a feedstuff for livestock has been documented from as early 1900 (Henry, 1900). Successful use of DDGS have been reported for feedlot cattle (Ham et al., 1994), grazing cattle (MacDonalds et al., 2007), creep-fed calves (Reed et al., 2006), and dairy cattle (Al-Suwaiegh et al., 2002). In a meta-analysis of 4 studies (Klopfenstein et al., 2007) reported that the feeding value of DDGS was 123% of that of corn when the level of inclusion in a finishing diet was 20%, and declined to 100% with increasing the level of inclusion to 40%. However, information on uses of DDGS as feedstuff for lambs is limited. Therefore the objective of this study was to evaluate the effect of level of DDGS inclusion on 80% concentrate diets based on dry rolled-corn on serum metabolic hormone concentrations.

## MATERIALS AND METHODS

Twenty four spring-born Rambouillet wether lambs born on the main campus at New Mexico State University were used in this study. All procedures were approved by the Institutional Animal Care and Use Committee. Lambs were docked at 1 d of age, castrated and vaccinated against tetanus and enterotoxemia at 28 d of age and again at weaning when lambs were approximately 60 d of age. During the preweaning period, lambs had free access to alfalfa hay and cracked corn was offered at levels appropriate for age and BW. After weaning, lambs were fed alfalfa hay and cracked corn until they were approximately 80 d of age at which time they began an adaptation period to the basal experimental diet. When lambs weighed 27.6 ± 0.5 kg and were 103 ± 1.2 d of age, they were stratified by BW and randomly assigned to 1 of 4 dietary treatments. During the 63-d experiment, lambs were maintained outdoors in individual pens (2 x 4 m) and had free access to water and their experimental diet.

All diets were composed of 15% sudangrass and 5% alfalfa has as the roughage sources. The basal diet contained 64.4% dry-rolled corn and 8% DDGS yielding a CP content of 11.94%. Dicalcium phosphate and limestone were added to balance the P and Ca contents among dietary treatments. The remaining diets had 16, 24, and 32% DDGS replacing dry-rolled corn, to yield respective CP (DM basis) levels of 13.4, 14.8, and 16.35%. Other dietary ingredients were incorporated in similar amounts in all diets and included molasses (3.5%), tallow (approximately 0.4%), ammonium chloride (0.9%), salt (0.9%), and a vitamin premix (0.5%; 2,200 IU/g vitamin A, 1,200 IU/g Vitamin $D_3$, and 2.2 IU/g vitamin E). The ratio of degradable intake protein to TDN was 0.09, 0.10, 0.11, and 0.12 for the diets containing 8, 16, 24, and 32% DDGS, respectively. Fresh feed was offered daily in amounts to stimulate ad libitum intake and orts were recorded daily. Orts were pooled weekly and DM was determined. Lambs were weighed at 21-d intervals.

Blood samples were collected from each lamb at d 0, 21, 42, and 43 before feeding. Samples were obtained by

jugular venipuncture into sterile vacuum tubes (Corvac Serum Separator, Kendall, St. Louis, MO). Blood was allowed to clot at room temperature for approximately 30 min after which serum was harvested by centrifugation at 1,500 g for 15 min at 4º C. Serum was transferred to plastic vials and stored frozen until analyzed. Hormone concentrations were determined by RIA. Concentrations of IGF-1 and PRL were quantified by double antibody RIA as described by Berrie et al. (1995) and Spoon and Hallford (1989), respectively. Serum T3 was determined by solid phase RIA using components of commercial kits (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) and validated for ruminant serum in our laboratory as described by Wells et al. (2003). Insulin was quantified by solid phase RIA using components of a commercial kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA) as described by Reimers et al. (1982).Within and between assay CV for all determinations were less than 15%.

Serum hormone concentrations responses (PRL, T3, insulin, IGF-I) were subjected to ANOVA appropriate for a randomized complete block design with animal as the experimental unit. Analyses were computed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Because treatments were arranged with increasing levels of dietary CP, linear, quadratic, and cubic responses were also examined.

## RESULTS AND DISCUSSION

Increasing level of DDGS had no effect on PRL ($P$ = 0.47; 370.8, 362.2, 321.4, and 268.2 ± 49.7 ng/mL for 8, 16, 24, and 32% DDGS, respectively). Several workers have suggested that PRL may be involved in growth (McAtee and Trenkle, 1971; Bauman et al., 1982). The lack of effect on serum PRL observed in this study agrees with the lack of effects on growth traits in response to increasing levels of DDGS observed in the present study and reported somewhere else (Diaz et al., 2008). Serum T3 was not affected ($P$ = 0.38) by increasing DDGS level (1.30, 1.35, 1.47, and 1.43 ± 0.07 ng/mL for 8, 16, 24, and 32% DDGS, respectively). Thyroid activity exerts an influence on general and energetic metabolism in mammals (Pipes et al., 1963). Efficiency of goitrogens in improving feed efficiency in ruminants has generally been attributed to a decreased in basal metabolic rate and a subsequent increase in energy availability (Burroughs et al., 1958). Therefore, in the present study, growth traits were most likely not influenced by basal metabolic rate and energy availability due to increasing DDGS levels. Likewise, insulin was similar ($P$ = 0.80) among DDGS replacement levels (1.06, 0.92, 1.00, and 1.13 ± 0.16 ng/mL for 8, 16, 24 and 32% DDGS, respectively). Changes in insulin usually correspond to changes in circulating glucose concentration (Busato et al., 2002). Therefore, the lack of changes in serum insulin concentrations was most likely due to a lack of DDGS level effect on glucose concentration. Serum IGF-I was also not affected ($P$ = 0.49) by increasing DDGS level (254, 240, 239, and 221, ± 14.7 ng/mL for 8, 16, 24, and 32% DDGS, respectively). The lack of effect of DDGS level on serum IGF-I concentration may be indicative of similar nutritional status across experimental diets (Zulu et al., 2002). We conclude that lamb serum hormones of feedlot lambs are not altered by level of DDGS replacement.

## IMPLICATIONS

These results imply that replacing dry-rolled corn with DDGS does not change the metabolic status of feedlot lambs. Therefore, DDGS can be used in finishing diets for lambs up to 32% without negatively affecting metabolic hormone status.

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# EFFECTS OF CORN CONDENSED DISTILLERS SOLUBLES SUPPLEMENTATION ON PERFORMANCE AND DRY MATTER INTAKE OF BEEF COWS CONSUMING FORAGE-BASED DIETS

**L. R. Coupe, G. P. Lardy, A. M. Meyer, B. W. Neville, J. L. Leupp, and J. S. Caton**
Department of Animal Sciences, North Dakota State University, Fargo

**ABSTRACT:** Eighty crossbred cows (avg initial BW = 607 kg ± 10 kg, avg initial BCS = 5.0 ± 0.1) in mid to late gestation were used in a randomized complete block design to determine effect of feeding method and level of corn condensed distillers solubles (CCDS) supplementation on performance of beef cows fed forage-based diets. Cows were housed in a dry lot, blocked by BW and projected calving date, and allocated to 1 of 5 treatments (4 replicates per treatment). Treatments were arranged in a 2 x 2 + 1 factorial design; main effects were feeding method (mixed vs. fed separately) and level of CCDS (0.2 vs. 0.4% BW; 29.7% CP, 24.3% EE, DM basis). The resulting 5 treatments were a negative control (no supplement), 0.2% BW CCDS (DM basis) mixed with the forage, 0.4% BW CCDS (DM basis) mixed with the forage, 0.2% CCDS (DM basis) supplement fed separately, and 0.4% BW CCDS (DM basis) fed separately. All treatments were offered ad libitum forage (7.9% CP, 65.3% NDF, 41.6% ADF; DM basis) which consisted of a mixture of 40% grass hay and 60% corn stover. The trial lasted for 48 d, cows were weighed every 14 d and BCS was evaluated at the beginning and end of the trial. Supplemented cows had greater ($P < 0.001$) BW gains than non-supplemented cows. Cows supplemented 0.4% CCDS had greater ($P = 0.005$) weight gains than cows fed 0.2% CCDS. There was no treatment effect ($P = 0.87$) on BCS change. Non-supplemented cows had greater ($P = 0.006$) forage DMI than all supplemented treatments. Mixing CCDS with the forage resulted in lower ($P = 0.004$) forage DMI compared to diets where CCDS was fed separately. Total (forage and CCDS) DMI was increased ($P < 0.001$) in treatments with CCDS fed separately compared to those treatments where CCDS was mixed with forage. Corn condensed distillers solubles appear to be an effective supplement for cows fed forage-based diets.

**Key words:** beef cows, corn condensed distillers solubles, forage intake

## Introduction

With the expansion of the ethanol industry and steady rise of feed costs, alternative feeds and use of byproducts are becoming more important. The ethanol industry is expanding, and consequently producers have the option to utilize these byproducts. Corn condensed distillers solubles (CCDS) is one ethanol byproduct which is currently available. Corn condensed distillers solubles are relatively high in CP and fat, which makes this product appealing for supplementing wintering beef cows. Low-quality forages and crop residues are a plentiful and economical resource that can be an important asset in ruminant animal diets (NRC, 1983). However, to achieve an acceptable level of animal production, energy and/or protein supplementation must be provided. Research has indicated that grain supplementation may cause a decrease in forage intake but increase livestock performance (Caton and Dhuyvetter, 1997). Protein supplementation can increase forage intake, utilization, and subsequently increase cattle performance (Sanson et al., 1990; Bodine et al., 2001). Specifically, rumen degradable protein has been reported to improve forage intake and animal performance when low-quality forages are fed (Guthrie and Wagner, 1988; Del Curto et al., 1990; Köster et al., 1996).

Corn condensed distillers solubles are high in both protein and fat (20 to 30% CP and 4 to 20% fat, DM basis; Gilbery et al., 2006; Rust et al., 1990). A recent study by Gilbery et al. (2006) reported forage DMI was not affected by increasing levels of CCDS when fed separately from forage. However, when CCDS was mixed with forage, DMI increased quadratically with the greatest DMI at 10% CCDS. The objectives of this study were to evaluate the effects of CCDS supplementation on cow performance, body condition score, and feed intake.

## Materials and Methods

*Animals and Diets.* All animal care and handling techniques were approved by the North Dakota State University Animal Care and Use Committee prior to initiation of research. Eighty crossbred cows, in their third trimester of gestation, were used in a randomized complete block design. Cows were weighed and assigned a body condition score (BCS) on two consecutive days at the initiation and conclusion of the trial. Cows were housed in a dry lot and assigned to 1 of 20 pens by BW and projected calving date and were weighed every 14 d. Cows were offered ad libitum access to a basal diet consisting of 40% grass hay and 60% corn stover, which was chopped and mixed (Table 1). Cows had free access to water, mineral (minimum 9.0% of Ca, 21,120 ppm of Zn, 7,000 ppm of Cu, 28,000 ppm of Mn, 75 ppm of Co, 350 ppm of I, and 175 ppm of Se; Interstate Vet Clinic, Mandan, ND), and trace mineralized salt (minimum 93.0% of NaCl, 0.008% of Co, 0.039% of Cu, 0.008% of I, 0.2% of Fe, 0.19% of Mn, 0.38% of Zn, and 0.0053% of Se; Trouw Nutrition, Highland, IL). Orts were collected twice weekly, weighed, subsampled, and analyzed.

Treatments were arranged in a 2 x 2 factorial design with main effects of CCDS level (0.2% BW vs. 0.4% BW CCDS, DM basis) and feeding method (either mixed with the forage or fed separately). This resulted in the following treatments: negative control (no supplement), 0.2% BW

CCDS mixed with the forage (0.2 % MIX, DM basis), 0.4% BW CCDS mixed with the forage (0.4% MIX, DM basis), 0.2% CCDS supplement fed separately in tanks (0.2% SEP, DM basis), and 0.4% BW CCDS fed separately (0.4% SEP, DM basis).

*Laboratory Analysis.* Diet and ort 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 through a 2-mm screen. Samples were then analyzed for DM, ash, and CP (Procedure numbers: 930.15, 942.05, 4.2.10, respectively; AOAC, 1990). Concentrations of NDF and ADF were determined using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Corn condensed distillers solubles were analyzed for nutrient content at Midwest Laboratories (Omaha, NE).

*Statistical Analysis.* Data were analyzed as a 2 x 2 + 1 factorial using MIXED procedures of SAS. The model included treatment and stage of gestation. Pen was used as the experimental unit. Orthogonal contrasts included control vs. supplemented treatments, MIX vs. SEP, 0.4% level vs. 0.2% level, and the interaction of method of feeding x level of CCDS. When the overall F-test for treatment was significant ($P \leq 0.10$) means were separated using least significant difference, and were considered significant at $P < 0.10$.

## Results and Discussion

Cow performance and BCS data are reported in Table 2. There were no significant level x feeding method interactions for any variable measured ($P > 0.54$). There was no effect ($P = 0.66$) of treatment on initial BW. There was an effect ($P < 0.001$) of treatment on BW change. Cows supplemented with CCDS had greater ($P < 0.001$) weight gain than control cows. This agrees with a previous study by Sanson et al. (1990) that indicated protein and energy supplementation resulted in increased weight gain in cows grazing winter range or fed grass hay. Also, cows supplemented CCDS at the 0.4% level had greater weight gains ($P = 0.005$) than cows supplemented CCDS at the 0.2% level. There was a treatment effect ($P = 0.047$) on final BW. Supplemented cows weighed more ($P = 0.05$) than control cows at the conclusion of the trial. Furthermore, cows supplemented CCDS at the 0.4% level weighed more ($P = 0.042$) than cows supplemented CCDS at the 0.2% level. There was no effect ($P > 0.326$) of treatment on initial or final BCS.

Control cows had greater forage DMI than supplemented cows, when expressed as kg/d ($P = 0.006$) or % BW ($P = 0.003$). Cows fed CCDS separately from forage had greater ($P < 0.004$) forage DMI than cows fed CCDS mixed with forage. This conflicts with the results found in a recent study by Gilbery et al. (2006). Reasons for the differences between these studies are unclear. Gilbery et al. (2006) reported that feeding CCDS and forage together likely results in improved synchrony and release of nutrients. However, more research is needed to better understand the differences in DMI which occur when CCDS is fed mixed with forage vs. separately. Cows fed 0.2% level of CCDS had greater ($P = 0.084$) forage DMI than cows fed 0.4% level of CCDS.

By design there was a significant effect of treatment on CCDS DMI ($P < 0.001$). There was also a treatment effect on total DMI, when expressed on a kg/d ($P < 0.037$) and % BW basis ($P < 0.016$). Cows fed CCDS supplement mixed with forage had lower ($P < 0.002$) total DMI than cows fed CCDS separately.

Results of this study suggest that CCDS are an effective supplement for beef cows that can improve performance of gestating cows fed a forage-based diet. More research is needed to fully determine and understand the effects of CCDS on DMI.

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**Table 1.** Analyzed nutrient content of forage and corn condensed distillers solubles (CCDS)

| Item, % | Forage[1] | CCDS[2] |
|---|---|---|
| DM | | 24.6 |
| | %, DM Basis | |
| Fat | ND[3] | 24.3 |
| Ash | 12.1 | 7.4 |
| CP | 7.9 | 29.7 |
| NDF | 65.3 | ND |
| ADF | 41.6 | 0.5 |
| Ca | 0.6 | 0.1 |
| P | 0.1 | 1.3 |
| S | ND | 1.7 |

[1]Forage consisted of 40% grass hay and 60% corn stover.
[2]CCDS = corn condensed distillers solubles
[3]ND = not determined.

**Table 2.** Effects of corn condensed distillers solubles (CCDS) supplementation on cow performance while consuming a forage-based diet.

| | Treatment[1] | | | | | | | Contrast[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | CON | 0.2% MIX | 0.4% MIX | 0.2% SEP | 0.4% SEP | SEM[3] | *P*-value[4] | CON vs. SUP | MIX vs. SEP | HIGH vs. LOW | METH x LEV |
| BW, kg | | | | | | | | | | | |
| Initial | 608.4 | 612.8 | 613.6 | 594.4 | 607.4 | 10.41 | 0.659 | 0.900 | 0.220 | 0.490 | 0.539 |
| Final | 674.6 | 698.3 | 716.9 | 677.9 | 706.7 | 12.01 | 0.047 | 0.050 | 0.188 | 0.042 | 0.655 |
| Change | 66.1 | 85.5 | 103.3 | 83.5 | 99.3 | 5.85 | <0.001 | <0.001 | 0.612 | 0.005 | 0.865 |
| BCS | | | | | | | | | | | |
| Initial | 4.84 | 5.03 | 4.99 | 5.05 | 5.02 | 0.12 | 0.701 | 0.157 | 0.808 | 0.772 | 0.990 |
| Final | 5.35 | 5.63 | 5.61 | 5.63 | 5.47 | 0.12 | 0.326 | 0.068 | 0.450 | 0.551 | 0.551 |
| Change | 0.51 | 0.45 | 0.61 | 0.57 | 0.60 | 0.13 | 0.871 | 0.719 | 0.653 | 0.437 | 0.594 |

[1]CON = forage only, 0.2% MIX = forage mixed with 0.2% BW corn condensed distillers solubles supplement, 0.4% MIX = forage mixed with 0.4% BW corn condensed distillers solubles supplement, 0.2% SEP = forage with 0.2% BW corn condensed distillers solubles supplement fed separately, 0.4% MIX = forage with 0.4% BW corn condensed distillers solubles supplement fed separately.
[2]CON vs. SUP = control treatment vs. all supplemented treatments, MIX vs. SEP = forage and corn condensed distillers solubles mixed vs. forage and corn condensed distillers solubles fed separately, HIGH vs. LOW = 0.4% BW corn condensed distillers solubles level vs. 0.2% BW corn condensed distillers solubles level, METH vs. LEV = method of feeding (mixed and fed separately) and corn condensed distillers solubles supplementation level interaction.
[3]n = 4 observations per treatment.
[4]Probability value for the *F*-test of overall treatment.

**Table 3.** Effects of corn condensed distillers solubles (CCDS) on DMI on cows consuming forage-based diet.

| | Treatment[1] | | | | | | | Contrast[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | CON | 0.2% MIX | 0.4% MIX | 0.2% SEP | 0.4% SEP | SEM[3] | *P*-value[4] | CON vs. SUP | MIX vs. SEP | HIGH vs. LOW | METH x LEV |
| Intake, kg/d | | | | | | | | | | | |
| Forage | 14.02 | 11.73 | 11.30 | 13.55 | 12.67 | 0.55 | 0.002 | 0.006 | 0.004 | 0.232 | 0.680 |
| CCDS[5] | 0.00 | 1.12 | 2.22 | 1.20 | 2.35 | 0.02 | <0.001 | <0.001 | <0.001 | <0.001 | 0.165 |
| Total | 14.02 | 12.84 | 13.51 | 14.74 | 15.02 | 0.55 | 0.037 | 0.986 | 0.002 | 0.392 | 0.723 |
| Intake, % BW | | | | | | | | | | | |
| Forage | 2.24 | 1.85 | 1.75 | 2.19 | 1.98 | 0.09 | <0.001 | 0.003 | 0.001 | 0.084 | 0.501 |
| CCDS[4] | 0.00 | 0.18 | 0.34 | 0.19 | 0.36 | 0.004 | <0.001 | <0.001 | <0.001 | <0.001 | 0.410 |
| Total | 2.24 | 2.02 | 2.10 | 2.38 | 2.35 | 0.09 | 0.016 | 0.803 | <0.001 | 0.829 | 0.532 |

[1]CON = forage only, 0.2% MIX = forage mixed with 0.2% BW corn condensed distillers solubles supplement, 0.4% MIX = forage mixed with 0.4% BW corn condensed distillers solubles supplement, 0.2% SEP = forage with 0.2% BW corn condensed distillers solubles supplement fed separately, 0.4% MIX = forage with 0.4% BW corn condensed distillers solubles supplement fed separately.
[2]CON vs. SUP = control treatment vs. all supplemented treatments, MIX vs. SEP = forage and corn condensed distillers solubles mixed vs. forage and corn condensed distillers solubles fed separately, HIGH vs. LOW = 0.4% BW corn condensed distillers solubles level vs. 0.2% BW corn condensed distillers solubles level, METH vs. LEV = method of feeding (mixed and fed separately) and corn condensed distillers solubles supplementation level interaction.
[3]n = 4 observations per treatment.
[4]Probability value for the *F*-test of overall treatment.

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# THE EFFECTS OF CO-ENSILING WET DISTILLER'S GRAINS PLUS SOLUBLES WITH CORN SILAGE ON GROWTH PERFORMANCE OF BRED BEEF HEIFERS DURING LATE PREGNANCY

**R. P. Arias, R. P. Lemenager, L. Unruh-Snyder, and S. L. Lake**
Purdue University, West Lafayette, IN.

**ABSTRACT:** The objective of this study was to evaluate the effects of co-ensiling wet distiller's grains (WDG) with corn silage on growth performance of bred heifers during the last trimester of gestation. Ninety-six commercial Angus, bred heifers (two year-old; 522 ± 49.1 kg of initial BW; 5.3 ± 0.1 initial BCS) were blocked by weight and randomly assigned to one of four diets on a 62-d trial; 1) A corn silage and soybean meal control (**CON**), 2) Corn silage co-ensiled with wet distiller's grains with solubles (3:1 corn silage:WDG on a DM basis; **CO-EN**), 3) Corn silage mixed with dry distiller's grains with solubles (DDG) added at feeding time (**CS+DDG**) and, 4) Corn silage mixed with WDG added at feeding time (**CS+WDG**). All the diets were formulated to be iso-caloric and iso-nitrogenous and to meet NRC requirements of two year-old heifers in the third trimester of gestation. Cows were weighed and body condition scored on two consecutive days at the beginning and the end of the trial. Initial and final BW was corrected for fetal weight according to day of gestation. By design, there was no difference in initial BW ($P$ = 0.39) and initial BCS ($P$ = 0.36) between treatments. Heifers fed the CS+DDG diet had decreased DMI ($P$ < 0.01) compared to all other diets. Heifers fed the CO-EN treatment had a greater ADG ($P$ = 0.03) and tended to have a greater G:F ($P$ = 0.06) compared to other treatments. Heifers fed the CO-EN treatment had greater change in BW ($P$ = 0.03) compared to the CON and the CS+DDG treatments, while the CS+WDG treatment was intermediate. There was no significant differences in BCS ($P$ = 0.35), final BCS ($P$ = 0.40), or final BW ($P$ = 0.14) due to dietary treatment. Results from this study suggest that co-ensiling corn silage with WDG creates a viable feedstuff for growing heifers and improves growth performance compared to a traditional corn silage diet or the addition of either WDG or DDG at the time of feeding. Additionally, co-ensiling provides extended shelf life and increased feeding flexibility for smaller production units.

**Key words:** Beef heifers, Distiller's grains, Co-ensiling

## Introduction

The beef industry serves as one of the most important value-added enterprises in the U.S. with over a million farms and ranches benefiting directly from the sales of cattle (NCBA, 2006). In 2002, gross receipts from the sale of cattle and calves totaled over $45 billion and accounts for over 21% of all agricultural receipts. This makes the beef sector the single largest agricultural enterprise in the U.S. (USDA, 2006). It has been estimated that although the U.S. beef industry has less than 10% of the world's cattle population, it provides nearly 25% of the world's beef supply (USDA, 2002). Interestingly, small and medium-sized beef producers (less than 200 cows) account for 96.5% of the beef cow operations and 67 % of the U.S. beef cow inventory (USDA, 1997).

Despite increased consumption and growth within the industry, production agriculture is at a crossroads. Government subsidies given to the bio-fuel industries have contributed to the growth in the corn-based ethanol industry which, in turn, has resulted in future corn prices of over $4/bushel. The ramifications of the shift towards ethanol production are far reaching. The sudden increase in corn prices during the fall of 2006 has placed a heavy burden on beef producers. Small and medium-sized producers currently are not capable of utilizing commodity feeds with limited 'shelf-life', like wet distiller's grains (WDG), and this places them at a severe disadvantage compared to larger operations. The increasing cost of traditional feed grains (especially corn) which have been traditionally used in beef production has the potential to drive them out of business.

Garcia and Kalscheur (2004) reported successful storage and co-ensiling of WDG with corn silage, soybean hulls, and wet beet pulp. The challenge is that WDG are naturally low in pH and may inhibit the fermentation process, especially in residues lacking readily fermentable carbohydrate sources (IBC, 2005). Furthermore, how the ensiling process of the mixed ingredients affects the rate of oxidation of the feed (spoilage at the face of the open silo structure and in the feed bunk) is not known. Additionally, questions regarding performance of animals fed these mixtures, maximal inclusion rates to determine optimal end-product quality, and how these mixtures fit into small to medium-sized farm operations have not been answered.

Therefore, the objectives of the current study are to evaluate the effects of co-ensiling corn silage and WDGS on performance of heifers during the third trimester of gestation.

## Materials and Methods

Ninety six, two year-old, commercial Angus heifers (initial BW of 522 ± 49.1 kg; initial BCS of 5.3 ± 0.1) in their last trimester of gestation were blocked according to BW and BCS (24 pens with 4 heifers per pen; 6 replicas per treatment) and assigned in a completely randomized design, to one of four diets (Table 1): 1) a control diet (**CON**) consisting of corn silage plus soybean meal; 2) co-ensiled corn silage with WDG added at 25% DM basis (**CO-EN**); 3) corn silage plus 25% DDG added at

mixing (**CS+DDG**); and 4) corn silage plus 25% WDG added at mixing (**CS+WDG**).

Diets were formulated to meet requirements (NRC, 1996) for Angus heifers during the last trimester of gestation and to be iso-caloric and iso-nitrogenous (Table 2). The diets were offered on a limited-fed basis, once daily, at 0900 with free access to water throughout the 62-d trial.

At the beginning and the end of the trial, animal's BW and BCS were recorded on two consecutive days. Initial and final BW were corrected for day of gestation with the following equation (Ferrell et al., 1976):

$$GU = 743.9e^{(0.02000-0.0000143t)t}$$

Where,

GU stands for *Gravid uterus* (fetus, fetal membranes, fetal fluids and uterus), *e* is a constant and *t* is the status of pregnancy in days.

Table 1. Ingredient composition of diets fed to heifers

| | Diets[1] (% of DM) | | | |
|---|---|---|---|---|
| Ingredient | CON | CO-EN | CS+ DDG | CS+ WDG |
| Corn Silage[2] | 88.8 | — | 73.6 | 73.6 |
| Soybean meal | 10.2 | — | — | — |
| Co-ensiled[3] | — | 98.1 | — | — |
| DDG[4] | — | — | 24.5 | — |
| WDG[5] | — | — | — | 24.5 |
| Mineral premix[6] | 1.8 | 1.9 | 1.9 | 1.9 |

[1] CON = control (corn silage with soybean meal), CO-EN = co-ensiled, CS+DDG = corn silage plus DDG added at mixing, CS+WDG = corn silage plus WDG with solubles added at mixing.
[2] Corn silage: 35% DM, 9.1% CP, 40% NDF (DM basis).
[3] Co-ensiled corn silage with WDG 3:1 (DM basis).
[4] DDG = Dry distillers grains with solubles.
[5] WDG = Wet distillers grains with solubles.
[6] 70% $CaCo_3$, 11.5% inorganic mix, 18.5% NaCl.

Table 2. Composition of diets (DM basis) fed to heifers

| | Diets[1] | | | |
|---|---|---|---|---|
| Ingredient | CON | CO-EN | CS+ DDG | CS+ WDG |
| NEg, Mcal/Kg [2] | 1.06 | 1.12 | 1.12 | 1.12 |
| CP, % | 12.2 | 12.5 | 12.4 | 12.4 |
| Prot. Sol., % CP | 44.7 | 41.6 | 38.3 | 34.5 |
| aNDF, % | 38.4 | 37.7 | 39.7 | 37.8 |
| ADF, % | 21.7 | 19.2 | 21.6 | 20.1 |
| DM, % | 38.3 | 36.1 | 38.8 | 40.5 |

[1] CON = control (corn silage with soybean meal), CO-EN = co-ensiled corn silage with wet distiller's grains 3:1 (DM basis), CS+DDG = corn silage plus dry distiller's grains with solubles added at mixing, CS+WDG = corn silage plus wet distiller's grains with solubles added at mixing.
[2] Dietary energy and protein were formulated using tabular values (NRC, 1982).

Heifer performance data was analyzed using the Proc GLM procedures of SAS (SAS Inst. Inc., Cary, NC) for a completely randomized design with pen as the experimental unit. Significant means ($P < 0.05$) were separated using the LSD method.

## Results and Discussion

By design, there was no difference in initial BW ($P = 0.39$) and initial BCS ($P = 0.36$) between treatments (Table 3). Heifers fed the CS+DDG diet had decreased DMI ($P < 0.01$) compared to all other diets. Heifers fed the CO-EN treatment had greater ADG ($P = 0.03$) than those fed the CON and CS+DDG diets. The CO-EN fed heifers also tended to have greater G:F ($P = 0.06$) compared to those fed the CON and CS+WDG. Similar results were reported by Larson et al. (1993) and Ham et al. (1994) when evaluating WDG in finishing steers and by Klopfenstein et al. (2007) when comparing WDG to corn-based diets. Heifers fed the CO-EN treatment had greater overall gain in BW ($P = 0.03$) compared to the CON and the CS+DDG treatments, while the CS+WDG treatment was intermediate. There was no significant differences in BCS ($P = 0.35$), final BCS ($P = 0.40$), or final BW ($P = 0.14$) due to dietary treatment.

The increased performance (ADG and BW change) observed with heifers fed the CO-EN treatment compared to CON and CS+DDG treatments may be due, in part, to differences in DMI. It is interesting to note, however, that there were no differences in performance between heifers fed the CO-EN and CS+WDG diets, but there was a tendency ($P < 0.06$) for the CO-EN heifers to be more efficient.

Results from this study suggest that WDG co-ensiled with corn silage have equal or greater feeding value when fed to heifers in the last trimester of gestation compared to corn silage based diets supplemented with soybean meal, DDG or WDG at feeding time. This creates an opportunity for smaller production units to utilize the WDG in their feeding management plans.

## Implications

Co-ensiling WDG with corn silage not only appears to enhance animal performance but also provides an economically viable feed source.

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Table 3. Effect of treatments on performance of Angus heifers during the last trimester of gestation

| | Treatments[1,2] | | | | | |
|---|---|---|---|---|---|---|
| Item | CON | CO-EN | CS+DDG | CS+WDG | SEM | *P* |
| DMI, kg | 7.83[a] | 8.04[a] | 6.99[b] | 8.14[a] | 0.10 | 0.01 |
| Initial BW, kg | 521.2 | 521.3 | 524.2 | 523.2 | 1.90 | 0.39 |
| Initial BCS | 5.43 | 5.36 | 5.33 | 5.27 | 0.09 | 0.36 |
| ADG, kg | 0.83[b] | 1.05[a] | 0.89[b] | 0.95[ab] | 0.07 | 0.03 |
| G:F | 0.106 | 0.130 | 0.127 | 0.117 | 0.01 | 0.06 |
| Final BW, kg | 572.8 | 586.2 | 579.5 | 582.0 | 5.40 | 0.14 |
| Final BCS | 5.62 | 5.73 | 5.54 | 5.48 | 0.15 | 0.40 |
| Change in BW, kg | 51.5[b] | 65.0[a] | 55.0[b] | 58.8[ab] | 4.15 | 0.03 |
| Change in BCS | 0.19 | 0.38 | 0.21 | 0.21 | 0.11 | 0.35 |

[1] CON = control (corn silage with soybean meal), CO-EN = co-ensiled corn silage with wet distiller's grains plus solubles 3:1 (DM basis), CS+DDG = corn silage plus dry distiller's grains with solubles added at mixing, CS+WDG = corn silage plus wet distiller's grains with solubles added at mixing.

[2] Means within a row lacking a common superscript differ ($P < 0.05$)

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# EFFECTS OF CRUDE GLYCERIN ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF MARKET LAMBS

**A.F. Musselman, M.L. Van Emon, P.J. Gunn, C.P. Rusk, M.K. Neary, R.P. Lemenager, and S.L. Lake**
Department of Youth Development and Agricultural Education and Department of Animal Sciences,
Purdue University, West Lafayette, IN 47977

**ABSTRACT:** The objectives of this study were to determine the effects of feeding crude glycerin on feedlot performance and carcass characteristics of market lambs. Forty-eight Southdown x Suffolk lambs (31.9 ± 5.4 kg; 24 ewes, 24 wethers) were blocked by weight and assigned randomly to one of four dietary treatments (1 ewe and 1 wether/pen; 8 pens/treatment): 1) 25% dried distiller's grains with solubles (CON), 2) 15% glycerin (15GLYC), 3) 30% glycerin (30GLYC), and 4) 45% glycerin (45GLYC). Crude glycerin (approximately 90% glycerin) replaced corn in the diet on a one to one basis. Weights were taken every 21 days to monitor BW change. Lambs were harvested when wethers reached an approximate 12th rib fat depth of 0.51 centimeters. Lambs fed CON and 15GLYC diets had greater DMI ($P < 0.001$), ADG ($P < 0.001$), G:F ($P < 0.001$), and had fewer days on feed ($P < 0.001$) compared with both the 30GLYC and 45GLYC treatments. No differences were detected in final body weight ($P = 0.76$), HCW ($P = 0.78$), LM area ($P = 0.44$), body wall thickness ($P = 0.41$), flank streaking ($P = 0.24$), or leg score ($P = 0.21$) due to dietary treatment. Lambs fed CON and 15GLYC diets also had greater dressing percentage ($P = 0.01$), 12th rib fat depth ($P = 0.002$), and yield grade ($P = 0.003$) compared with both the 30GLYC and 45GLYC diets; however, lambs on the 30GLYC and 45GLYC treatments tended to have greater LM ether extract ($P = 0.09$) compared with lambs on the CON and 15GLYC treatments. These results imply glycerin can be added at up to 15% DM in the diet of market lambs without decreasing feedlot performance or carcass characteristics.

**Keywords**: Feedlot, Glycerin, Lamb

## Introduction

The drastic increase in ethanol and biodiesel production has led to elevated prices of traditional feedstuffs; leaving livestock producers searching for alternative feeds to lower production costs and maintain performance. Production of biodiesel in the U.S. over the next decade is expected to yield an estimated 1.4 billion pounds of glycerin. The price of crude glycerin is expected to drop from $ 0.20-0.25 cents per pound to $0.05 cents per pound, potentially providing a cost-effective alternative energy feed resource for livestock.

In ruminant animals, glycerol can be rapidly converted to propionic acid and readily absorbed through the rumen wall (Kijora et al., 1997). Of the primary volatile fatty acids, propionate is the only one which is directly gluconeogenic. Feeding glycerol increased ruminal propionate and subsequently increased circulatory glucose concentration in cattle (Chung et al., 2007; Trabue et al., 2007). Similarly, total organic matter digestibility was not influenced due to varying levels of glycerol in diets containing low levels of starch (Schröder and Südekum, 1999). Schröder and Südekum (1999) found that feeding glycerol decreased the acetate:propionate ratio and stimulated water intake, both of which were beneficial to transition dairy cows. Johns (1953) reported that adding glycerol to sheep rumen contents resulted in the formation of propionic acid. DeFrain et al. (2004) reported that substitution of corn with glycerol resulted in similar plasma glucose concentrations in dairy cattle, suggesting that glycerol has the potential to act as an energy substitute for ruminant animals. Our hypothesis was that glycerol may act as a viable energy substitute in finishing lamb rations. Therefore, our objectives were to determine feedlot performance and carcass characteristics of feedlot lambs.

## Materials and Methods

### *General*

All protocols for this study were approved by the Purdue Animal Care and Use Committee. The experiment was conducted from May through September 2007 at the Purdue University Animal Science Research and Education Center. Forty-eight crossbred lambs (31.9 ± 5.4 kg; 24 ewes, 24 wethers) were blocked by weight and randomly assigned (1 ewe and 1 wether/pen; 8 pens/treatment) to one of four dietary treatments (Table 1): 1) corn-based feedlot ration (CON), 2) CON diet with 15% crude glycerin (approximately 90% glycerol) added to replace corn (15GLYC), 3) CON diet with 30% crude glycerin added to replace corn (30GLYC), and 4) CON diet with 45% crude glycerin added to replace corn (45GLYC).

### *Laboratory Analysis*

Weights for the lambs were collected every 21 days to monitor change in BW. Wether lambs were harvested when they obtained an approximate back fat thickness of 0.51 cm and the respective paired ewe lamb was removed from the study and returned to the Purdue University flock. Wether lambs were harvested at the Purdue University Meats Laboratory where HCW, flank streaking scores, LM area (taken by tracing the muscle at the 12th rib), and leg score conformations were evaluated 24 h after harvest and recorded. A 12th rib sample was also taken at this time, frozen in liquid nitrogen and stored for later ether extract analysis.

Table 1. Composition of diets fed to finishing lambs[a] (DM %)

| Item | CON | 15GLYC | 30GLYC | 45GLYC |
|---|---|---|---|---|
| Alfa-grass hay | 10.8 | 10.7 | 10.6 | 19.9 |
| Corn | 59.9 | 24.5 | 8.1 | – |
| DDGS | 25.1 | 25.0 | 25.2 | 25.1 |
| Mineral | 3.0 | 3.0 | 3.0 | 3.0 |
| Molasses | 1.2 | 1.2 | 1.2 | 1.2 |
| Gluten | – | 1.2 | 3.4 | 6.1 |
| Soyhulls | – | 19.5 | 17.9 | – |
| Crude Glycerin | – | 14.9 | 30.6 | 44.7 |

[a] Dietary Treatments: 1) corn-based feedlot ration (CON), 2) CON diet with 15% crude glycerin (approximately 90% glycerol) added to replace corn (15GLYC), 3) CON diet with 30% crude glycerin added to replace corn (30GLYC), and 4) CON diet with 45% crude glycerin added to replace corn (45GLYC).

***Statistical Analysis***

Data was analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Treatment was tested against the dependent variables. The model statement included the effect of treatment on specific characteristics to calculate the variations from the mean. The lsmeans statement was used to compute the adjusted means for the different treatments.

## Results and Discussion

The effects of crude glycerin on feedlot performance of market lambs are presented in Table 2. Lambs fed the CON and 15GLYC treatments had greatest DMI ($P$ <0.0001), ADG ($P$ <0.0001), G:F ($P$ <0.0001) and fewer days on feed (P <.0001, 105 ± 37 d) compared with both the 30GLYC and 45GLYC treatments. The lambs on the CON and 15GLYC treatments finished an average of 28 d earlier than those on the 30GLYC treatment and an average of 63 d earlier than the lambs on the 45GLYC treatment. The increased number of days on feed when glycerol is fed at levels greater than 15% could be a significant economic disadvantage. Similar results for ADG and feed efficiency observed in this study for the 15GLYC lambs were reported when 10% glycerin was added to the finishing diet of Angus-cross steers (Pyatt et al., 2007). Similarly, Schröder and Südekum (1999) reported no difference in DMI when glycerol was fed at 10% of DM as a replacement for fermentable starch in the diet of dairy cows.

The effects of crude glycerin on carcass characteristics of market lambs are presented in Table 3. No differences were detected in final BW ($P$ = 0.76), HCW ($P$ = 0.78), 12th rib LM area ($P$ = 0.44), body wall thickness ($P$ = 0.41), flank streaking ($P$ = 0.24), or leg score ($P$ = 0.21) due to dietary treatment. However, lambs on the CON and 15GLYC treatments had the greatest dressing percentage ($P$ = 0.01), back fat thickness ($P$ = 0.002), and yield grade ($P$ = 0.003) compared to the 30GLYC and 45GLYC treatments. However, lambs fed the 30GLYC and 45GLYC treatments tended to have greater ether extract ($P$ = 0.09) compared with the lambs fed the CON and 15GLYC treatments.

Though the lambs on the CON and 15GLYC treatments outperformed the lambs on the 30GLYC and 45GLYC, it was determined that glycerin can be used by the tissues as an energy source and it can be used to replace a portion of dietary corn in the lamb finishing diets.

## Implications

These results suggest that crude glycerin (90%) can be added at up to 15% of the DM in finishing diets of lambs without decreasing feedlot performance or carcass characteristics.

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Table 2. Effects of dietary treatment on finish lamb performance[a]

| Item | CON | 15GLYC | 30GLYC | 45GLYC | SEM[b] | P value |
|---|---|---|---|---|---|---|
| Lambs, no. | 12 | 12 | 12 | 12 | | |
| InitialWt, kg | 29.0 | 28.7 | 29.2 | 29.1 | 29.1 | 0.89 |
| StartWt, kg | 33.6 | 32.8 | 33.5 | 32.5 | 0.7 | 0.64 |
| EndWt, kg | 54.83 | 53.85 | 56.40 | 54.60 | 1.70 | 0.76 |
| DMI, kg | 2.80[cd] | 2.92[c] | 2.56[d] | 2.13[e] | 0.08 | < 0.001 |
| ADG, kg/d | 0.32[c] | 0.25[d] | 0.21[e] | 0.15[f] | 0.01 | < 0.001 |
| Feed efficiency, G:F, kg | 0.12[c] | 0.08[d] | 0.08[de] | 0.06[e] | 0.01 | < 0.001 |
| Days | 82.67[e] | 82.50[e] | 110.83[d] | 145.67[c] | 7.89 | < 0.001 |

[a] Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% crude glycerin (approximately 90% glycerol) added at the expense of corn (15GLYC), CON diet with 30% crude glycerin added at the expense of corn (30GLYC), and CON diet with 45% crude glycerin added at the expense of corn (45GLYC).
[b] Greatest SEM is presented.
[c-f] Means within a row lacking a common superscript differ

Table 3. Effects of dietary treatment on carcass characteristics of finishing lambs[a]

| Item | CON | 15GLYC | 30GLYC | 45GLYC | SEM[b] | P value |
|---|---|---|---|---|---|---|
| Lambs, no. | 6 | 6 | 6 | 5 | | |
| Hot Carcass Wt, kg | 32.4 | 32.5 | 31.80 | 30.9 | 1.3 | 0.78 |
| Dressing Percentage | 57.7[c] | 58.5[c] | 55.5[d] | 55.3[d] | 0.8 | 0.01 |
| Fat Depth, cm | 0.67[c] | 0.65[c] | 0.38[d] | 0.34[d] | 0.07 | 0.001 |
| Ribeye Area, $cm^2$ | 57.2 | 54.7 | 68.3 | 46.2 | 10.0 | 0.45 |
| Body Wall, cm | 2.7 | 2.7 | 2.3 | 2.3 | 0.25 | 0.41 |
| Yield Grade | 2.96[c] | 2.99[c] | 1.88[d] | 1.84[d] | 0.26 | 0.003 |
| Quality Grade, Flank Streaking | 20.7 | 19.8 | 20.0 | 19.4 | 0.5 | 0.24 |
| Leg Score | 13.0 | 13.0 | 13.3 | 12.2 | 0.4 | 0.21 |
| Ether Extract % Fat | 9.4 | 7.2 | 4.7 | 4.2 | 1.5 | 0.09 |

[a] Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% crude glycerin (approximately 90% glycerol) added at the expense of corn (15GLYC), CON diet with 30% crude glycerin added at the expense of corn (30GLYC), and CON diet with 45% crude glycerin added at the expense of corn (45GLYC).
[b] Greatest SEM is presented.
[c,d] Means within a row lacking a common superscript differ

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# EFFECT OF MATERNAL DIETARY SELENIUM AND NUTRIENT RESTRICTION ON FETAL JEJUNAL PROLIFERATION AND VASCULARITY

J.J. Reed*[1], M.A. Ward[1], K.A. Vonnahme[1], T.L. Neville[1], P.P. Borowicz[1], J.B. Taylor[2], D.A. Redmer[1], A.T. Grazul-Bilska[1], L.P. Reynolds[1], and J.S. Caton[1]

[1]Center for Nutrition and Pregnancy, Department of Animal Sciences, North Dakota State University, Fargo
[2]USDA-ARS, U. S. Sheep Experiment Station, Dubois, ID

**ABSTRACT:** In Experiment (Exp.) 1, western whiteface ewes (n = 32; BW = 45.6 ± 2.2 kg) were allotted to one of four treatments in a completely randomized design to examine effects of maternal dietary Se and source on fetal jejunal crypt cell proliferation. Maternal diets contained (DM basis) no added Se (control) or supranutritional Se included as high-Se wheat (**SW**) and sodium selenate (**S3** and **S15**). Treatments were initiated at 50 ± 5 d of pregnancy. The control, SW, S3, and S15 diets provided 2.5, 75, 75, and 375 µg Se/kg BW, respectively. On day 134 ± 10 of pregnancy, ewes and lambs were necropsied and tissues were harvested. In Exp. 2, western whiteface ewes (n = 36; BW = 53.8 ± 1.3 kg) were allotted to treatments in a 2 x 2 factorial arrangement to examine effects of maternal nutrition and dietary Se on fetal jejunal crypt cell proliferation and vascularity. Treatments were maternal nutrition (100% of requirements [**CON**] vs. 60% of CON [**RES**]) and dietary Se (6 µg Se/kg BW/d [**ASe**] and 80 µg Se/kg BW/d [**HSe**]) from Se enriched yeast. Selenium treatments were initiated 21 d before breeding and maternal nutrition treatments began on d 64 of gestation. On d 135 ± 5 of gestation, ewes and lambs were necropsied and tissues harvested. In Exp. 1, maternal nutrition did not affect ($P$ = 0.96) percentage of proliferating nuclei in jejunal crypts. In Exp. 2, compared to RES fetuses, CON had greater ($P$ = 0.01) small intestinal mass. HSe fetuses tended ($P$ = 0.14) to have greater small intestinal mass than ASe. Total microvascular volume was greater ($P$ = 0.002) in CON compared to RES fetuses; a Se effect was not detected ($P$ = 0.37). Neither maternal nutrition nor Se affected percent proliferating jejunal nuclei ($P$ = 0.99) or percent vascularity and capillary area ($P$ > 0.32). Maternal nutrition appears to impact fetal intestinal mass and total microvascular volume, but not percent vascularity or crypt cell proliferation.

**Key words**: nutrient restriction, pregnancy, selenium

## Introduction

The lifelong regulation of normal growth, development, and nutrient utilization are likely programmed *in utero* (Wu et al., 2006). Visceral tissues utilize a disproportional amount of energy in relationship to their contribution to overall body mass (Ferrell, 1988; Reeds et al., 1999). Specifically, the gastrointestinal tract consumes approximately 20% of maintenance energy (Webster, 1989; Eisemann and Nienaber, 1990). Therefore, changes in gut metabolism can have a profound affect on overall animal energy expenditure and thus, growth efficiency. Recently, our laboratory has evaluated the effects of dietary Se on intestinal growth in fast growing animals. Jejunal mass was increased, and consequently, total number of jejunal proliferating cells was almost double in finishing steers fed high-Se wheat (Soto-Navarro et al., 2004). In addition, supranutritional Se increased percentage of proliferating crypt cells in the jejunum and total proliferating crypt cells within the jejunal mucosa when fed to rapidly gaining (282 g/d), pregnant ewe lambs (Neville et al., 2008). Hammer et al. (2007) reported altered ability to absorb IgG in lambs due to maternal dietary Se and plane of nutrition. Therefore, our objectives were to determine if maternal dietary Se and nutrient restriction effected fetal small intestinal mass and jejunal proliferation and vascularity at approximately 130 d of gestational age.

## Materials and Methods

For both experiments, the North Dakota State University Animal Care and Use Committee approved the care and use of the animals. Ewes were individually housed in 0.91 x 1.2-m pens in a temperature controlled (12°C) and ventilated facility for the duration of the study. Lighting within the facility was automatically timed to mimic ambient daylight. Ewes were provided free access to water and a trace mineralized salt (containing no additional Se; American Stockman, Overland Park, KS).

In Exp. 1, thirty-two pregnant Targhee ewe lambs (45.6 ± 2.2 kg) were allotted randomly to 1 of 4 treatments in a completely randomized design. Treatments (initiated on d 50 ± 5 d gestation) were as follows: control (0.1 ppm Se), Se wheat (SW; 3 ppm Se), selenate fed at 3 ppm (S3), and selenate fed at 15 ppm (S15). The SW and S3 diets provided 75 µg/kg of BW of Se, whereas the S15 treatment provided 375 µg/kg of BW of Se. Diets contained 5% soybean hulls, 33.5% beet pulp, 2.5% soybean meal, 27% alfalfa, and 32% wheat (DM basis). The SW diet was formulated by replacing wheat in the control diet with a high (9 ppm)-Se wheat from a seleniferous region near Pierre, South Dakota. Ewes on selenate treatments received control pellets top-dressed with a tap water-based selenate solution. Diets (DM basis) were similar in CP (15.5%) and energy (2.68 Mcal of ME/kg) and were fed to meet or exceed the NRC requirements (NRC, 1985). All diets were delivered in a complete pelleted form (0.48-cm diam.) and were fed twice daily. Ewes were fed at a rate of 2.5% BW (as fed) of their respective treatment diets daily, with BW measured every 14 d.

In Exp. 2, twenty-one days before breeding, ewes were assigned randomly to Se treatments (adequate vs. high Se), and Se supplementation began and was continuous throughout the study. On d 64 of gestation, 36 pregnant ewe lambs (53.8 ± 1.3 kg) were assigned randomly to 1 of 4 treatments in a completely randomized design, with the treatments arranged as a 2 x 2 factorial. Main effects evaluated were dietary levels of Se (adequate vs. high Se), and plane of nutrition [100% (control) vs. 60% (restricted) of the NRC (1985) requirements for gestating ewe lambs]. The high-Se group received 80 µg/kg of BW (81 µg/kg of BW for control-high-Se and 78 µg/kg of BW for restricted-high-Se ewes) and the adequate group received 6 µg/kg of BW (7 µg/kg of BW for control- adequate-Se and 4 µg/kg of BW for restricted-adequate-Se ewes). Coupling ewe BW with Se intake values yielded total Se intakes that were 0.37, 4.41, 0.21, and 4.17 mg/d for control-adequate-Se, control-high-Se, restricted-adequate-Se, and restricted-high-Se ewes, respectively. Diets (DM basis) were similar in CP (16.0%) and ME (2.12 Mcal of ME/kg), were fed individually, and consisted of alfalfa hay (chopped, 3.8 cm in length), 0.42 ppm (mg/kg) of Se, whole corn (offered when additional energy was needed to meet ME requirements), and pelleted (0.48-cm diameter) supplements. The adequate-Se supplement (0.32 ppm of Se) contained 96% corn and 4% molasses, whereas the HSe supplement (43.2 ppm of Se) contained 88% corn, 4% molasses, and 8% Se-enriched yeast (DM basis; Sel-Plex, Alltech Inc., Nicholasville, KY). Chopped alfalfa was top-dressed with supplement and corn. Nutrient requirements were based on the NRC (1985) recommendations for 60-kg pregnant ewe lambs during mid to late gestation (weighted ADG of 140 g). Intake of the respective supplements and corn were calculated based on BW, ME requirements, and supplement ME and Se concentrations.

*Slaughter and Tissue Collection.* In Exp. 1 on day 134 ± 10 of gestation, and in Exp. 2 on d 135 ± 5 of gestation, ewes and lambs were necropsied and tissues were harvested as described by Reed et al. (2007).

*Jejunal Proliferation and Vascularity*. To measure cellular proliferation in jejunal tissue, cross sections of fresh intestinal tissue were made from a section of jejunum. Tissue sections were immersed in Carnoy's solution (60% ethanol, 30% chloroform, and 10% glacial acetic acid, vol/vol/vol) for 3 h. The tissues were subsequently transferred to a 70% (vol/vol) ethanol solution until embedded in paraffin (Reynolds and Redmer, 1992). Tissue sections (4-µm thick) were made from the paraffin blocks, mounted on glass slides, and prepared for counterstaining procedures, as described by Fricke et al. (1997) and Soto-Navarro et al. (2004). Tissue sections were treated with blocking buffer consisting of PBS and 1.5% (vol/vol) normal horse serum (Vector Laboratories, Burlingame, CA) for 20 min. Sections of fixed tissues were incubated with mouse anti-proliferating cell nuclear antigen monoclonal antibody (Clone PC-10; Roche Diagnostics Corp., Indianapolis, IN) at 1 µg/mL in blocking buffer (Fricke et al., 1997; Scheaffer et al., 2003). Primary antibody was detected by using a biotinylated secondary antibody (horse anti-mouse immunoglobulin G, Vectastain; Vector Laboratories) and Avidin-Biotin Complex system (Vectastain; Vector Laboratories). Tissue sections were counterstained with Nuclear Fast red to visualize unlabeled nuclei. Number of cells proliferating was calculated by dividing total jejunal DNA by 6.6 x $10^{-12}$ g and then multiplying by the percentage of cell proliferation (Baserga, 1985; Zheng et al., 1994).

To measure the vascularity of the small intestine, a portion of the freshly excised gastrointestinal tract was perfusion-fixed. Procedures used for tissue preparation were similar to those previously described (Scheaffer et al., 2004; Soto-Navarro et al., 2004), except that epoxy casting resin [Mercox, consisting of 0.8 mL of catalyst, 5 mL of diluent (methyl methanylate), and 5 mL of resin, which were all from Ladd Industries, Williston, VT] was allowed to set for 30 min rather than for 75 min. Cross sections of perfused intestinal tissue were processed in a similar fashion as the jejunal tissues (described above). The process of measuring vascularity of the jejunal tissue required 4-µm-thick tissue sections that were stained using periodic acid-Schiff's staining procedures (Luna, 1968) to provide contrast to the vascular tissue. Measurements were made using image analysis software (Image Pro Plus 5.0, MediaCybernetics), as previously described (Borowicz et al., 2007).

*Statistics*. In both studies, fetal number was included in the model and was retained when significant ($P \leq 0.10$) and dropped when not significant ($P > 0.10$). For Exp. 1, data were analyzed using ANOVA (PROC GLM, SAS Inst. Inc., Cary, NC). Contrasts were used to evaluate differences between level and source of dietary Se. Specifically, contrasts were made between control vs. Se treatments (SW, S3, and S15), SW vs. S3, and S3 vs. S15 and were considered different at $P \leq 0.10$. For Exp. 2, data were analyzed as a completely randomized design with a 2 x 2 factorial arrangement of treatments using PROC GLM (SAS Inst. Inc., Cary, NC. The model contained effects for nutrition (control vs. restricted), level of Se (adequate vs. high), and the nutrition x Se interaction. When interactions were present ($P < 0.10$), means were separated by the least significant difference test. Main effects were considered significant when $P < 0.10$.

## Results

In Exp. 1, level and source of maternal dietary Se did not affect ($P = 0.96$) percentage of proliferating nuclei in jejunal crypts (Table 1). In Exp. 2, compared to RES fetuses, CON had greater ($P = 0.01$) small intestinal mass (Table 2). Ewes fed HSe had fetuses that tended ($P = 0.14$) to have greater small intestinal mass when compared with those fed ASe. Total jejunal microvascular volume was greater ($P = 0.002$) in fetuses from CON compared with RES ewes. No effect of maternal dietary Se was detected ($P = 0.37$) in Exp. 2. Neither maternal nutrition nor Se affected percent proliferating jejunal nuclei ($P = 0.99$) or percent vascularity and capillary area ($P > 0.32$).

## Discussion

Similar to Exp. 2, Osgerby et al. (2002) reported decreased gut weight (g and as % BW) at d 135 of gestation due to 70% maternal diet restriction initiated at d 22 of gestation, though no differences were found in gut weight at

d 45 or 90. Wallace et al. (2000), reported that ewes fed ad libitum intake had fetuses (d 128 of gestation) with decreased gut weight, but when expressed relative to fetal body weight, gut weight was greater than in fetuses from control ewes. Thus, undernutrition and overnutrition have been shown to affect gut weight.

Nutrient restriction and Se have been shown to alter maternal proliferation in jejunum and jejunal mucosa of pregnant ewe lambs (Reed et al., 2007; Neville et al., 2008). In recent data from our laboratory (unpublished data), no differences were observed for percent proliferating nuclei, however total number of cells proliferating was greater in offspring from control compared to restricted or high nutrition ewes. However, in the current studies jejunal proliferation was not affected by maternal nutrition.

Recently, maternal Se intake has been shown to change lamb IgG absorption, digestion, and growth. Hammer et al. (2007) reported that maternal nutrition and high dietary Se alter IgG absorption after birth. Absorption of IgG was 2276, 1586, and 1214 mg/dl for lambs from restricted, moderate, and high nutrition, respectively and was 1912 and 1472 mg/dl for lambs from adequate Se and high Se ewes, respectively.

Lambs born to restricted or overfed ewes were lighter at birth compared to lambs born to control ewes (Caton et al., 2007). Caton et al. (2007) reported that total tract digestion of DM, OM, NDF and ADF were reduced by high maternal Se intake at 13 and 19 wk of age. Additionally, offspring from overfed, adequate Se ewes had increased ADG and gain:feed (Caton et al., 2007) at 13 and 19 wk of age. Changes in fetal gut due to maternal nutrition were expected, since maternal nutrition has been shown to affect IgG absorption, digestion, and growth.

Changes in fetal small intestinal weight due to maternal nutrition were reported in the current study, as well as others (Wallace et al., 2000; Osgerby et al., 2002). Other researchers have reported changes in IgG absorption (Hammer et al., 2007) digestion, and gain:feed (Caton et al., 2007) in lambs due to maternal nutrition. Therefore, changes in fetal gut proliferation and vascularity may be expected. In the current study, there was an increase in total microvascular volume in control compared with restricted fetuses, which was driven by increased small intestinal weight; however, there were no differences in fetal jejunal percent proliferation, percent vascularity, or capillary area.

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Table 1. Effect of source and level of maternal dietary Se on fetal jejunal proliferation (Exp. 1)

| | Treatments[1] | | | | | Contrasts[2] | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | SW | Se3 | Se15 | SEM | Con vs Se | SW vs S3 | S3 vs S15 |
| Proliferating nuclei area, $\mu^2$ | 8407 | 9042 | 8120 | 9249 | 634 | 0.55 | 0.30 | 0.18 |
| Nonproliferating nuclei area, $\mu^2$ | 5971 | 7505 | 6137 | 7130 | 1083 | 0.41 | 0.36 | 0.49 |
| % proliferation[3] | 54.7 | 52.0 | 53.0 | 52.8 | 4.0 | 0.62 | 0.86 | 0.97 |

[1]CON = control, SW = selenium wheat, 3ppm Se, Se3 = selenate, 3 ppm Se, and Se15 = selenate, 15 ppm Se. Treatments were applied from d 50 to d 134 of gestation and samples were collected at d 134 of gestation.
[2]Con vs Se = control vs Se treated ewes (SW, Se3, and Se15); SW vs S3 = Se wheat vs 3 ppm selenate; S3 vs S15 = 3ppm selenate vs 15 ppm selenate .
[3]% proliferation = proliferating nuclei area/(proliferating nuclei area + nonproliferating nuclei area).

Table 2. Effect of level of maternal nutrition and Se on fetal small intestine mass and jejunal proliferation and vascularity (Exp. 2)

| | Nutrition[1] | | Selenium[2] | | | *P*-Value[3] | | |
|---|---|---|---|---|---|---|---|---|
| Item | CON | RES | ASe | HSe | SEM | Nut | Se | Nut*Se |
| Small intestine mass, g | 62.4 | 53.8 | 55.6 | 60.5 | 2.4 | 0.01 | 0.14 | 0.70 |
| Proliferating nuclei, % | 51.7 | 52.2 | 52.3 | 51.6 | 2.4 | 0.89 | 0.85 | 0.97 |
| Small Intestine | | | | | | | | |
| Total cells x $10^{11}$ | 364.0 | 305.5 | 337.7 | 331.7 | 28.0 | 0.13 | 0.87 | 0.43 |
| Total cell proliferation x $10^{11}$ | 188.0 | 159.5 | 172.2 | 175.3 | 16.9 | 0.22 | 0.90 | 0.74 |
| Capillary area density[4], % | 18.8 | 17.6 | 18.5 | 17.9 | 0.57 | 0.13 | 0.41 | 0.40 |
| Capillary number density[5], $mm^2$ | 998.8 | 1011.0 | 1019.2 | 990.6 | 35.2 | 0.80 | 0.55 | 0.15 |
| Capillary surface density[6], ($\mu m/\mu m^2$) | 0.1002 | 0.1006 | 0.0983 | 0.1024 | 0.0026 | 0.90 | 0.26 | 0.14 |
| Area/capillary[7], µm2 | 199.2 | 189.8 | 197.9 | 191.1 | 12.3 | 0.58 | 0.69 | 0.91 |
| Total microvascular volume, mL[8] | 11.58 | 8.77 | 9.97 | 10.37 | 0.64 | 0.002 | 0.37 | 0.40 |

[1]Nutritional treatments (applied from d 60 to d 135 of gestation were control (CON) and restricted (RES; 60% of controls).
[2]Selenium treatments (applied from 21 d before breeding until d 135 of gestation) were daily intake of organically-bound Se; adequate Se (ASe; 6 µg/kg BW) and high Se (HSe; 80 µg/kg BW).
[3]Probability values for effects of nutrition (Nut), selenium (Se), and the interaction. Absence of Nut x Se interactions ($P > 0.10$) allowed for presentation of main effects.
[4]Capillary area density = (capillary area /tissue area evaluated) x 100.
[5]Capillary number density = (capillary number/tissue area evaluated)*1,000,000. This calculation provides number of capillaries per $mm^2$ of tissue area analyzed.
[6]Capillary surface density = (total capillary circumference/tissue area evaluated)*10.
[7]Area per capillary = capillary area/capillary number per sample area.
[8]Total microvascular volume = capillary area density (%) x jejunal mass (g).

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# IMPACT OF NUTRIENT IMBALANCE ON RUMEN AND BLOOD METABOLITES

**M. B. Horvath*, S. L. Ivey, C. M. Gardner, J. D. Walsh, and D. E. Hawkins**
Department of Animal Science, New Mexico State University 88003

**ABSTRACT:** Nutrient balance is key in optimizing performance and production potential in livestock. Adverse effects resulting from imbalances between CP and carbohydrate are often ignored when cattle graze dormant forage. Objectives of this study were to determine ruminal and metabolic changes in cattle fed forage diets with varying CP:NDF ratios. Eighteen 2 yr old crossbred heifers (428 ± 44 kg) were stratified by weight and assigned to 1 of 3 treatments (trt). Treatments were: 1) 50% Buffalo Straw-50% Old World Blue Stem; CP:NDF = 0.07), 2) 50% Buffalo Straw:Old World Blue Stem 50% Sudan; CP:NDF = 0.13, and 3) 100% Sudan; CP:NDF = 0.17. Heifers were given ad libitum access to trt for 29 d. Serum and plasma were collected every other d and rumen fluid (RF) daily. Daily ruminal samples collected for VFA analysis were pooled by wk. Total VFA and acetate did not differ by trt P > 0.07), however, total VFA, acetate to propionate ratio (A:P) increased by wk with the greatest difference being between wk 1 and wk 5 (38.3, 41.8, and 41.7% respectively; P < 0.01). A linear increase in propionate and A:P ratios was observed with decreasing nutrient imbalance (P < 0.03). Ruminal ammonia increased with increasing CP:NDF (linear, P < 0.01). Ruminal ammonia differed by d (P < 0.01) with the lowest level observed on d 15 (0.49 mmol) and the highest on d 25 (2.07 mmol). Serum NEFA decreased with decreasing CP:NDF (linear, P < 0.01). During the study d influenced (P = 0.02) NEFA concentration, such that d 3 displayed the highest NEFA and d 26 the lowest. Serum glucose was not different for trt (P = 0.95) or d (P = 0.55). These data indicate that ruminal microbial populations can adapt to maintain fermentation of feedstuffs in light of nutrient imbalances. Knowledge of mechanisms controlling adaptation of the microbial population may allow for better understanding of nutrient imbalance impacts on livestock productivity.

Keywords: Beef cattle, nutrient imbalance, methylglyoxal

## Introduction

Nutrient balance pertains to proportions of nutrients to one another in ruminant diets. These nutrients can be used by the rumen microbial population to meet their needs and supply energy and protein to the host animal. Equally important is nutrient synchrony, which takes into account the fermentation pattern of the dietary nutrients specifically the time it takes to breakdown of CP and carbohydrate. When dietary crude protein and carbohydrate release is synchronized become available for utilization at the same time, allowing for improved nutrient balance (Hall and Huntington, 2007). Because livestock cannot perform above or beyond the most limiting dietary nutrient (Beames, 1959; Wallace, 1987) a balance is necessary but equally important is the synchrony of release.

Valkeners and co-workers (2004, 2006) have shown that short term imbalances between CP and carbohydrate minimally impact performance of cattle. This is further supported by work of Dawson (1999) which showed ruminal recycling of urea aids microbes in overcoming N deficiencies, enabling ruminal and metabolic systems to remain stable for periods of time. In vitro work by Russell and Strobel (1987), found ammonia could be transported into microbes reaching high intracellular concentrations despite low concentrations in media. These authors suggested this mechanism of active ammonia transport is likely present in vivo, allowing cellulolytic microbes to obtain ammonia from the ruminal environment, despite low N levels.

Nutrient balance and synchrony of nutrient release are often overlooked in grazing ruminants. Ruminants are fortunately able to modify grazing habits which offset potential imbalances and arrhythmic breakdown of CP in relation to carbohydrate. Thus, slowly digested carbohydrate from the first grazing bout becomes available for utilization at the same time as readily degradable CP from the second or third grazing bout. Changes in grazing behavior allow for re-establishment of nutrient synchronization, allowing maximal utilization of available nutrients (Hall and Huntington, 2007) and partial re-establishment of nutrient balance.

Experimental objectives of the current study were to determine effects of imbalances between CP and carbohydrate on rumen and blood metabolites in heifers fed diets of differing CP to carbohydrate ratios for 5 wk to simulate the different forage qualities consumed by grazing ruminants. It was hypothesized that ruminal and metabolic systems enable ruminants to manage nutrient imbalances by adaptation of microbial populations to changing forage nutrient status.

## Materials and Methods

A study was conducted at the New Mexico State University, Campus Farm, in Las Cruces, NM. Animal handling and experimental procedures were conducted in accordance with guidelines of the Institutional Animal Care and Use Committee of New Mexico State University. Eighteen, two yr old crossbred heifers (428 ± 96 kg) were stratified by weight into one of three treatments, penned and fed separately. Treatments were designed to provide differing CP:NDF ratios and consisted of (As fed basis) 1) 50% Buffalo Straw: 50% Old World Blue Stem (**LOW**; CP:NDF = 0.07 2) 50% BS-OW: 50% Sudan (**MID**; CP:NDF = 0.13 3) 100% Sudan (**HIGH;** CP:NDF = 0.17).

No adaptation to diets was allowed with data collection beginning immediately at the start of the experiment. This was done in an effort to determine which ruminal and metabolic impacts incurred from inception of nutrient imbalance and what was the duration of these changes. Animals were allowed ad libitum access to treatments for 5 wk, with feed weighed and placed in each pen at 0700, then removed and weighed back at 1900, to determine daily intake.

Individual diets were sampled daily and refusal samples were collected each evening upon feed removal. Diet and ort samples were pooled by wk, sub-sampled and analyzed for CP, NDF, ADF, and ash. Rumen fluid was collected daily for ammonia quantification, and separate samples of rumen fluid were composited by wk for VFA analysis. Prior to feeding, serum was collected every other d at 0600 into serum separator tubes (Corvac, Sherwood Medical, St. Louis, MO), by coccygeal venapuncture for quantification of glucose, NEFA, and BUN.

Ruminal ammonia was analyzed using the phenol–hypochlorite procedure of Broderick and Kang (1980), adapted to a microtiter plate. Volatile fatty acid concentration of weekly composites was determined by gas chromatography (Star 3400, Varian, Walnut Creek, CA) utilizing methods of May and Galyean (1996). Serum samples were analyzed using commercially available kits to measure glucose (Sigma Diagnostics, St. Louis, MO), NEFA (Wako Chemicals USA, Inc., Richmond, VA), and BUN (Sigma Diagnostics)

Statistical analysis was performed using the MIXED procedure of SAS. Model included cow, trt, and d (wk in the case of VFA) as well as treatment • day (wk in the case of VFA) interactions. The repeated statement was used for analysis of serum samples which tested fixed effects of treatment, day (or week), treatment • day (or week) interactions. Observed level of significance was $P < 0.05$.

## Results and Discussion

Ruminal ammonia displayed a difference by trt ($P < 0.01$). LOW was different from MID ($P < 0.01$) and HIGH ($P < 0.01$), yet MID and HIGH did not differ ($P = 0.22$). Ammonia values obtained for LOW, MID, and HIGH (0.70, 1.15, and 1.33 ± 0.10 mM) were below those reported by Satter and Slyter (1974), in which forage or concentrate was provided as a substrate. These authors reported that 3 mM of ammonia was required for maximal microbial protein synthesis. Our data indicates ruminal ammonia levels were inadequate for optimal microbial protein synthesis according to levels defined by Satter and Slyter (1987). The low ammonia values reported agree with findings of Horvath et al (2007), and are not atypical of cattle consuming dormant forage. Concentrations greater than 0 mM could be interpreted as being in excess of microbial needs, resulting in excretion of urea by the animal and subsequent waste of valuable protein N. Low levels of ruminal ammonia may not be deleterious to the rumen environment, as long as microbial needs are met. Ammonia concentrations also differed by d ($P < 0.01$) dropping from 1.97 mM on d 1 to 0.55 mM on d 4, and remained low until d 27 when concentrations returned to similar levels (1.98 mM) observed on d 1. Diets did not supply the 3 mM proposed by Satter and Slyter (1974) for maximal microbial growth, yet d results show that populations were able to adapt to low ammonia and regain efficiency.

Stern and coworkers (1978) concluded that a major factor affecting utilization of degraded dietary N was the type and rate of carbohydrate availability. It is possible that slower breakdown and availability of carbohydrate on the high NDF containing LOW decreased immediate microbial need for ammonia. Conversely, the readily fermentable HIGH with its lower NDF content, increased immediate microbial demand for ammonia, which was met by higher levels of dietary CP. Thus, although nutrient imbalance broadened from HIGH to MID to LOW, a relative ruminal balance was evident due to carbohydrate breakdown patterns over time in relation to protein supply and microbial ammonia needs.

Total VFA concentration, which serves as an indicator of ruminal fermentation and in the current study showed no effect for trt ($P = 0.07$). Total VFA did vary by wk ($P < 0.01$). Total VFA concentrations exhibited a 61% decrease from 49.15 mM prior to initiation of the experiment, to 29.88 mM during wk 1 on trial, then increasing by 62% back to 48.41 mM by wk 5. Acetate increased ($P < 0.01$) from wk 1 (20.99 mol/100mol) to wk 5 (36.05 mol/100 mol), and propionate ($P < 0.01$) followed a similar trend from wk 1 (4.91 mol/100 mol) to wk 5 (8.40 mol/100 mol). Because of similarities in acetate and propionate change, there was no difference in A:P ratios throughout the trial ($P = 0.38$), which remained stable at 4.40 ± 0.33. The immediate decrease and subsequent increase in VFA concentrations further supports the idea of microbial adaptation to low quality, dormant forage diets. Rumen microbial populations capable of scavenging ample ammonia, and those which could digest and utilize available carbohydrate beyond maintenance requirements, prevailed and thrived. Time favored these populations and as the ruminal environment shifted, more efficient populations were capable of faster growth and greater turnover rates, while those which grew slower and required vast resources to meet maintenance needs declined (Russell et al, 1992). Subsequent growth and turnover rates of these more readily adaptable population's increased total VFA concentration. The energy gained from increased propionate flow to the small intestine inhibited need for tissue mobilization, allowing long term animal performance to stabilize and adapt to the provided trt.

NEFA levels decreased linearly ($P <0.01$) across LOW, MID, and HIGH. There was also a difference in NEFA levels by d ($P = 0.02$), heifers increased fat mobilization from d 1 (226 μM) to d 2 (325 μM), then declined to an average of 174 μM for the duration of the study. These data suggest that by wk 4, steadily increasing VFA concentrations (38.32 mM) produced by shifting microbial populations alleviated need for increased fat mobilization thus, NEFA levels recessed during wk 4 (170 μM) as heifers once again turned to VFA production as the primary energy source.

Blood urea nitrogen was not different by trt ($P = 0.14$) but differences were observed by d ($P < 0.01$).

Concentrations were greatest for d 1 (15.09 mg/dL), decreased to 6.56 mg/dL by d 2, then dropped below 4.99 mg/dL for the remainder of the trial. For nitrogen recycling to decrease and remain minimal is verification that dietary nitrogen may have been insufficient. Thus, the majority of dietary CP was broken down to ammonia in the rumen for incorporation into MCP. This resulted in minimal portal transport of excess N to the liver for urea synthesis. Coupled with the theory of Russell and Strobel (1987) which suggests ammonia can be transported into microbes and reach high intracellular concentrations, even when ruminal N levels are low. Support is lent to low BUN levels observed, as amino acid mobilization and portal transport of N and or urea to the rumen may not have been necessary to satisfy microbial needs.

Serum glucose showed no difference for trt ($P = 0.95$) or d ($P = 0.55$). As nutrient imbalances between CP and NDF broadened, dietary energy became insufficient as a result of decreasing propionate production. However, because heifers on trial were penned and had minimal production oriented need for glucose, low propionate levels may have been sufficient to fulfill maintenance glucose needs. This idea is confirmed by extremely low BUN concentrations after d 3, which reveal no significant N recycling, indicative of minimal amino acid utilization from mobilization of muscle. The fact that heifers were able to maintain constant glucose concentrations necessary for requirements, even with propionate production less than optimal, lends support to the concept of heifers being somewhat insulin insensitive. Insulin insensitivity would allow serum glucose levels to remain elevated, decreasing need for additional propionate, and eliminating demand for gluconeogenic amino acids from muscle breakdown. This would have resulted in consequent lack of significance between treatment and day, as glucose concentrations remained stable.

## Implications

Broad imbalances between CP and NDF are not uncommon in dormant forage scenarios and often result in mobilization of energy reserves, subsequent weight loss, and temporary depressions in individual animal performance. Mobilization of stored energy provides vital nutrients for fulfillment of maintenance requirements, allowing microbial populations time to adapt to changing diet and shifting CP:NDF ratios. This population deflection ultimately leads to more efficient utilization of forage. As populations shift and efficiency is regained, ruminal and metabolic systems once again normalize, allowing the animal to derive the majority of energy from grazed forage, and subsequent microbial byproducts. This achieved, the slow process of repletion may begin, as the cow redirects excess nutrients towards storage. Further work is necessary to gain a better appreciation of the intricate workings of nutrient balance, microbial adaptation, and subsequent effects resulting from imbalances. Better understanding such processes would allow for improvements upon low input supplementation strategies commonly utilized by commercial cow-calf enterprises, which would be of considerable worth in maximizing returns during formidable economic times.

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# SULFUR-INDUCED POLIOENCEPHALOMALACIA IN ROUGHAGE-FED FEEDLOT STEERS ADMINISTERED HIGH-SULFUR WATER.

**C.W. Knight*[1], K.C. Olson[2], C.L. Wright[2], K.J. Austin[1], K.M. Cammack[1], R. Cockrum[1]**
University of Wyoming, Laramie, WY[1], South Dakota State University, Brookings, SD[2]

**ABSTRACT:** Sulfur-induced polioencephalomalacia (PEM) is a neurological disorder affecting ruminants and is commonly associated with high dietary sulfur (S). The incidence of Sulfur-induced PEM is more prevalent in the U.S. where high concentrations of S compounds exist in stock water. Producers typically do not have access to cost-effective alternative water sources in these areas. In addition to high-sulfur water, sulfur-rich feedstuffs, such as byproducts of the ethanol industry, increase the probability of Sulfur-induced PEM. The purpose of this study was to confirm previously observed variation in individual response to increased concentrations of dietary S and determine if the mineral compound zeolite effectively binds excess hydrogen ions in the rumen, limiting hydrogen sulfide production, and therefore reducing the risk of Sulfur-induced PEM. Steers (n = 96) were assigned to one of four treatment groups for a 77 d period: control (CTRL; ≤ 400 ppm S); high-sulfur water (HS; ≥ 3000 ppm S); high-sulfur water plus high zeolite (HSHZ; ≥ 3000 ppm S + 5% zeolite); or high-sulfur water plus low zeolite (HSLZ; ≥ 3000 ppm S + 2.5% zeolite). The HSLZ treatment group had 5 confirmed cases and 4 suspected cases of Sulfur-induced PEM, the HSHZ treatment group had 3 confirmed cases and 1 suspected case, and the HS treatment had 1 confirmed case and 3 suspected cases. There were no differences ($P > 0.05$) in the number of PEM cases among S treatment groups. No cases of PEM were observed among CTRL steers. Of the 17 steers that demonstrated symptoms of Sulfur-induced PEM, 9 were euthanized. Total body weight gain over the 77 d trial period did not differ ($P > 0.05$) among treatment groups. The CTRL steers gained 79.9 kg compared to 68.2, 71.9, and 68.4 kg for HSHZ, HSLZ and HS groups, respectively. These data demonstrate the variation in individual response to elevated dietary S and suggest that zeolite is not an effective ameliorator of Sulfur-induced PEM at either 5% or 2.5% of the diet DM.

Key Words: Sulfur, Polioencephalomalacia, Hydrogen Sink, Steers

## Introduction

Sulfur toxicity is becoming more of a problem due to high concentrations of sulfate ($SO_4^-$) in stock water in the United States, especially in the western and Great Plains regions (Wright, 2007). Drought conditions coupled with the feeding of sulfur-rich feedstuffs, such as byproducts of the ethanol industry, has led to an increase in the incidence of sulfur-induced PEM. Reported industry values of distillers dried grains and wet distillers grains vary from 0.35% to 1.00% and 0.34% to 0.93%, respectively (Pritchard, 2008), which greatly exceeds current NRC (2005, 1996) recommendations of 0.15% dietary S with a maximum tolerable dose of 0.4% S for beef cattle on a diet containing more than 40% forage.

Hydrogen sulfide ($H_2S$) gas produced in the rumen via reduction of $SO_4^-$ by rumen bacteria (Figure 1) has been proposed to inhibit cytochrome oxidase in the electron transport chain which decreases ATP production and leads to brain necrosis (Gould, 1998). Kandylis (1983) asserts that $H_2S$ becomes toxic to the animal when it is eructated and inhaled into the lungs. Daugherty et al. (1962) showed that sheep infused with $H_2S$ gas into their rumen showed no signs of toxicity when their trachea was blocked, but animals with an open trachea collapsed after a few eructations. Hydrogen sulfide gas has a direct route to the heart and brain from the lungs which will bypass any detoxification pathways in the liver and exert its toxic effects on the respiratory, circulatory, and nervous systems (Bird, 1972; Wright, 2007).

Recent data suggest that inorganic S in the form of $SO_4^-$ poses a more direct animal health threat because $SO_4^-$ is more readily reduced to sulfide which combines with hydrogen ions ($H^+$) to create poisonous $H_2S$ gas (Gould, 2002; Kung, 2008). The clay mineral zeolite was hypothesized to bind excess $H^+$ in the rumen and prevent greater than normal production of $H_2S$ gas. Our objective was to examine the incidence of sulfur-induced PEM in forage-fed steers due to consumption of high-sulfur water, and investigate whether feeding zeolite, as a H sink, may ameliorate the reduction of sulfate to $H_2S$ and lead to subsequent development of sulfur-induced PEM.

## Materials and Methods

This study was conducted at the South Dakota State University Cottonwood Range and Livestock Research Station near Phillip, SD. Steers (n = 96) were blocked by weight and randomly assigned to one of four treatment groups: control (CTRL; ≤ 400 ppm S), CTRL plus high sulfur-water (HS; ≥ 3000 ppm) , high-sulfur water plus high zeolite (HSHZ; ≥ 3000 ppm S + 5% zeolite); or high-sulfur water plus low zeolite (HSLZ; ≥ 3000 ppm S + 2.5% zeolite), with three pens of eight animals per treatment. Steers remained on trial for 77 d, and water and feed records were recorded daily to track S consumption. Stock water was mixed using a Dosatron® (Dosatron - North America, Clearwater, FL) with sodium sulfate ($Na_2SO_4$) as the S source. Water was administered daily with the morning feeding and in the evening when necessary. All diets contained 50% ground crested wheat grass hay and 44% wheat middling pellets. The CTRL and HS treatment

groups contained 6% ground limestone, which was reduced with addition of zeolite (Table 1). Water and feed were given on an ad libitum basis.

Table 1. Diet composition (DM basis)

| | CTRL[1] | HSLZ | HSHZ |
|---|---|---|---|
| Ingredients, % of DM | | | |
| Crested wheat grass hay | 50.0 | 50.0 | 50.0 |
| Pelleted wheat middlings | 44.0 | 44.0 | 44.0 |
| Ground limestone | 6.0 | 3.5 | 1.0 |
| Zeolite | | 2.5 | 5.0 |

[1]CTRL = control (≤ 400 ppm S), HSHZ = high-sulfur water plus high zeolite (≥ 3000 ppm S + 5% zeolite), HSLZ = high-sulfur water plus low zeolite (≥ 3000 ppm S + 2.5% zeolite)

Steers were monitored daily for signs of sulfur-induced PEM, including blindness, ataxia, muscle tremors, diarrhea, anorexia, weight loss, lethargy, and in severe cases recumbency or seizure. Animals showing any signs of sulfur-induced PEM were immediately removed from their pen and treated with penicillin, thiamine, and dexamethasone intramuscularly (IM). They were then placed in a small pen with low-sulfur water and free choice hay and wheat middling pellets. Intramuscular injections of thiamine and dexamethasone were administered twice daily for 7 d as therapy to alleviate symptoms caused by brain edema. Steers that went recumbent, seized, or had worsening symptoms were euthanized. Surviving steers were given the probiotic Probios® (Bomac Vets Plus Inc., Knapp, MN) after 7 d, and moved to a pasture supplied with low-sulfur water and free-choice crested wheat grass hay.

The brains of euthanized/dead steers were immediately removed and dissected sagitally into two equal portions, one preserved in formalin. Samples were then placed on frozen cold packs and sent to the South Dakota Animal Disease Research and Diagnostic Laboratory in Brookings, SD for sulfur-induced PEM diagnosis. Diagnosis was made by the presence of necrotic lesions on the cortical region of the brain which fluoresce under ultraviolet light.

## Results

There were no differences ($P > 0.05$) in the number of PEM cases among S treatment groups (Table 2). The HSLZ treatment group had 5 confirmed cases and 4 suspected cases of S-induced PEM, the HSHZ treatment group had 3 confirmed cases and 1 suspected case, and the HS treatment had 1 confirmed case and 3 suspected cases. No cases of PEM were observed among CTRL steers. Of the 17 steers that demonstrated symptoms of Sulfur-induced PEM, 9 were euthanized. These data suggest that the clay mineral zeolite is ineffective at ameliorating Sulfur-induced PEM in feedlot steers fed a roughage diet using the levels fed in this trial.

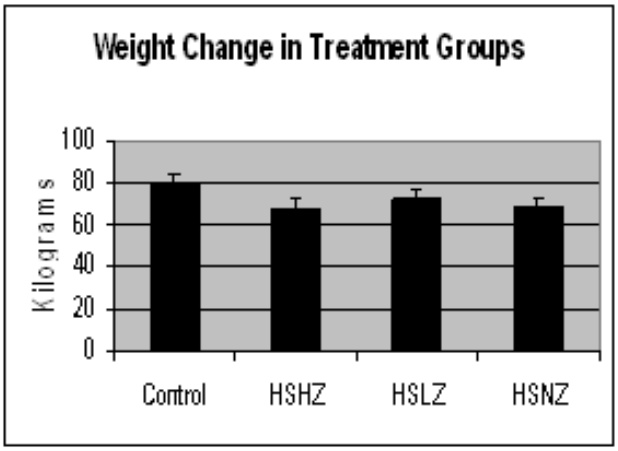
Table 1. Number of sulfur-induced PEM cases.

| Treatment | Confirmed | Suspected |
|---|---|---|
| CTRL[1] | 0 | 0 |
| HS | 1 | 3 |
| HSLZ | 5 | 4 |
| HSHZ | 3 | 1 |

[1]CTRL = control (≤ 400 ppm S), HSHZ = high-sulfur water plus high zeolite (≥ 3000 ppm S + 5% zeolite), HSLZ = high-sulfur water plus low zeolite (≥ 3000 ppm S + 2.5% zeolite)

Total body weight gain over the 77 d trial period did not differ ($P > 0.05$) among treatment groups (Figure 2). The CTRL steers gained 79.9 kg compared to 68.2, 71.9, and 68.4 kg for HSHZ, HSLZ and HS groups, respectively.

Figure 1. Weight change in treatment groups.




## Discussion

Individual variation in response to high dietary S, causing sulfur-induced PEM, is reported by Gould et al. (1991) when the authors fed (n = 9) Holstein steers toxic levels of $Na_2SO_4$. Of those nine steers, five developed sulfur-induced PEM, although all had a significant increase in ruminal fluid sulfide concentrations. Case studies reported by Gould (1998) demonstrate that not all animals that consume greater than recommended doses of S exhibit clinical signs of sulfur-induced PEM which is consistent with the data of this trial wherein only seventeen of the seventy-two animals exposed to high-sulfur water displayed symptoms of sulfur-induced PEM.

Reduced performance has been consistently reported with increasing levels of dietary S, suggesting that the numerically lower gain of high-sulfur treated cattle is attributed to the high levels of dietary S (Bolsen et al., 1973; Zinn et al., 1999; Loneragan et al., 2001; Spears and Lloyd, 2005) Mammals are incapable of reducing $SO_4^-$ to sulfide to create necessary sulfur-containing amino acids, sulfur-containing B-vitamins, and coenzyme A. Some ruminant microbes can reduce $SO_4^-$ and are termed sulfate-reducing bacteria (SRB). These SRB are capable of producing organic sources of S which can be utilized by all rumen bacteria. Early studies have shown that both organic

and inorganic sources of S are adequate for synthesis of essential sulfur-containing amino acids (Loosli et al., 1949; Block et al., 1951). However, more recent data suggest inorganic S in the form of $SO_4^-$ poses a more direct animal health threat because $SO_4^-$ is more readily reduced to sulfide which combines with hydrogen ions ($H^+$) to create poisonous $H_2S$ gas (Gould, 2002; Kung, 2008). The clay mineral zeolite was hypothesized to bind excess $H^+$ in the rumen and prevent greater than normal production of $H_2S$ gas. Both 2.5% and 5% inclusion of the DM diet of the clay mineral zeolite appear to be insufficient at reducing $H_2S$ production in the rumen. Kung, Jr. (2008) states that aluminum content of clay minerals may actually reduce the activity of SRB instead of inhibiting production of $H_2S$ by acting as a hydrogen sink and binding excess $H^+$.

## Implications

When formulating diets, more attention needs to be paid to the source of S, and not just overall concentration of S in the diet. Also, $SO_4^-$ concentrations in water sources need to be taken into consideration, especially when high-sulfur feedstuffs are fed in conjunction with high-sulfur water. It is recommended that water sources be tested, and S concentration of feedstuffs, such as ethanol by-products, be monitored on a regular basis.

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# EXTRACT FROM *LARREA* INFLUENCES RUMEN FERMENTATION

**J. Petersen*, S. L. Lodge-Ivey, J. Browne-Silva, and M. B. Horvath**

New Mexico State University, Las Cruces, New Mexico

**ABSTRACT:** *Larrea* plant extract (LPE) has antimicrobial properties and may have potential as a rumen modifier. Modification of rumen microbial fermentation due to consumption of LPE has not been documented. An in vitro experiment (48 h) was conducted to evaluate additions of LPE on IVDMD and VFA production. Three dietary substrates simulating different resources available for livestock production and five concentrations of LPE were compared to monensin (47.5 µg/mL, MON). Substrates included (DM basis) 100% meadow hay (100), 50% alfalfa-50% ground corn (50:50), and 90% ground corn-10% alfalfa (90:10). Treatments were 0 (Control; CON), 20, 40, 60, 80 µg/mL LPE and MON. Treatment means were compared using two single degree of freedom contrasts (0 µg/mL LPE vs MON and LPE vs MON) and orthogonal polynomial contrasts within LPE levels. MON fermented with 100 had the lowest ($P < 0.01$) IVDMD. A linear increase in IVDMD was observed for 50:50 ($P < 0.01$) but not 90:10 or 100 ($P > 0.40$). Total VFA decreased from 0 to 20 µg/mL additions of LPE then leveled (quadratic; $P < 0.01$) for 50:50 and 90:10 while 100 linearly decreased from 0 to 80 µg/mL LPE ($P < 0.02$). LPE addition to all substrates decreased acetate and acetate:propionate ratio (A:P) and increased propionate (quadratic; $P < 0.01$). Butyrate increased linearly for 90:10 ($P < 0.03$) while 50:50 and 100 increased quadratically ($P < 0.01$). Propionate showed the greatest increase with addition of MON and was dependent on diet vs CON (100 vs 50:50 vs 90:10; 22.5%, 44.4%, and 30.2%, respectively). LPE results for propionate production varied with the greatest increase (10.5%) caused by addition of 60 µg/mL to 50:50 ($P < 0.01$). The lowest overall A:P was obtained with addition of MON to 90:10 (1.35) and the highest ratio resulted from adding 60 µg/mL LPE to 100 (3.63). The greatest decline in molar percentage of butyrate was caused by adding MON to 50:50 vs CON (6.01 % vs 11.78 %). LPE can change rumen fermentation and is dependent on diet; with different results than MON. LPE has potential as a rumen modifier in a natural meat production system.

**Key words:** *Larrea*, rumen modifiers, plant extracts

## Introduction

Use of antibiotic growth promoters has proven to be a useful means to improve feed efficiency and prevent rumen acidosis in ruminants fed diets containing starch. Subtherapeutic use of antibiotics is controversial and legislators in Europe have moved to prohibit their use in animal feeds (Wallace, 2004). As a result, several research groups in Europe and the U.S. are looking for natural products to replace antibiotics used for growth promotion in livestock; of particular interest are antibiotics that are also used in human health (Wallace, 2004). Desert plants in the *Larrea* family are considered invasive on 19 million ha of the Mojave, Sonoran, and Chihuahuan Desert regions of the southwestern United States and contribute to the degradation of desert grasslands (Whitford et al., 2001). Extracts from this plant have shown antimicrobial properties and may have potential use as positive natural ruminal modifiers similar to antibiotics used for growth promotion. Using extracts from invasive plants for enhancement of livestock growth and production may provide economic incentives to remove invasive plants from desertified grasslands; thus, improving overall productivity of once desert grasslands for their forage, water, and recreational potential. The objective of this study was to determine in vitro the effects of extract from *Larrea* on total and individual volatile fatty acid production and dry matter digestibility.

## Materials and Methods

The effects of *Larrea* plant extract (LPE) on rumen microbial fermentation and diet digestibility were evaluated using IVDMD procedures (Tilley and Terry, 1963) and subsequent VFA production analysis (Goetsch and Galyean, 1983). Treatments for this study were arranged as a 3 X 6 factorial with three levels of substrate and six levels of LPE or monesin. Three different substrates were used as fermentation substrate, each simulating a common ruminant production diet (DM basis); 100% meadow hay (**100**), 50% corn: 50% alfalfa (**50:50**), and 90% corn: 10% alfalfa (**90:10**). Each substrate was ground with a Wiley Mill to pass a 2 mm screen. Samples were weighed out (0.5 g) and placed in 30 mL in vitro tubes. Five different doses of LPE: 0 (**CON**), 20, 40, 60, 80 µg/mL and one dose level of monensin (47.5 µg/mL) were added to the three substrates in triplicate at each substrate X dose level. Ruminal fluid was collected from a ruminally cannulated cows weighing approximately 750 kg. These cows were allowed ab libitum access to diets comparable to substrates used in the IVDMD experiment 10 days prior to rumen fluid collection. Animal care and management practices were approved by the Institutional Animal Care and Use Committee at NMSU. A suction strainer was used to collect rumen fluid at the mat layer interface into a collection thermos that had been heated to 37°C. Rumen fluid was combined with equal parts of McDougall's buffer (Tilley and Terry, 1963) to make the IVDMD inoculum. In

vitro tubes with substrate were filled with 15mL of inoculum, sealed with a plastic cap and incubated (37°C) in an anaerobic glove box with a 95% $CO_2$: 5% $H_2$ atmosphere for 48 h. Tubes were manually agitated 10 times at the beginning of the experiment and were then agitated every two hours for twelve hours. Upon removal from the anaerobic glove box tubes were stored at -80°C until IVDMD and VFA production analysis was performed. In vitro dry matter digestibility was calculated from the amount of substrate remaining after digestion with rumen fluid inoculum at 48 h. Gas chromatography was used to quantify VFA production (Goetsch and Galyean, 1983).

Data were analyzed using GLM procedure of SAS (1999). Treatment means were compared using two single degree of freedom contrasts (0 µg/mL LPE vs MON and LPE vs MON) and orthogonal polynomial contrasts within LPE levels. A probability of less than 0.05 was considered significant.

## Results and Discussion

The plant extract used in this experiment has not been completely characterized but preliminary data suggests that it is a phenolic compound. Because phenolic compounds have antimicrobial properties, the effect of LPE on IVDMD and VFA production was evaluated to determine the usefulness of LPE as a rumen modifier. Results of this experiment are presented in Table 1.

In vitro dry matter digestibility increased linearly ($P < 0.01$) when LPE was added to 50:50 and 100 or 90:10 were effected ($P > 0.23$). When compared to MON, LPE had greater IVDMD for 50:50 and 100 ($P < 0.01$) with no effect for 90:10 ($P > 0.98$). MON fermented with 100 resulted in the lowest (26.5%) IVDMD of all treatment combinations. This treatment simulated a medium quality diet composed of 100% forage. Poos et al. (1979) found that feeding monensin reduced feed intake and digestibility of high fiber diets while Simpson (1978) found monensin to inhibit cellulose degrading bacteria in vitro. Our results indicate that unlike MON, LPE may not reduce ruminal diet digestibility. Furthermore, LPE at 80 µg/mL in rations that average 50% concentrate and 50% forage may improve diet digestibility over MON in vivo. Monensin is currently being used in dairy production systems to alleviate subacute acidosis during the period when cows transition from the dry period to early lactation. The rations used in the dry period are predominately composed of forage while early lactation rations contain an increasing level of starch (Fairfield et al., 2007) given the data from the current study LPE may also be of value in this scenario.

Volatile fatty acids are the end product of rumen microbial fermentation and represent the main supply of metabolizable energy for ruminants (Van Soest, 1982). Therefore, a reduction in VFA production would be energetically unfavorable for the nutrition of the animal (Busquet et al., 2006). Total VFA concentrations were greatest for CON and were highest for 50:50 and lowest for 100. Addition of LPE or MON decreased total VFA quadratically ($P < 0.01$) for 50:50 and 90:10 when compared to CON. This reduction in total VFA production with addition of LPE or MON is not surprising given the antimicrobial nature of these compounds

Butyrate production increased linearly with LPE for 90:10 ($P < 0.03$) while 50:50 and 100 increased quadratically ($P < 0.01$). The greatest decline in butyrate was caused by adding MON to 50:50 vs CON (6.01 % vs 11.78 %). Acetate decreased quadratically for all substrates tested with LPE ($P < 0.01$) and adding LPE vs MON resulted in higher acetate values ($P < 0.01$) by as much as 9% with 80 µg/mL LPE added to 100. A 13% reduction in acetate production was observed when MON was added to 90:10, when compared to CON. Propionate production increased by addition of MON compared to CON which was opposite of for acetate. Propionate showed the greatest increase with addition of MON and was dependent on diet vs CON (100 vs 50:50 vs 90:10; 22.5%, 44.4%, and 30.2%, respectively). LPE results for propionate production varied with the greatest increase (10.5%) caused by addition of 60 µg/mL to 50:50 ($P < 0.01$). The lowest overall A:P was obtained with addition of MON to 90:10 (1.35) and the highest ratio resulted from adding 60 µg/mL LPE to 100 (3.63).

In vitro screening of plant extracts as rumen modifiers has become an active area of research of late. Plant extracts that are capable of increasing propionate production, decreasing acetate and methane production without reducing total VFA are being targeted. LPE in the current study was able to decrease acetate and increase propionate while not adversely affecting IVDMD. LPE does appear to have potential as a rumen fermentation modifier and may target a different rumen microbial population than MON.

In the context of the current study LPE does change rumen fermentation in vitro. In general, reductions in A:P ratio and increases in butyrate production are related to inhibition of methanogensis. In vitro experiments provide an effective method to screen compounds for potential as rumen modifiers. However, the utility of these resulting modifications still remains in questions. To determine the true value of plant extracts as ruminal modifiers these experiments must be conducted in vivo. The benefits of ruminal modifiers on ruminal retention times of liquids and solids, microbial proteolysis, and the subsequent additive effects on other systems that may result in increases in animal performance cannot be measured in vitro.

## Implications

Addition of LPE to an in vitro system utilizing differing substrates resulted in changes in acetate and propionate production as well as the total amount of VFA produced while having little effect on IVDMD. These results indicate that LPE can favorably change rumen fermentation and the results are dependent on diet; with different results than MON. LPE has potential as a rumen modifier in a natural protein production system.

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Table 1. Effects of *Larrea* plant extract (LPE) and monensin on IVDMD and VFA production when fermented with different ruminant production diets.[a]

| | Treatments[b] | | | | | | | Contrasts[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 20 | 40 | 60 | 80 | Monensin | P value | Linear | Quadratic | Con vs MON | LPE vs MON | SE |
| IVDMD, % | | | | | | | | | | | | |
| 100 | 32.7 | 32.0 | 32.6 | 34.3 | 32.7 | 26.5 | <0.01 | 0.45 | 0.83 | <0.01 | <0.01 | 5.69 |
| 50:50 | 43.2 | 43.5 | 44.2 | 44.3 | 45.3 | 43.1 | <0.01 | <0.01 | 0.48 | 0.81 | 0.003 | 0.66 |
| 90:10 | 57.3 | 56.8 | 56.4 | 56.4 | 56.7 | 56.6 | 0.71 | 0.26 | 0.24 | 0.25 | 0.98 | 1.09 |
| Acetate, mol/100 mol | | | | | | | | | | | | |
| 100 | 69.5 | 68.9 | 69.1 | 70.7 | 71.1 | 64.7 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.09 |
| 50:50 | 61.1 | 59.1 | 57.3 | 56.7 | 60.0 | 55.0 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.64 |
| 90:10 | 48.1 | 46.8 | 45.9 | 47.5 | 47.9 | 41.5 | <0.01 | 0.65 | <0.01 | <0.01 | <0.01 | 0.36 |
| Propionate, mol/100 mol | | | | | | | | | | | | |
| 100 | 20.9 | 21.0 | 21.0 | 19.5 | 19.9 | 26.9 | <0.01 | <0.01 | 0.05 | <0.01 | <0.01 | 0.08 |
| 50:50 | 24.8 | 26.1 | 27.1 | 27.7 | 26.3 | 35.6 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.43 |
| 90:10 | 17.0 | 16.7 | 17.5 | 17.1 | 17.0 | 30.7 | <0.01 | 0.38 | 0.21 | 0.10 | <0.01 | 0.09 |
| Butyrate, mol/100 mol | | | | | | | | | | | | |
| 100 | 8.2 | 8.5 | 8.6 | 8.4 | 7.8 | 7.1 | <0.01 | 0.02 | <0.01 | <0.01 | <0.01 | 0.03 |
| 50:50 | 11.8 | 12.4 | 12.9 | 13.0 | 11.5 | 6.0 | <0.01 | 0.84 | <0.01 | <0.01 | <0.01 | 0.06 |
| 90:10 | 22.3 | 21.8 | 22.4 | 22.5 | 22.6 | 14.2 | <0.01 | 0.02 | 0.19 | <0.01 | <0.01 | 0.014 |
| Total VFA, m*M* | | | | | | | | | | | | |
| 100 | 127.3 | 107.1 | 109.5 | 59.2 | 49.3 | 103.7 | <0.01 | <0.01 | 0.41 | 0.11 | 0.06 | 25.2 |
| 50:50 | 222.3 | 187.7 | 190.2 | 181.2 | 178.0 | 186.0 | <0.01 | <0.01 | 0.05 | <0.01 | 0.82 | 16.52 |
| 90:10 | 144.1 | 117.0 | 127.9 | 153.35 | 158.19 | 152.7 | <0.01 | <0.01 | <0.01 | 0.20 | 0.01 | 11.55 |

[a]Three substrates were used as fermentation substrate, each simulating a common ruminant production diet (DM basis); 100% meadow hay (**100**), 50% corn: 50% alfalfa (**50:50**), and 90% corn: 10% alfalfa (**90:10**).

[b]Five doses of LPE: 0 (**CON**), 20, 40, 60, 80 µg/mL and one dose level of monensin (**MON**; 47.5 µg/mL) were added to the three substrates in triplicate at each substrate X dose level.

[c]Linear and quadratic contrasts were used to compare treatment means within LPE level; MON vs CON compared monensin to no additive; LPE vs MON contrast compared addition of monensin to LPE addition averaged across all LPE levels.

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# RUMINAL DEGRADATION KINETICS OF DIETS OF MANZARINA AND CORN SILAGE FOR SHEEP WITH THE *IN VITRO* TECHNIQUE OF GAS PRODUCTION

**C. Arzola*[1], A. Muro[2], M.R. Murphy[3], O. Ruiz[1], C. Rodriguez-Muela[1], J. Salinas[4], F. Salvador[1];**

Universidad Autónoma de Chihuahua, Chihuahua,Chihuahua,Mexico[1], Universidad Autónoma de Zacatecas, Zacatecas,Zacatecas,Mexico[2], University of Illinois, Urbana,Il,USA[3], Universidad Autónoma de Tamaulipas, Cd. Victoria, Tamaulipas, Mexico[4]

**ABSTRACT:** Ruminal degradation kinetics of four isoproteic and isoenergetic diets composed of corn silage and manzarina (an apple by-product) or cottonseed meal formulated for male sheep (NRC, 1985) was carried on, using the in vitro gas production technique. Diets were corn silage and a mixture of manzarina and cottonseed meal as protein concentrate, with manzarina proportions of 0, 33, 66 and 100% and the rest cottonseed meal. The diets had ADF means 8.77, 12.93, 14.65 and 16.05% for the diets with 0, 33, 66 and 100% of manzarina respectively ($P<0.05$). NDF means were 21.84, 26.04, 27.61 and 27.66% for the diets with 0, 33, 66, and 100% of manzarina, respectively ($P<0.05$). Crude protein (CP) means were 8.48, 11.17, 9.53 and 8.01% for the diets with 0, 33, 66, and 100% of manzarina respectively ($P<0.05$). Crude protein (CP) of the 33% manzarina diet was larger than the rest ($P<0.05$). Four rams, diets and periods were used in a 4X4 latin square design. In each period ruminal fluid was drawn immediately prior to the feeding of the animals then were strained through cheese cloth and mixed with artificial saliva. 30ml of the mixture were placed into 50ml flasks, adding 200mg of diet ground in sieves of 1mm, and incubated at 39°C during 96 hours. Readings of the pressure were taken at 0, 3, 6, 12, 24, 48, 72 and 96h. An ANOVA analysis showed differences for volumes of gas production at 72 and 96 hours between diets with 0 and 33% of manzarina, respectively ($P<0.05$). For the gas production profiles, data was analyzed with PROC MIXED of SAS, performing a tendency analysis when the treatment (diet) effect was significant, using polynomial contrasts. The parameters of the profiles of gas production using the single-phase model of Groot did not show differences between treatments ($P>0.05$). No significant differences were found on linear, quadratic and cubic polynomial contrasts. It was concluded that manzarina can be used in combination of corn silage as a protein concentrate, replacing cottonseed meal without adverse effects on ruminal degradability kinetics.

**Key words:** Manzarina, Corn Silage, In vitro gas production

## Introduction

Corn silage is a common forage in dairy and meat production. In Mexico it can comprise from 30-40% of TMR (Chalupa, 1995). A key issue in dairy nutrition is to accomplish a good balance in the ration. The objective of this work was to evaluate the value of manzarina as a protein source as compared with cotton seed meal, using the in vitro rumen gas production technique with sheep.

**Hypothesis.** The in Vitro ruminal degradation kinetics are influenced by FDN, FDA and CP concentration in diets. The evaluation of kinetics can be used to compare any differences of degradation when using manzarina and/or cotton seed meal as part of a corn silage-based diet.

## Materials and Methods

The research was carried on at Facultad de Zootecnia of the Universidad Autónoma de Chihuahua, located in Chihuahua, México. Four male sheep fitted with a ruminal cannulae and placed in individual cages were offered rations comprised of corn silage as basal forage and different proportions or manzarina and/or cottonseed meal, plus minerals, to cover nutritional needs for maintenance (NRC, 1985).

Dry matter and crude protein were determined according to AOAC (1984); Neutral detergent fiber (NDF) and Acid detergent fiber (ADF) were analyzed according to Goering and Van Soest (1970).

Gas production technique. The Menke and Steingass (1988) technique was used. Readings of gas production were made at 3, 6, 9, 12, 24, 48, 72 and 96h. Data was fitted with the logistic, Gompertz and Groot´s model, (Groot *et al.,* 1996) using PROC NLIN of SAS 9.0 (2002) to adjust the data. A 4x4 latin square design, corresponding to the 4 diets, animals and four periods of 96h of gas measurements, plus an 11 day adaptation period was used to test the gas production response based in the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

Where

$Y_{ijk}$ = Response variable

$\mu$ = General mean

$\alpha_i$ = Diet effect

$\beta_j$ = Sheep (animal) effect

$\varepsilon_{ijk}$ = Error

## Results and Discussion

Table 1 shows ADF concentration of the diets. Significant differences were found among diets ($P<0.05$), having treatment 4 the highest concentration (16.05 **%**) and treatment 1 the lowest.(8.77**%**) ($P<0.05$). NDF also showed statistical differences in the diets, treatment 3 presented the highest value (27.66%), and treatment 1 a low value ($P<0.05$).

Gas production. Least square means for volumes of gas production up to 96 h are shown in table 2. There were not found significant differences among diets ($P>0.05$).

Several models were used in order to select the most appropriate accordingly with our data. The models evaluated were the Logistic (Schofield et al., 1994), the Gompertz (Lavrencic et al., 1997) and Groot´s monophasic model. The best model found with our data was the Groot monophasic model (Groot et al., 1996). We can notice that various models describe the kinetics degradability for the variability of the parameters of gas production profiles, but the adjustment of this parameters, apart of the substrates evaluated, will depend of work conditions (Beuvink and Kought, 1993).

Table 3 shows the last square means of the parameters of ruminal kinetics obtained with the monophasic model. Similarly for the volume of gas production no significant differences were found among treatments ($P>0.05$). The highest value for parameter A (68.4) corresponded to diet 3, followed by diet 4, then diet 1 and finally diet 2. For parameter B, highest value was for diet 4, followed by diet 3, diet 2 and finally treatment 1. For parameter C, higher value was for diet 2, then diet 3, followed by diet 1 and diet 4. No significant differences were found on linear, quadratic and cubic polynomial contrasts.

## Conclusions and Recomendations

Under the experimental conditions of this trial, we can conclude, that "manzarina" can be used as protein ingredient in diets in substitution of other ingredients such us cotton seed meal.

When we use "manzarina" in feed diets we have to take into account the FDA and FDN values, these values were increased when "manzarina" was added to the diet.

"Manzarina" as protein ingredient in diets using corn silage did not show in vitro difference gas production volumes, therefore, did not affect the digestibility under the work conditions in this trial. Numerical differences were found but they were not significant.

The ruminal fermentation parameters adjusted with the monophasic model did not show differences maybe due to the low frequency of readings in the in vitro gas production.

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Table 1. Chemical composition of the diets (DMB)

| DIET | DM (%) | ADF (%) | NDF (%) | CP (%) |
|---|---|---|---|---|
| 1 | 94.65 | 8.77 | 21.84 | 8.48 |
| 2 | 97.27 | 12.93 | 26.04 | 11.17 |
| 3 | 94.82 | 14.65 | 27.71 | 9.53 |
| 4 | 94.70 | 16.05 | 27.66 | 8.01 |

1 = Manzarina 0%, cottonseed meal 100%, 2 = Manzarina 33%, cottonseed meal 66%, 3 = Manzarina 66%, cottonseed meal 33%, 4 = Manzarina 100%, cottonseed meal 0%.
DM = dry matter, ADF= acid detergent fiber, NDF = neutral detergent fiber, CP =crude protein

Table 2. Least square means for gas production (ml/200 mg DM) for the four diets

| DIET | Hours of incubation | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 6 | 12 | 24 | 48 | 72 | 96 |
| 1 | 9.30[a] | 18.02[a] | 29.72[a] | 40.92[a] | 49.80[a] | 54.59[a] | 57.44[a] |
| 2 | 6.39[a] | 13.26[a] | 24.02[a] | 35.38[a] | 44.24[a] | 48.24[a] | 50.61[a] |
| 3 | 7.28[a] | 15.63[a] | 27.61[a] | 38.29[a] | 47.47[a] | 52.88[a] | 56.18[a] |
| 4 | 6.38[a] | 13.17[a] | 24.26[a] | 35.77[a] | 46.08[a] | 52.65[a] | 56.61[a] |
| S.E. | 0.81 | 1.10 | 1.50 | 1.82 | 2.10 | 2.01 | 2.28 |

1 = Manzarina 0%, 2 = Manzarina 33%, 3 = Manzarina 66%, 4 = Manzarina 100%.
S.E. = Standard error of the mean DM = Dry matter.
[ab] Means in the same row with the same superscript are significantly different (P<0.05).

Table 3. Least square means for the estimated parameters of DM ruminal degradability and polynomial contrasts of the diets using the monophasic model

| Diets/ Contrasts | Parameters | | |
|---|---|---|---|
| | A | B | C |
| 1 | 66.14 | 15.81 | 1.14 |
| 2 | 58.16 | 17.54 | 1.26 |
| 3 | 68.40 | 19.53 | 1.17 |
| 4 | 68.32 | 24.41 | 1.08 |
| E.E. | 3.26 | 3.92 | 0.71 |
| Linear | NS | NS | NS |
| Quadratic | NS | NS | NS |
| Cubic | NS | NS | NS |

1 = Manzarina 0%, cottonseed meal 100%, 2 = Manzarina 33%, cottonseed meal 66%, 3 = Manzarina 66%, cottonseed meal 33% 4 = Manzarina 100%, cottonseed meal 0%.
S.E. = Standard error of the mean.
A = Asymptotic gas production. B = Time (h) after incubation at which half of the gas has been formed C= Constant determining the sharpness of the switching characteristic of the profile.
[a]= Columns with different literal are significantly different (P<0.05).
NS = Not significant

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# CHROMIUM METHIONINE SUPPLEMENTATION ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF BULLS: II. RESULTS INCLUDING TROUGH HOT AND HUMIDITY SEASON IN THE NORTHWEST OF MEXICO

**R. Barajas [1]*, B. J. Cervantes[1, 2], E. A. Velazquez[1], J. A. Romo[1], F. Juarez[1], P. J. Rojas[1, 3] and F. R. Peña [1]**
[1] FMVZ-Universidad Autónoma de Sinaloa, México. [2] Ganadera Los migueles, S.A. de C.V. [3] Tecnologia de Máxima Producción, S.A. de C.V.
E-mail: rubar@uas.uasnet.mx

**ABSTRACT**: To determine the influence of chromium methionine supplementation on feedlot performance and carcass characteristics of bulls including trough hot and humidity season, a 215-days feedlot experiment involving forty eight Brahman cross bulls 210.93 kg was conducted. Experiment was conducted from May 1 to December 8, 2007. Animals were blocked by starting weight and in groups of four placed in ground flour pen (2 x 12 m). Experiment was conducted Agreement with a randomized complete block design were assigned to receive or not supplementary chromium. Chromium supplementary level was equivalent to 0.30 mg of Cr/kg of DM, and was provided from a chromium methionine premix (MiCroplex; Zinpro Corp. Eden Prairie, MN). Blood samples were taken in day 28 and at killed time for cortisol determination.
Chromium supplementation increased ($P < .05$) ending weight (490 vs. 508 kg), and average daily gain (1.30 vs. 1.39 kg/d). DMI was not affected ($P = .69$) by treatments, and feed/gain ratio was not affected ($P = .12$) by treatments. Hot carcass weight was augmented ($P < .10$) in 2.29% by Chromium (310 vs. 318 kg). Carcass dressing was similar ($P = .13$) between treatments. Back fat thickness was increased ($P = 0.8$) by Cr. Loin muscle area and marbling were similar ($P > .15$) in both treatments. KPH fat was decreased ($P = .08$) in 10% by Cr. USDA grade, Preliminary Cuts yield, and meat pH were unaltered by treatments ($P > .50$). It is concluded, that chromium methionine supplementation improves weight gain and carcass weight of feedlot-bulls, when feedlot period include the hot and humidity season in the Northwest of Mexico.

Key words: Chromium, bulls, feedlot-performance.

## Introduction

The organic chromium supplementation has been reduced blood cortisol (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Chang et al., 1995), improved weight gain during receiving period (Monsie-Shager et al., 1993; Kegley and Spears, 1995); and sometimes during growing period (Kegley et al., 1997). Pollard et al. (1999) not found effect of high-Cr yeast supplementation on performance of feedlot cattle.

Chromium methionine supplementation reduce cortisol (Almeida and Barajas, 2002), improved weight gain during receiving (Barajas and Almeida, 1999; Barajas et al., 1999; Barajas et al., 2005a) and growing period (Barajas et al., 2005b). However there is not information about the effect of chromium methionine supplementation during complete feedlot period of cattle.
This experiment was conducted with the objective of determine the influence of chromium methionine supplementation on feedlot performance and carcass characteristics of bulls including trough hot and humidity season.

## Material and Methods

*Location*

The experiment was conducted from May 1 to December 8, 2007 at Experimental Station for Beef Cattle in Dry Tropic Weather of the Universidad Autonoma de Sinaloa. The research facilities are located at Ganadera Los Migueles feedlot, S.A. de C.V. in Culiacan, Sinaloa situated in Northwest Mexico (24º 51' N. and 107º 26' W. ; 57 m o.m.s.l.; mean temperature 25 ºC, and 645 mm annual rainfall).

*Animals Management*

Animals used in the experiment were managed according to the recommended guidelines in *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (1988). Two hundred Brahman-cross bull calves were acquired from different farms and transported 250 km via truck to Ganadera Los Migueles feedlot. Upon arrival at the feedlot, calves were weighed and vaccinated to prevent infections by *Pasteurella sp*. (One Shoot®; Pfizer Ltd.). Cattle were then placed in a large dirt lot. They were fed alfalfa hay and a 40:60 forage:concentrate diet, and had free access to fresh clean drinking water.

Three days later, all the animals were weighed and 48 calves weighing between 200 and 220 kg live weight were selected for the 215 day feedlot experiment. Selected calves were identified with a numbered ear tag, implanted

(Component TES with Tylan®;ELANCO Co.), vaccinated with *Clostridium* and *Haemophilus somnus* (Ultrabac-Somnobact®; Pfizer ), dewormed (Albendaphorte®; Lab. Salud y Bienestar ), and injected with vitamins A, D and E (ADEphorte®; Lab. Salud y Bienestar ). Groups of four calves were randomly placed in 12 pens (6 x 12 m), each fitted with a 2.4 m feed bunk and 0.6 m waterer. Animals had *ad libitum* access to feed and water.

*Treatments assignation*

In accordance to a randomized complete blocks design described by Hicks (1973), the calves were randomly assigned to receive one of two treatments: 1) Control, no Cr supplementation (**CON**); or 2) Chromium methionine supplementation, 2.75 mg Cr/head/day, during first 84 days in the feedlot, and then switched to 2.2 mg Cr head/day during remainder 131 days (**CrMet**). Supplemental Cr was in the form of chromium methionine (MiCroPLEX®; Zinpro Corporation, Eden Prairie, MN).

*Experimental procedure*

During the first 84 days, Cr supplementation was provided in the following manner. For each CrMet designated pen, 11 g MiCroPLEX was thoroughly mixed with 1 kg ground corn, mixture was top dressed in the feed bunk and hand mixed with the diet. During remainder 131 days 8.8 g MiCroPLEX was thoroughly mixed with 1 kg ground corn. For calves in the CON treatment pens, 1 kg of ground corn was top dressed and hand mixed with the diet in the feed bunk, to homogenize daily ration composition in respect to calves receiving supplemental Cr. Diet composition is presented in Table 1. Cattle had *ad libitum* access to the diets that were offered once daily (1600 h). Feed intake was measured as feed offered minus weekly refusals. Feed samples (4 kg) were collected weekly directly from mixer wagon, oven dried (105 °C for 24 h), and dry matter intake calculated. Thirty three days before slaughter date, all diets were supplemented with 6 mg/kg of the beta-adrenergic zilpaterol chloride (Zilmax®; Intervet), three days previously to slaughter, zilpaterol was take out of the diet. Animals were weighed on days 1, 28 and end of the experiment, and 4% of weight was subtracted as a correction for digestive tract fill (NRC, 1984).

*Carcass Measurements*

Upon complete the feedlot experiment time, the bulls were sacrificed in a slaughter house supervised by the sanitary authority of Culiacan City. Hot carcass weights were recorded, and after 24 hours chilling period in a cold room (2 ºC), left carcass side longissimus muscle was cross sectioned between the 12th and 13th rib, back fat (cm) and longissimus muscle area **(LMA)** was measured by direct grid reading, marbling score and percentage of KPH fat was visually estimated. Preliminary yield grade and cutability was estimated using procedures proposed by USDA (1996). Meat pH was measured in *pectoralis profundus* muscle using a pH-meter fitted with a penetration electrode (HI8314 membrane pHmeter; Hanna Instruments).

Table 1. Composition of basal diets used in feedlot performance experiment

| Ingredients | Diets | | | |
|---|---|---|---|---|
| | Receiving | Starting | Growing | Finishing |
| Corn straw | 16.80 | 7.86 | 18.22 | 13.18 |
| Corn silage | 43.57 | 28.82 | - | - |
| Ground corn | 9.34 | 47.16 | 57.18 | 51.71 |
| Corn DDG | - | - | - | 12.44 |
| Soybean meal | 23.34 | 8.65 | 7.08 | - |
| Pork Meat and bone meal | 2.80 | 3.14 | 4.05 | - |
| Sugar cane molasses | - | - | 8.43 | 10.98 |
| Tallow | - | - | 2.23 | 2.79 |
| Premix [1] | 3.11 | 3.49 | 2.81 | 2.82 |
| Buffer blend [1] | 1.04 | 0.87 | - | - |
| Total | 100% | 100% | 100% | 100% |
| | Calculated Analyses (DM basis) [2] | | | |
| DM, % | 48.2 | 57.25 | 88.93 | 88.82 |
| CP, % | 20.66 | 15.77 | 14.64 | 16.43 |
| NEm, Mcal/kg | 1.610 | 1.833 | 1.920 | 2.032 |
| NEg, Mcal/kg | 1.02 | 1.215 | 1.274 | 1.367 |

[1] Ganamin Total ® (Vitamins and mineral premix containing 25 g of sodium-monensin from Rumensin 200 ® (Elanco), and Ganabuffer ® (Buffering agents premix), are trademarks (Técnica Mineral Pecuaria, S.A. de C.V.; Guadalajara, Jal., México).

[2] Calculated from tabular values (NRC, 2000).

*Serum determinations*:

Twelve bull-calves (one from each pen; six by treatment), were randomly selected to be blood sampled. Bull-calves were bled by jugular venipuncture on d 28, using plain glass vacuum tubes (Vacutainer 6431; Becton Dickinson, Rutherford, NJ). At death time, blood samples were taken again. Serum was obtained for cortisol, measurements. Serum cortisol was determined by radioimmunoassay using antibody-coated tubes (Diagnostic Products Corp., Los Angeles, CA).

*Statistical Analysis*

Performance and serum data was analyzed as a randomized complete blocks design (Hicks, 1973), considering each pen as the experimental unit. General AOV/AOCV procedure of Statistix® 8 program (Analytical Software, Tallahassee, FL) was used to perform the analyses, and *P*-value for F-test was obtained.

## Results and Discussion

Mean chromium intake in complete experiment was equivalent to 0.3 ppm. The influence of chromium methionine supplementation on feedlot performance of bulls is shown in Table 2, and its influence on carcass characteristics is presented in Table 3. Chromium methionine supplmentnation increased ($P < .05$) 3.7%

ending weight and 6.6% average daily gain. This response is in correspondence with previously observed in receiving cattle fed chromium methionine (Barajas and Almeida, 1999; Barajas et al., 1999; Barajas et al., 2005a), and is attributable to effect of chromium that improves insulin signal (Vincent, 2000), and consequently its anabolic effect. The increment ($P < .10$) in 2.2% of carcass weight is interpreted as augment in body mass by better nutriment utilization via anabolic insulin activity. The 17% percentage increase on back fat thickness indicates that anabolic activity of insulin is manifested with a general increment in tissue deposition that includes fat syntheses, so that is not expected that chromium supplementation have a lipolysis activity. Treatment have not influence on serum cortisol ($P = .75$).

## Implications

Results of this experiment suggests that chromium methionine supplementation improves body mass accretion, manifested as daily weight gain and carcass weight increment, in feedlot bulls, include when part of its permanence is during the hot and humidity season in the Northwest of Mexico. Even, when chromium supplementation improves feedlot performance there are not reason for expecting lipolysis activity.

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Table 2. Influence of chromium methionine supplementation on feedlot performance of bulls.

| Variables | Treatments | | SEM[2] | *P-value* |
|---|---|---|---|---|
| | Control | Chromium[1] | | |
| Animals, n | 24 | 24 | | |
| Pens, repetitions, n | 6 | 6 | | |
| Days in trial | 214.67 | 214.67 | 1.48 | .99 |
| Starting weight, kg [3] | 210.98 | 210.87 | 0.51 | .88 |
| Ending weight, kg [3] | 490.17 | 508.32 | 5.56 | < .05 |
| All experiment weight gain, kg | 279.19 | 297.45 | 5.32 | < .05 |
| Average daily gain, kg/d | 1.299 | 1.385 | 0.03 | < .05 |
| Dry matter intake, kg/d | 8.106 | 7.990 | 0.20 | .69 |
| Feed/gain, kg/kg | 6.271 | 5.772 | 0.28 | .12 |

[1] Experiment mean chromium intake was 0.3 ppm
[2] Standard Error of the mean
[3] Four percent was pencil discounted as digestive tract fill (NRC, 1984)

Table 3. Influence of chromium methionine supplementation on carcass characteristics of bulls.

| Variables | Treatments | | SEM[2] | *P-value* |
|---|---|---|---|---|
| | Control | Chromium[1] | | |
| Hot carcass weight, kg | 310.44 | 317.56 | 2.76 | .10 |
| Hot carcass dressing, % | 63.35 | 62.48 | 0.36 | .13 |
| Back fat thikness, cm | 0.74 | 0.87 | 0.05 | .08 |
| Loin muscle area, $cm^2$ | 78.78 | 81.17 | 1.09 | .15 |
| Marbling [3] | 458 | 460 | 12.4 | .94 |
| KPH fat, % | 2.16 | 1.94 | 0.08 | .08 |
| USDA Yield grade | 2.35 | 2.38 | 0.07 | .78 |
| Cutability, % | 51.57 | 51.52 | 0.15 | .83 |
| Meat pH | 5.95 | 5.91 | 0.04 | .57 |

[1] Experiment mean chromium intake was 0.3 ppm
[2] Standard Error of the mean
[3] Code: traces = 300; slight = 400; small = 500; modest = 600, etc.

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# EFFECT OF NUTRIENT RESTRICTION IN EARLY GESTATION BEEF COWS ON MATERNAL AND FETAL INTESTINAL MASS AND JEJUNAL PROLIFERATION, VASCULARITY, AND MORPHOLOGY

**A. M. Meyer*, J. J. Reed*, K. A. Vonnahme*, L. P. Reynolds*, S. A. Soto-Navarro‡, B. W. Hess†, S. P. Ford†, and J. S. Caton***

*North Dakota State University, Fargo; †University of Wyoming, Laramie; ‡New Mexico State University, Las Cruces

**ABSTRACT:** Thirty multiparous beef cows (BW = 571 ± 63 kg, BCS = 5.4 ± 0.7) carrying female fetuses were allocated to receive either a diet of native grass hay (CON; 12.1% CP, 70.7% TDN) to meet NRC recommendations for gain during early gestation or a nutrient restricted diet of millet straw (NR; 9.9% CP, 54.5% IVDMD) to provide 68.1% of $NE_m$ and 86.7% of MP requirements for early gestation. On d 125 of gestation, 10 CON and 10 NR cows were necropsied and tissues collected. Five remaining CON cows received the CON diet, and 5 NR cows were realimented with a concentrate supplement (13.2% CP, 77.6% IVDMD) added to the NR diet to achieve a BCS similar to CON cows by d 220 of gestation. Remaining cows were necropsied on d 250 of gestation. At necropsy maternal and fetal viscera were collected. Cow BW and eviscerated BW (EBW) were less ($P < 0.01$) for NR than CON at d 125 but did not differ ($P \geq 0.64$) at d 250. There was no effect ($P \geq 0.21$) of treatment or day of gestation on cow small intestine (SI) length or weight. Duodenal DNA was greater ($P = 0.01$) and jejunal RNA was less ($P = 0.02$) for cows at d 250 vs. 125, but there were no effects ($P \geq 0.16$) on intestinal protein content. Jejunal cell proliferation (% total cells) was decreased ($P = 0.08$) in cows at d 250 vs. 125. Cow jejunal capillary area density was increased for NR vs. CON ($P = 0.04$) and on d 250 vs. 125 ($P = 0.04$), while jejunal villus morphology was unaffected ($P \geq 0.21$) by treatment or day of gestation. Fetal BW and EBW were unaffected by treatment ($P \geq 0.41$), but were greater ($P < 0.01$) at d 250 vs. 125. Length and weight of fetal SI increased ($P < 0.01$) with day of gestation, but were similar ($P = 0.50$) per unit of EBW. Fetal jejunal DNA was less ($P = 0.09$) at d 250 vs. 125. Fetal jejunal cell proliferation (% total cells) was similar ($P = 0.14$) at d 125 and 250, despite a decrease ($P = 0.07$) in number of proliferating:non-proliferating nuclei at d 250 vs. 125. Results of this study indicate that maternal and fetal intestine undergo changes during gestation, which can be affected by nutrient restriction in early gestation and by later realimentation.

**Key words**: gestation, intestine, nutrient restriction

## Introduction

Beef cows often are undernourished during gestation due to limiting forage quality and quantity, although this may be prevented with supplementation. Maternal undernutrition during gestation can cause intrauterine growth retardation (**IUGR**), resulting in impaired development in utero and ultimately low birth weight offspring with poor long-term performance and health (Wu et al., 2006). Intrauterine growth restricted offspring have been shown to have decreased small intestinal development (Trahair et al., 1997; Wang et al., 2005). In addition, the maternal gastrointestinal tract has been shown to respond to both pregnancy status and nutritional level (Scheaffer et al., 2004a; Scheaffer et al., 2004b), suggesting that the dam may compensate for nutritional insults, thus sparing her offspring. Our objectives were to determine the effects of nutrient restriction during early gestation and subsequent realimentation upon the maternal and fetal small intestine.

## Materials and Methods

This study was conducted at the University of Wyoming and was approved by the Institutional Animal Care and Use Committee.

Thirty multiparous Angus × Gelbvieh cows (initial BW, 571 ± 63 kg; initial BCS, 5.4 ± 0.7) gestating female fetuses were blocked by initial BW, BCS, and age, and assigned to 1 of 2 nutritional treatments. Control (**CON**) cows were fed native grass hay (12.1% CP, 70.7% TDN; DM basis) fortified with vitamins and minerals at NRC (2000) recommendations for a mature cow to gain 0.72 kg/d during the first 125 d of gestation. Nutrient restricted (**NR**) cows were fed millet straw (9.9% CP. 54.5% IVDMD; DM basis) to provide 68.1% of the $NE_m$ and 86.7% of the MP requirements during the first 125 d of gestation (NRC, 2000), in addition to minerals and vitamins at 50% of the amount provided to CON cows. Cows were weighed every 14 d to adjust the rations for changes in BW throughout the experiment.

On d 125 of gestation, 10 CON and 10 NR cows were necropsied. The remaining CON cows (n = 5) were fed the control diet to maintain a BCS of 5.75 from d 125 to d 250 of gestation, while the NR cows (n = 5) were fed millet straw in addition to a concentrate supplement (79.6% corn, 6.1% soybean meal, 5.3 % sunflower meal, 4.2% molasses, 2.7% safflower meal, 1.6% dried skim milk; 13.2% CP, 77.6% IVDMD; DM basis) and CON minerals and vitamins. The realimentation diet was formulated to provide 2.15 Mcal more $NE_m$/d than the control diet so that NR cows would achieve a BCS equal to their CON contemporaries by d 220 of gestation. Realimented NR cows had a BCS of 5.6, whereas CON cows had a BCS of 5.7 on d 192 of gestation (Miller et al., 2004). On d 250 of gestation, the remaining CON and NR cows were necropsied.

***Tissue Collection.*** On the day of slaughter, cows were stunned with a captive bolt and exsanguinated. The gravid

uterus was immediately collected and weighed, and the fetus was removed and weighed. Both maternal and fetal viscera were removed, trimmed of fat, and stripped of digesta. Small intestines (**SI**) were then measured and weighed. Demarcations of the duodenum, jejunum, ileum, cecum, and colon were made as described by Soto-Navarro et al. (2004). Eviscerated BW (**EBW**) was considered to be the maternal BW minus the gravid uterus and viscera or the fetal BW minus viscera.

***Cellularity Estimates.*** Maternal and fetal duodenum and jejunum were harvested and preserved for RNA, DNA, and protein analysis. The samples were wrapped in foil, snap-frozen in liquid nitrogen, and stored at -80°C. Tissues were then thawed and homogenized before being analyzed for DNA and RNA using diphenylamine (Johnson et al., 1997) and orcinol procedures (Reynolds et al., 1990) and for protein using Coomassie brilliant blue G (Bradford, 1976). The concentration of DNA was used as an index of hyperplasia, with protein:DNA and RNA:DNA ratios used as indexes of hypertrophy (Soto-Navarro et al., 2004).

***Proliferation, Vascularity, and Morphology.*** Fresh jejunal tissue sections were immersed in Carnoy's fixative. Tissues were embedded in paraffin (Reynolds and Redmer, 1992) and 4-µm tissue sections were mounted on glass slides, and prepared for staining procedures (Fricke et al., 1997; Soto-Navarro et al., 2004). Cellular proliferation was quantified using Image-ProPlus 5.0 software (MediaCybernetics Inc., Silver Spring, MD). A 150-cm section of jejunum was immediately removed for vascular perfusion according to Soto-Navarro et al. (2004). Cross-sections of perfused intestinal tissue were stained using periodic acid-Schiff's staining procedures to contrast the vascular tissue. Capillary area density measurements were made in the intestinal villi using the Image-Pro Plus software. Jejunal morphology was determined on the histological sections by computerized image analysis as previously described (Jin et al., 1994). A total of 20 villi and their associated length, width, and crypt depth were measured for each animal.

***Statistical Analysis.*** Maternal and fetal data were analyzed using the MIXED procedure of SAS version 9.1 (SAS Institute, Inc., Cary, NC) with day of gestation, nutritional treatment, and their interaction included in the model. Means were separated using the LSMEANS option of SAS and were considered significant when $P < 0.10$.

## Results and Discussion

***Maternal Effects.*** As expected, NR cows had reduced ($P < 0.01$) BW and eviscerated BW (EBW) compared with CON cows at d 125 (Table 1). At d 250 after realimentation, these were not different ($P \geq 0.64$). Weight and length of the cows' SI, when expressed alone or per unit of EBW, were not affected ($P \geq 0.21$) by treatment or day of gestation. However, in this same study, NR cows had reduced stomach complex and liver weights at d 125 (Molle et al., 2004). In contrast to the current study, reports by Scheaffer et al. (2004b) and Reed et al. (2007) indicate that nutrient restriction in gestating ewes resulted in reduced SI mass in addition to reduced stomach complex and liver masses.

Duodenal RNA:DNA was decreased in NR vs. CON cows ($P = 0.09$), indicating reduced synthetic capacity. This is in agreement with work using restricted pregnant ewe lambs (Reed et al., 2007), although their ewes were not realimented. Cow duodenal DNA was greater ($P = 0.01$) while RNA:DNA ($P = 0.06$) and protein:DNA ($P = 0.04$) were less at d 250 vs. 125. Jejunal RNA ($P = 0.02$) and RNA:DNA ($P = 0.09$) were also less at d 250 vs. 125. Scheaffer et al. (2003) also observed an effect of gestational day on intestinal DNA, RNA, and protein content in pregnant and nonpregnant heifers.

There was no effect of treatment on cow jejunal cell proliferation, which is in agreement with previous work showing no differences in jejunal cell proliferation of nutrient restricted or control pregnant ewes (Scheaffer et al., 2004a; Reed et al., 2007). When expressed as a % of total cells or number of proliferating:non-proliferating nuclei proliferation was decreased ($P \leq 0.08$) at d 250 vs. 125 (Table 2). Scheaffer et al. (2003) also observed an effect of gestational day on jejunal cell proliferation in pregnant heifers. Capillary area density of cow jejunum was increased for NR vs. CON ($P = 0.04$) and on d 250 vs. 125 ($P = 0.04$), in agreement with work of Scheaffer et al. (2004a) but in contrast to work by Reed et al. (2007). Jejunal villous morphology was not affected ($P \geq 0.21$) by treatment or day of gestation in the current study, which agrees with previous work investing the effect of day of gestation (Scheaffer et al., 2003).

***Fetal Effects.*** There was no effect of treatment ($P \geq 0.41$) on fetal BW or EBW, although both were greater ($P < 0.01$) at d 250 vs. 125 (Table 3). Fetal SI weight and length increased ($P < 0.01$) with d of gestation, but were unaffected by treatment ($P \geq 0.49$) and similar ($P = 0.50$) per unit of EBW. This is in contrast to previous research observing reduced SI mass and/or length in IUGR fetal or neonatal lambs and pigs (Trahair et al., 1997; Wang et al., 2005; Reed et al., 2007). Offspring in all of these studies showed signs of IUGR related weight or growth restriction, which is in contrast to the current study.

There was no effect of treatment on fetal SI cellularity estimates. Fetal jejunal DNA was decreased ($P = 0.09$) and RNA:DNA ($P = 0.05$) was increased at d 250 vs. 125. Fetal jejunal cell proliferation was unaffected ($P \geq 0.19$) by treatment. Although proliferation when expressed as a percentage of total cells was similar ($P = 0.14$) at d 125 and 250, number of proliferating:non-proliferating nuclei were decreased ($P = 0.07$) at d 250 vs. 125 (Table 4), supporting the hypothesis that hyperplasia is decreased at d 250.

Results of this study suggest that nutrient restriction in early gestation may have limited effects upon the offspring intestine when followed by realimentation during mid to late gestation. Although both maternal and fetal intestinal changes were observed in this study, day of gestation had a much greater effect than maternal nutritional treatment.

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**Table 1.** Effect of nutritional treatment and day of gestation on cow BW and small intestinal weight, length, and cellularity estimates

| | Treatment[1] | | | Day of Gestation[1] | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | NR | SEM | 125 | 250 | SEM | Trt | Day | Trt x Day |
| BW, kg | 624.5 | 578.5 | 11.5 | 557.5 | 645.5 | 13.3 | 0.009 | 0.001 | 0.05 |
| d 125 BW, kg | 596.6[a] | 517.2[b] | 13.3 | -- | -- | -- | -- | -- | -- |
| d 250 BW, kg | 651.0 | 638.3 | 18.8 | -- | -- | -- | -- | -- | -- |
| Eviscerated BW[3], kg | 367.6 | 333.5 | 10.5 | 353.3 | 347.8 | 12.1 | 0.03 | 0.71 | 0.01 |
| d 125 EBW, kg | 389.5[a] | 316.3[b] | 12.1 | -- | -- | -- | -- | -- | -- |
| d 250 EBW, kg | 344.9 | 349.9 | 17.1 | -- | -- | -- | -- | -- | -- |
| Small intestine | | | | | | | | | |
| Length, cm | 3,633.3 | 3,981.6 | 246.8 | 3,583.5 | 4,031.3 | 285.0 | 0.33 | 0.21 | 0.51 |
| Weight, g | 4,026.6 | 3,904.9 | 234.4 | 3,894.7 | 4,036.8 | 270.6 | 0.72 | 0.67 | 0.29 |
| g/kg EBW | 11.01 | 11.90 | 0.73 | 11.21 | 11.71 | 0.85 | 0.40 | 0.63 | 0.82 |
| Duodenum | | | | | | | | | |
| DNA, mg/g | 3.16 | 3.45 | 0.22 | 2.89 | 3.72 | 0.26 | 0.36 | 0.01 | 0.47 |
| RNA, mg/g | 3.48 | 3.13 | 0.28 | 3.23 | 3.38 | 0.33 | 0.40 | 0.72 | 0.62 |
| Protein | 78.2 | 78.1 | 2.4 | 75.8 | 80.6 | 2.7 | 0.98 | 0.16 | 0.50 |
| RNA:DNA | 1.14 | 0.93 | 0.08 | 1.15 | 0.92 | 0.10 | 0.09 | 0.06 | 0.64 |
| Protein:DNA | 26.1 | 24.4 | 1.9 | 28.1 | 22.4 | 2.2 | 0.54 | 0.04 | 0.35 |
| Jejunum | | | | | | | | | |
| DNA, mg/g | 1.83 | 1.74 | 0.11 | 1.87 | 1.70 | 0.13 | 0.57 | 0.30 | 0.42 |
| RNA, mg/g | 2.91 | 2.83 | 0.28 | 3.35 | 2.38 | 0.32 | 0.84 | 0.02 | 0.75 |
| Protein | 94.3 | 98.7 | 2.4 | 94.7 | 98.3 | 2.8 | 0.21 | 0.31 | 0.49 |
| RNA:DNA | 1.63 | 1.68 | 0.19 | 1.89 | 1.42 | 0.22 | 0.84 | 0.09 | 0.82 |
| Protein:DNA | 53.7 | 58.4 | 3.3 | 53.2 | 58.9 | 3.8 | 0.32 | 0.23 | 0.55 |

[a, b] Within a row, means for nutritional treatment differ ($P \leq 0.05$).

[1] On d 125 of gestation, 10 control (CON) and 10 nutrient restricted (NR) cows were necropsied. Remaining NR cows were realimented with a concentrate supplement, and on d 250 the remaining cows (5 CON and 5 NR) were necropsied.

[2] Probability values for effects of nutritional treatment (Trt), day of gestation (Day), and their interaction (Trt x Day).

[3] Eviscerated BW = final BW – (gravid uterus + viscera).

**Table 2.** Effect of nutritional treatment and day of gestation on maternal and fetal jejunal proliferation (prolif), vascularity, and morphology

| | Treatment[1] | | | Day of Gestation[1] | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | NR | SEM | 125 | 250 | SEM | Trt | Day | Trt x Day |
| Cow Jejunal Proliferation | | | | | | | | | |
| % proliferation[3] | 17.0 | 17.7 | 1.3 | 19.1 | 15.7 | 1.5 | 0.73 | 0.08 | 0.76 |
| # Prolif:#Non-prolif Nuclei | 0.12 | 0.13 | 0.01 | 0.14 | 0.11 | 0.01 | 0.77 | 0.07 | 0.80 |
| Cow Jejunal Vascularity | | | | | | | | | |
| Capillary Area Density[4] | 9.90 | 11.75 | 0.60 | 9.92 | 11.73 | 0.69 | 0.04 | 0.04 | 0.21 |
| Cow Jejunal Villi Morphology | | | | | | | | | |
| Villi Length, µ | 432.4 | 418.4 | 20.2 | 407.0 | 443.8 | 23.4 | 0.63 | 0.21 | 0.34 |
| Villi Width, µ | 113.7 | 121.3 | 11.8 | 123.5 | 111.5 | 13.6 | 0.65 | 0.48 | 0.69 |
| Crypt Depth, µ | 423.7 | 410.9 | 44.2 | 414.3 | 420.4 | 51.1 | 0.84 | 0.92 | 0.82 |
| Fetal Jejunal Proliferation | | | | | | | | | |
| % proliferation[a] | 17.1 | 22.7 | 2.9 | 23.0 | 16.8 | 3.2 | 0.19 | 0.14 | 0.76 |
| # Prolif:#Non-prolif Nuclei | 0.16 | 0.22 | 0.04 | 0.24 | 0.14 | 0.04 | 0.25 | 0.07 | 0.13 |

[1] On d 125 of gestation, 10 control (CON) and 10 nutrient restricted (NR) cows were necropsied and fetuses harvested. Remaining NR cows were realimented with a concentrate supplement, and on d 250 the remaining cows (5 CON and 5 NR) were necropsied and fetuses harvested.
[2] Probability values for effects of nutritional treatment (Trt), day of gestation (Day), and their interaction (Trt x Day).
[3] % proliferation = Proliferating Nuclei Area/( Proliferating Nuclei Area + Nonproliferating Nuclei Area).
[4] Capillary area density = total capillary area / total tissue area.

**Table 3.** Effect of nutritional treatment and day of gestation on fetal small intestine weight, length, and cellularity estimates

| | Treatment | | | Day of Gestation[1] | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | NR | SEM | 125 | 250 | SEM | Trt | Day | Trt x Day |
| Fetal Weight, kg | 13.5 | 13.9 | 0.4 | 0.9 | 26.5 | 0.4 | 0.51 | <0.001 | 0.43 |
| Eviscerated BW[3], kg | 10.4 | 10.7 | 0.3 | 0.7 | 20.3 | 0.3 | 0.41 | <0.001 | 0.35 |
| Small intestine | | | | | | | | | |
| Length, cm | 1,019.2 | 991.9 | 27.7 | 488.8 | 1,522.4 | 32.0 | 0.49 | 0.001 | 0.27 |
| Weight, g | 262.0 | 263.6 | 9.2 | 17.4 | 508.2 | 10.6 | 0.90 | 0.001 | 0.87 |
| g/kg EBW | 24.7 | 25.1 | 1.0 | 24.5 | 25.4 | 1.1 | 0.78 | 0.50 | 0.94 |
| Duodenum | | | | | | | | | |
| DNA, mg/g | 3.99 | 4.22 | 0.23 | 3.98 | 4.23 | 0.26 | 0.48 | 0.44 | 0.60 |
| RNA, mg/g | 3.39 | 3.41 | 0.15 | 3.41 | 3.39 | 0.17 | 0.93 | 0.94 | 0.32 |
| Protein | 77.0 | 76.2 | 2.3 | 75.8 | 77.4 | 2.6 | 0.80 | 0.61 | 0.83 |
| RNA:DNA | 0.89 | 0.84 | 0.06 | 0.87 | 0.86 | 0.07 | 0.58 | 0.86 | 0.21 |
| Protein:DNA | 20.2 | 18.9 | 1.4 | 19.8 | 19.4 | 1.7 | 0.53 | 0.86 | 0.65 |
| Jejunum | | | | | | | | | |
| DNA, mg/g | 2.76 | 2.67 | 0.16 | 2.91 | 2.51 | 0.19 | 0.69 | 0.09 | 0.76 |
| RNA, mg/g | 1.64 | 1.80 | 0.12 | 1.68 | 1.77 | 0.13 | 0.33 | 0.58 | 0.91 |
| Protein | 96.0 | 98.8 | 1.9 | 99.3 | 95.5 | 2.1 | 0.30 | 0.16 | 0.47 |
| RNA:DNA | 0.60 | 0.69 | 0.04 | 0.59 | 0.71 | 0.05 | 0.16 | 0.05 | 0.69 |
| Protein: DNA | 35.5 | 38.8 | 2.1 | 35.7 | 38.6 | 2.4 | 0.27 | 0.32 | 0.57 |

[1] On d 125 of gestation, 10 control (CON) and 10 nutrient restricted (NR) cows were necropsied and fetuses harvested. Remaining NR cows were realimented with a concentrate supplement, and on d 250 the remaining cows (5 CON and 5 NR) were necropsied and fetuses harvested.
[2] Probability values for effects of nutritional treatment (Trt), day of gestation (Day), and their interaction (Trt x Day).
[3] Eviscerated BW = fetal weight – viscera.

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# CHROMIUM METHIONINE SUPPLEMENTATION ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF BULLS: I. RESULTS DURING COOL SEASON IN THE NORTHWEST OF MEXICO

**R. Barajas [1]*, B. J. Cervantes[1, 2], E. A. Velazquez[1], J. A. Romo[1], F. Juarez[1], and P. J. Rojas[1, 3]**
[1] FMVZ-Universidad Autónoma de Sinaloa, México. [2] Ganadera Los migueles, S.A. de C.V. [3] Tecnologia de Máxima Producción, S.A. de C.V.
E-mail: rubar@uas.uasnet.mx

**ABSTRACT**: To determine the influence of chromium methionine supplementation on feedlot performance and carcass characteristics of bulls during the cool season, a 174-days feedlot experiment involving Thirty Brahman cross bulls 240 ± 4.59 kg was performed. Experiment was conducted from October, 2006 to April, 2007. Animals were blocked by starting weight and in groups of five placed in ground flour pen (2 x 12 m). Experiment was conducted Agreement with a randomized complete block design were assigned to receive or not supplementary chromium. Chromium supplementary level was equivalent to 0.29 mg of Cr/kg of DM, and was provided from a chromium methionine premix (MiCroplex; Zinpro Corp. Eden Prairie, MN). Blood samples were taken in day 28 and at killed time for cortisol determination.
Chromium supplementation increased (P < .01) ending weight (466 vs. 500 kg), and average daily gain (1.28 vs. 1.47 kg/d). DMI was increased (P < .01) in 8% by Cr-Met, and feed/gain ratio was improved 5.6% (P = .08). Hot carcass weight was augmented (P < .05) in 6.8% by Chromium (308.9 vs. 329.8 kg). Carcass dressing was similar (P = .33) between treatments. Lon muscle area and KPH fat were increased (P < .10) by Cr-Met. Back fat thickness and marbling were similar (P >.10) in both treatments. It is concluded, that chromium methionine supplementation improves weight gain and carcass weight of feedlot-bulls.

Key words: Chromium, bulls, feedlot-performance.

## Introduction

The organic chromium supplementation has been reduced blood cortisol (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Chang et al., 1995), improved weight gain during receiving period (Monsie-Shager et al., 1993; Kegley and Spears, 1995); and sometimes during growing period (Kegley et al., 1997). Pollard et al. (1999) not found effect of high-Cr yeast supplementation on performance of feedlot cattle.
Chromium methionine supplementation reduce cortisol (Almeida and Barajas, 2002), improved weight gain during receiving (Barajas and Almeida, 1999; Barajas et al., 1999; Barajas et al., 2005a) and growing period (Barajas et al., 2005b). However there is not information about the effect of chromium methionine supplementation during complete feedlot period of cattle.
This experiment was conducted with the objective of determine the influence of chromium methionine supplementation on feedlot performance and carcass characteristics of bulls during the cool season in the Northwest of Mexico.

## Material and Methods

*Location*

The experiment was conducted from May, 2006 to April, 2007 at Experimental Station for Beef Cattle in Dry Tropic Weather of the Universidad Autonoma de Sinaloa. The research facilities are located at Ganadera Los Migueles feedlot, S.A. de C.V. in Culiacan, Sinaloa situated in Northwest Mexico (24º 51' N. and 107º 26' W. ; 57 m o.m.s.l.; mean temperature 25 ºC, and 645 mm annual rainfall).

*Animals Management*

Animals used in the experiment were managed according to the recommended guidelines in *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (1988). Hundred twenty Brahman-cross bull calves were acquired from different farms and transported 300 km via truck to Ganadera Los Migueles feedlot. Upon arrival at the feedlot, calves were weighed and vaccinated to prevent infections by *Pasteurella sp*. (One Shoot®; Pfizer Ltd.). Cattle were then placed in a large dirt lot. They were fed alfalfa hay and a 40:60 forage:concentrate diet, and had free access to fresh clean drinking water.

Seven days later, all the animals were weighed and 30 calves weighing 240 ± 4.59 kg were selected for the 174 day feedlot experiment. Selected calves were identified with a numbered ear tag, implanted (Component TES with Tylan®;ELANCO Co.), vaccinated with *Clostridium* and *Haemophilus somnus* (Ultrabac-Somnobact®; Pfizer ), dewormed (Albendaphorte®; Lab. Salud y Bienestar ), and injected with vitamins A, D and E (ADEphorte®; Lab. Salud y Bienestar ). Groups of five calves were randomly placed in 12 pens (6 x 12 m), each fitted with a 2.4 m feed

bunk and 0.6 m waterer. Animals had *ad libitum* access to feed and water.

*Treatments assignation*

In accordance to a randomized complete blocks design described by Hicks (1973), the calves were randomly assigned to receive one of two treatments: 1) Control, no Cr supplementation (**CON**); or 2) Chromium methionine supplementation, 2.2 mg of Cr/head/day, (**CrMet**). Supplemental Cr was in the form of chromium methionine (MiCroPLEX®; Zinpro Corporation, Eden Prairie, MN).

*Experimental procedure*

Chromium supplementation was provided in the following manner. For each CrMet designated pen, 11 g MiCroPLEX was thoroughly mixed with 1 kg ground corn, mixture was top dressed in the feed bunk and hand mixed with the diet. For calves in the CON treatment pens, 1 kg of ground corn was top dressed and hand mixed with the diet in the feed bunk, to homogenize daily ration composition in respect to calves receiving supplemental Cr. Diet composition is presented in Table 1. Cattle had *ad libitum* access to the diets that were offered once daily (1600 h). Feed intake was measured as feed offered minus weekly refusals. Feed samples (4 kg) were collected weekly directly from mixer wagon, oven dried (105 °C for 24 h), and dry matter intake calculated. Thirty three days before slaughter date, all diets were supplemented with 6 mg/kg of the beta-adrenergic zilpaterol chloride (Zilmax®; Intervet), three days previously to slaughter, zilpaterol was take out of the diet. Animals were weighed on days 1, 28 and end of the experiment, and 4% of weight was subtracted as a correction for digestive tract fill (NRC, 1984).

*Carcass Measurements*

Upon complete the feedlot experiment time, the bulls were sacrificed in a slaughter house supervised by the sanitary authority of Culiacan City. Hot carcass weights were recorded, and after 24 hours chilling period in a cold room (2 ºC), left carcass side longissimus muscle was cross sectioned between the 12$^{th}$ and 13$^{th}$ rib, back fat (cm) and longissimus muscle area (LMA) was measured by direct grid reading, marbling score and percentage of KPH fat was visually estimated. Preliminary yield grade and cutability was estimated using procedures proposed by USDA (1996). Meat pH was measured in *pectoralis profundus* muscle using a pH-meter fitted with a penetration electrode (HI8314 membrane pHmeter; Hanna Instruments).

*Serum determinations*:

Twelve bull-calves (one from each pen; six by treatment), were randomly selected to be blood sampled. Bull-calves were bled by jugular venipuncture on d 28, using plain glass vacuum tubes (Vacutainer 6431; Becton Dickinson, Rutherford, NJ). At death time, blood samples were taken again. Serum was obtained for cortisol, measurements. Serum cortisol was determined by radioimmunoassay using antibody-coated tubes (Diagnostic Products Corp., Los Angeles, CA).

Table 1. Composition of basal diets used in performance experiment

| Ingredients (%) | Diets | | |
|---|---|---|---|
| | Receiving | Growing | Finishing |
| Corn Silage | 43.57 | - | - |
| Corn straw | 16.80 | 18.22 | 12.20 |
| Ground corn | 10.27 | 57.18 | 68.61 |
| Soyeban meal | 22.41 | 7.08 | 6.01 |
| Pork meat and bone meal | 2.80 | 4.05 | 2.00 |
| Sugar cane molasses | - | 8.43 | 4.17 |
| Tallow | - | 2.23 | 4.41 |
| Premix [1] | 3.11 | 2.81 | 2.78 |
| Buffer blend[1] | 1.04 | - | - |
| Total | 100% | 100% | 100% |
| | Calculated Analyses (DM basis) [2] | | |
| DM, % | 48.2 | 88.93 | 89.74 |
| CP, % | 19.92 | 14.31 | 13.17 |
| NEm, Mcal/kg | 1.503 | 1.920 | 2.048 |
| NENg, Mcal/kg | 0.922 | 1.270 | 1.385 |

[1] Ganamin Total ® (Vitamins and mineral premix containing 25 g of sodium-monensin from Rumensin 200 ® (Elanco), and Ganabuffer ® (Buffering agents premix), are trademarks (Técnica Mineral Pecuaria, S.A. de C.V.; Guadalajara, Jal, México).

[2] Calculated from tabular values (NRC, 2000).

*Statistical Analysis*

Performance and serum data was analyzed as a randomized complete blocks design (Hicks, 1973), considering each pen as the experimental unit. General AOV/AOCV procedure of Statistix® 8 program (Analytical Software, Tallahassee, FL) was used to perform the analyses, and *P*-value for F-test was obtained.

**Results and Discussion**

Mean chromium intake in complete experiment was equivalent to 0.29 ppm. The influence of chromium methionine supplementation on feedlot performance of bulls is shown in Table 2, and its influence on carcass characteristics is presented in Table 3. Chromium methionine supplmentnation increased ($P < .01$) 7% ending weight and 14.5% average daily gain. This response is in correspondence with previously observed in receiving cattle fed chromium methionine (Barajas and Almeida, 1999;

Barajas et al., 1999; Barajas et al., 2005a), and is attributable to effect of chromium that improves insulin signal (Vincent, 2000), and consequently its anabolic effect. Cr-Met increased (P < .01) DMI, and improved (P = .08) 5.6% feed/gain ratio. However, retained NE from the diet was unchanged (P = .23) and observed/expected NE ratio was similar (P = .29) across treatments. This result suggests that feedlot performance improvement by chromium supplementation is due to incremented daily energy intake rather than to an useful of energy ingested.

The increment (P < .05) in 6.8% of carcass weight is in concordance with increased (P < .01) ending weight. Cr-Met increased (P = .07) in 2.8% loin muscle area, and in 13% (P = .08) KPH fat. These finding indicates that anabolic insulin activity enhanced by chromium supplementation improves fat synthesis joint with muscle accretion. Treatment have not influence on serum cortisol (P > .40).

## Implications

Results of this experiment suggest that chromium methionine supplementation improves body mass accretion, manifested as daily weight gain and carcass weight increment in feedlot bulls. And appears that chromium from available sources as chromium-methionine is essential by well performance of cattle intensively fed with high grain diets.

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Table 2. Influence of chromium methionine supplementation on feedlot performance of bulls during cool season.

| Variables | Treatments | | SEM[2] | *P-value* |
|---|---|---|---|---|
| | Control | Chromium[1] | | |
| Animals, n | 15 | 15 | | |
| Pens, repetitions, n | 3 | 3 | | |
| Days in trial | 174 | 174 | | |
| Starting weight [3] | 239.74 | 240.67 | 4.59 | .99 |
| Ending weight [3, 4] | 466.39 | 499.86 | 8.63 | <.01 |
| Average daily gain, kg/day | 1.282 | 1.468 | .04 | <.01 |
| Dry matter intake, kg/day | 7.023 | 7.596 | .15 | <.01 |
| Feed/gain, kg/kg | 5.478 | 5.167 | .06 | 0.08 |
| Observed diet net energy | | | | |
| NEm , Mcal/kg | 2.141 | 2.228 | .02 | .23 |
| NEg, Mcal/kg | 1.467 | 1.543 | .02 | .23 |
| Observed/expected net energy | | | | |
| NEm , Mcal/kg | 1.12 | 1.16 | .01 | .29 |
| NEg, Mcal/kg | 1.16 | 1.21 | .01 | .30 |

[1] Experiment mean chromium intake was 0.29 ppm
[2] Standard Error of the mean
[3] Four percent was pencil discounted as digestive tract fill (NRC, 1984)
[4] Animals were fed zilpaterol chloride 30 days during end-finishing period, three days before slaughter zilpaterol was removed from the diet.

Table 3. Influence of chromium methionine supplementation on carcass characteristics of bulls.

| Variables | Treatments | | SEM[2] | *P-value* |
|---|---|---|---|---|
| | Control | Chromium[1] | | |
| Hot carcass weight, kg | 308.70 | 329.77 | 4.49 | 0.05 |
| Carcass dressing, % [3] | 67.13 | 66.88 | .51 | .33 |
| Back fat thickness, cm | 0.73 | 0.94 | 0.09 | 0.14 |
| Rib eye area, $cm^2$ | 81.25 | 83.56 | 1.16 | 0.07 |
| KPH fat, % | 1.93 | 2.20 | 0.09 | 0.08 |
| Marbling [4] | 480 | 433 | 27.77 | 0.29 |
| Cortisol, µg/dL | 5.7 | 6.0 | .46 | .88 |
| Muscle pH | 5.70 | 5.58 | 0.05 | 0.11 |
| Muscle temperature, ºC | 2.25 | 2.38 | 0.20 | 0.74 |
| USDA Yield grade | 2.23 | 2.45 | .07 | .11 |
| Cutability, % | 51.80 | 51.25 | .14 | .08 |

[1] Experiment mean chromium intake was 0.29 ppm
[2] Standard Error of the mean
[3]Animals were fed zilpaterol chloride 30 days during end-finishing period, three days before slaughter zilpaterol was removed from the diet.
[4] Code: traces = 300; slight = 400; small = 500; modest = 600, etc.

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# FEEDING BARLEY BETA-GLUCANS TO STIMULATE THE IMMUNE SYSTEM OF CALVES CHALLENGED WITH BVDV[1]

**A.V. Grove, C.R. Kaiser, J.A. Wiley, A.G. Harmsen, and J.G.P. Bowman**
Montana State University, Bozeman 59717

**ABSTRACT :** Ten <1-wk-old dairy calves (avg BW 40 kg) were assigned to one of 2 treatments: corn-based starter pellet (0.12% beta-glucan) or a 'Valier' barley-based starter pellet (2.8% beta-glucan) in order to evaluate the effect of barley beta-glucans on immune response to bovine viral diarrhea virus (BVDV) challenge. After a 7-wk adaptation and weaning period, all calves were challenged with 6.6 x $10^6$ $TCID_{50}$ (BVDV) in 10 mL of sterile saline in each side of the lung (d 0). Serum and bronchoalveolar lavage (BAL) samples were collected on d 0, 3, 5, 7, 10, 14, and 21 post-infection and analyzed for relative antibody titers, presence of virus, and cell differential counts. Calves were monitored and scored twice daily for clinical signs of sickness and weighed weekly throughout the study. Prior to infection, pellet intake and ADG did not differ ($P > 0.35$) between treatments; however, post-infection ADG by calves fed corn was greater ($P = 0.03$) than ADG by calves fed barley (0.8 vs 0.6 kg/d). Rectal temperature of calves fed barley tended ($P = 0.11$) to be higher than temperature of calves fed corn (38.8 vs 38.7° C) while calves consuming corn had a higher ($P < 0.05$) pulse rate than barley-fed calves on 6 d. Respiration rate was lowest ($P < 0.05$) by barley-fed calves in the afternoon (33 breaths/min), intermediate by barley and corn-fed calves in the morning (36 breaths/min), and highest by corn-fed calves in the afternoon (40 breaths/min). Bovine viral diarrhea virus from BAL samples was detected in more ($P < 0.05$) wells from corn than barley-fed calves on d 10, 14, and 21. Only 1 calf in each treatment seroconverted by d 21. Differential cell counts in BAL samples did not differ ($P > 0.54$) between treatments. There were some improvements in immune response of calves consuming barley diets containing beta-glucans; however, this did not translate to improved animal performance.

Key Words: barley, beta-glucan, BVDV

## Introduction

Beta-glucans are naturally occurring carbohydrates found in yeast, fungi, oats, and barley that have been shown to stimulate the mammalian immune system (Williams, 1997). Mice consuming barley-based diets had increased levels of antibodies and improved weight recovery after a viral infection compared to mice consuming corn-based diets (Grove et al., 2007b). Sealey et al. (2006) also reported fish fed diets based on barley varieties containing 5.2 or 8.2% beta-glucan had improved survivability compared to fish fed wheat-based diets (~1% beta-glucan) after a viral challenge. Few researchers have evaluated the immune response of orally administered beta-glucans in cattle because beta-glucans are assumed to be completely digested in the rumen. It has been hypothesized that mucosal cells in the intestine may be able to pick up beta-glucan, or beta-glucan fragments, before digestion occurs and translocate them directly to the blood and immune system while preserving the original active conformation of beta-glucan (Delaney et al., 2003 based on work by Owen, 1999). Ninety-eight percent of orally administered SSG (soluble BG from *Sclerotinia sclerotiorum*) was found in the gastrointestinal tract suggesting that beta-glucan can act directly from the gut to stimulate the immune system (Miura, 2005). While the exact mechanism is unknown, we are assuming that it is necessary to deliver beta-glucan to the small intestine in order to stimulate the immune system of mammals.

The objective of this study is to determine the immune response of virus-challenged dairy calves fed a barley-based diet containing beta-glucans. We hypothesize that calves fed barley-based diets will have an improved ability to eliminate viral antigens resulting in improved health and weight gains.

## Materials and Methods

One-day old dairy calves (avg wt 40 kg) were purchased and screened for BVDV titers in blood serum in order to identify 10 calves that had no previous exposure to BVDV or low BVDV titers. Calves were assigned to one of 2 treatments: corn-based starter pellet (0.12% beta-glucan) or a 'Valier' barley-based starter pellet (2.8% beta-glucan) as previously described (Grove et al., 2007a).

Calves were fed only milk replacer the first week of life and offered their assigned treatment pellets beginning on d -46 (i.e. 7 d after purchase and 46 d prior to infection). Calves were weighed upon receipt and once a week throughout the study. Calves were weaned off milk replacer beginning on d -18 and were consuming only pellets by d -10. Samples of diet and feed refused were collected from each calf on d -23, -16, -9, and -2. Diet and feed refusals were dried in a 60° C forced-air oven, ground through a Wiley mill (1-mm screen), and analyzed for dry matter (AOAC, 1999), starch (Megazyme, Sydney, Australia), and beta-glucan (McCleary kits; Megazyme, Sidney, Australia) in order to calculate DM, starch, and beta-glucan intake.

On d 0, all calves were challenged with 6.6 x $10^6$ $TCID_{50}$ BVDV (Singer strain Type 1 cytopathic) in 10 mL

[1] The authors wish to thank Kerri Rask and Jim Thompson for their valuable animal care expertise on this project.

of sterile saline in each side of the lung. Serum and bronchoalveolar lung lavage (BAL) samples were also collected from each side of the lung by fiberoptic bronchoscopy performed on lightly anesthetized calves (Silflow et al., 2005) on d 0 (prior to infection) and on d 3, 5, 7, 10, 14 and 21 post-infection. Serum was analyzed for relative antibody titers using a serum neutralizing assay and for the presence of BVDV using a virus isolation technique (Pathobiology Diagnostic Services, College of Veterinary Medicine, Auburn Univ.). Bronchoalveolar lung lavage samples were analyzed for presence of virus by cytopathic assays and for cell differential counts. Calves were monitored and scored twice daily for clinical signs of sickness including: body temperature, respiration, and pulse rate.

All data were analyzed using SAS (SAS Inst. Inc., Cary, NC). Intake and cell differential count data were analyzed using the MIXED procedure with treatment, day, and their interaction in the model (Littell et al., 1998). Body weight and ADG data were analyzed using the GLM procedure. Viral recovery was analyzed using chi-square. Fisher's exact test was used to obtain the probability level for the chi-square analysis if a cell in the contingency table contained less than 5 observations. Means were separated using LSMEANS when $P < 0.05$.

## Results and Discussion

*Intake.* Pellet intake did not differ ($P = 0.36$) between treatments prior to infection averaging 815 g/d (Table 1). Pellet intake increased ($P < 0.001$) as the study progressed and calves were weaned from milk. By design beta-glucan intake was greater ($P < 0.001$) by calves fed barley than by calves fed corn. Using the 93% beta-glucan ruminal digestibility reported by Grove et al. (2007a), we estimated that the amount of beta-glucan entering the small intestine of barley-fed calves in the current study ranged from 0.01 to 0.04 g beta-glucan/kg BW. The current results agrees with our previous estimates of beta-glucan intake by young calves (Grove et al., 2007a); however, in both studies the amount of beta-glucan available at the small intestine of young calves is lower than levels other researchers have administered orally to stimulate the immune system of mice: 0.10 to 0.22 g BG/kg BW (Yun et al., 2003; Davis et al., 2004). Similar to research in ruminants, Dongowski et al. (2002) also detected no beta-glucan in the feces of rats suggesting that beta-glucan may be digested in the gastrointestinal tract of rodents as well. The amount of beta-glucan reaching the small intestine of mice is unknown as is the amount of oral beta-glucan needed for immune system stimulation.

*Average daily gain.* Pre-infection ADG did not differ ($P = 0.70$) between treatments averaging 0.44 kg/d (Table 2). All calves continued to gain weight after infection with BVDV; however, ADG by calves fed corn was greater ($P = 0.03$) than ADG by calves fed barley post-infection (0.8 vs 0.6 kg/d). Due to the higher starch content of corn, starch intake by corn-fed calves was greater ($P < 0.001$) than starch intake by calves fed barley pellets on d -9 and -2 (Table 1). We were unable to get an accurate estimate of pellet intake once calves were infected; however, if this difference in starch intake continued after infection, then corn-fed calves may have consumed more energy resulting in greater weight gain.

*Clinical signs.* Clinical signs of sickness associated with BVDV are usually mild and include: mild fever, diarrhea, leucopenia, and viremia (Brownlie et al., 1987; Brock, 1997). Rectal temperature of calves fed barley tended ($P = 0.11$) to be higher than temperature of calves fed corn (38.8 vs 38.7° C). Calves consuming corn had a higher ($P < 0.05$) pulse rate than barley-fed calves on 6 d. Respiration rate was lowest ($P < 0.05$) by barley-fed calves in the afternoon (33 breaths/min), intermediate by barley and corn-fed calves in the morning (36 breaths/min), and highest by corn-fed calves in the afternoon (40 breathes/min). It should be noted that all barley fed calves were housed on the east side of the building and all corn fed calves were housed on the west side; however, it's difficult to determine if differences in temperature, pulse, and respiration were confounded by the side of the building calves were housed in.

*Virus isolation.* No BVDV was detected in the serum of calves. In contrast to our results, others reported viremia with BVDV infection (Brownlie et al., 1987; Brock, 1997); however, Harding et al. (2002) suggested that viremia was not observed in cattle infected with cytopathic BVDV but was common in cattle infected with non-cytopathic BVDV. We used Singer strain Type 1 cytopathic BVDV in the current study.

Relative amounts of virus in BAL samples were determined by incubating the sample in quadruplicate (4 wells/BAL sample/calf) at various dilutions (1:2, 1:10, 1:100, 1:1000). Therefore, virus detected in a greater number of wells and at a higher dilution would imply more virus was in that calf. At 1:2 dilution, BVDV from BAL samples was detected in more ($P < 0.05$) wells from corn than barley-fed calves on d 10, 14, and 21 (Table 3). At 1:10, BVDV was detected in more ($P < 0.05$) wells from corn than barley-fed calves on d 14. At 1:100, BVDV was detected in more ($P < 0.05$) wells from corn than barley-fed calves on d 10. By 10 d post-infection, no barley calves had detectable virus at 1:100. Virus began clearing the lungs of barley-fed calves on d 10 and >50% of barley-fed calves were clear of virus by d 14. On d 14, twice as many corn-fed calves had virus at 1:2 compared to calves fed barley pellets. One corn-fed calf never cleared the virus completely from the lungs. Greater viral recovery in BAL from corn- than barley-fed calves on d 10, 14, and 21 suggests slower viral clearance from the lungs of calves fed corn compared to calves fed barley.

*Serum neutralizing antibody response.* BVDV neutralization via serum antibodies was evaluated in order to determine if any calves had seroconverted following the viral infection. A known viral challenge (Type 1 BVDV reference strains) was incubated with aliquots of serum dilutions and then inoculated onto cell monolayers. The cell monolayers were then monitored for cytopathic effect. Only 1 calf in each treatment seroconverted by d 21. The barley-fed calf exhibited a 4-fold increase in serum neutralizing activity while a 6-fold increase in activity was observed in the corn-fed calf. Brownlie et al. (1987) and Howard et al. (1992) reported that antibody responses to BVDV did not

occur until 2 to 3-wk post-infection with maximum antibody titers observed 8 to 12-wk after infection (Brownlie et al., 1987; Howard et al., 1989).

*Differential cell counts in BAL.* There were day effects ($P < 0.001$) but no treatment effects ($P > 0.54$) or treatment x day interactions ($P > 0.18$) for differential cell counts in BAL (Table 4). Increased numbers of total cells on d 5 and 14 compared to d 0 probably reflect increased numbers of neutrophils in BAL samples on these days. There was also a spike in lymphocytes on d 5 but increases in numbers of macrophages didn't occur until 14 d post-infection. Macrophages and neutrophils both phagocytose antigens and are the first and second lines of defense, respectively, in an infection. The highest numbers of total cells, lymphocytes, macrophages, and neutrophils were observed on d 21. Decreased white blood cell counts are often observed with BVDV infection (Brownlie et al., 1987; Brock, 1997).

## Implications

Calves consuming barley-based pellets had faster viral clearance from the lung and lower pulse rates, and tended to have a lower respiration rate. Corn-fed calves had higher pellet intake and ADG, and slightly lower rectal temperatures. Lack of seroconversion until d 21 post-infection suggests that calves should have been monitored for a longer period of time. There were some improvements in immune response with the barley-fed calves, however, this did not translate to improved animal performance.

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Table 1. Dry matter, starch, and beta-glucan intake (DM basis) by dairy calves consuming starter pellets based on corn or barley

| | Treatment | | Day (prior to infection) | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | Barley | Corn | -23 | -16 | -9 | -2 | SE | Trt | Day | Trt*Day |
| Intake | | | | | | | | | | |
| Milk replacer, g/d | 202 | 202 | 538 | 269 | 0 | 0 | | | | |
| Pellet, g/d | 722 | 908 | 298$^{a}$ | 557$^{b}$ | 1036$^{c}$ | 1370$^{d}$ | 99.2 | 0.36 | <0.001 | 0.12 |
| Barley | | | 284 | 474 | 908 | 1222 | | | | |
| Corn | | | 311 | 639 | 1163 | 1519 | | | | |
| Starch, g/d | 255 | 400 | 140$^{a}$ | 231$^{b}$ | 405$^{c}$ | 535$^{d}$ | 54.4 | 0.08 | <0.001 | <0.001 |
| Barley | | | 121$^{a}$ | 174$^{b}$ | 309$^{cA}$ | 416$^{dA}$ | | | | |
| Corn | | | 158$^{a}$ | 287$^{b}$ | 500$^{cB}$ | 654$^{dB}$ | | | | |
| Beta-glucan, g/kg BW | | | | | | | | | | |
| | 0.35$^{b}$ | 0.02$^{a}$ | 0.08$^{a}$ | 0.13$^{b}$ | 0.24$^{c}$ | 0.29$^{d}$ | 0.027 | <0.001 | <0.001 | <0.001 |
| Barley | | | 0.15$^{aB}$ | 0.24$^{bB}$ | 0.45$^{cB}$ | 0.55$^{dB}$ | | | | |
| Corn | | | 0.01$^{A}$ | 0.02$^{A}$ | 0.02$^{A}$ | 0.03$^{A}$ | | | | |

[a-d] Within a row and item, means without a common superscript letter differ ($P < 0.05$).
[AB] Within a column and day, means without a common superscript letter differ ($P < 0.05$).

Table 2. Body weight and ADG by dairy calves consuming starter pellets based on corn or barley and infected with BVDV.

| Item | Barley | Corn | SE | *P*-value |
|---|---|---|---|---|
| Start wt, kg | 40.3 | 38.9 | 2.80 | 0.74 |
| End wt, kg | 74.8 | 80.7 | 5.6 | 0.47 |
| ADG, kg/d | | | | |
| Overall | 0.51 | 0.62 | 0.050 | 0.16 |
| Pre-infect | 0.42 | 0.45 | 0.064 | 0.70 |
| Post-infect | 0.64 | 0.83 | 0.053 | 0.03 |

Table 3. Percent wells (number calves) testing positive for BVDV from bronchoalveolar lavage samples of dairy calves consuming starter pellets based on corn or barley and infected with BVDV

| | Day | | | | | | |
|---|---|---|---|---|---|---|---|
| Dilution | 0 | 3 | 5 | 7 | 10 | 14 | 21 |
| 1:2 | | | | | | | |
| Barley | 0 | 36(5) | 29(5) | 24(4) | 8$^{a}$(3) | 3$^{a}$(2) | 0$^{a}$ |
| Corn | 0 | 30(5) | 24(4) | 25(3) | 23$^{b}$(4) | 18$^{b}$(4) | 8$^{b}$(1) |
| 1:10 | | | | | | | |
| Barley | 0 | 26(4) | 16(5) | 20(5) | 9(3) | 1$^{a}$(1) | 0 |
| Corn | 0 | 24(5) | 24(5) | 20(3) | 13(3) | 10$^{b}$(1) | 5(1) |
| 1:100 | | | | | | | |
| Barley | 0 | 13(4) | 4(2) | 3(2) | 0$^{a}$ | 0 | - |
| Corn | 0 | 5(2) | 9(4) | 8(3) | 9$^{b}$(1) | 3(1) | 1(1) |
| 1:1000 | | | | | | | |
| Barley | 0 | 1(1) | 0 | 0 | 0 | 0 | - |
| Corn | 0 | 0 | 2(1) | 0 | 2(1) | 0 | 1(1) |

[ab] Within a day and dilution, means without a common superscript letter differ ($P < 0.05$)

Table 4. Numbers of total cells, macrophages, lymphocytes and neutrophils in bronchoalveolar lavage samples from dairy calves consuming starter pellets based on corn or barley and infected with BVDV

| | Day | | | | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 3 | 5 | 7 | 10 | 14 | 21 | SE | Trt | Day | Trt*Day |
| | ----------1 x $10^{6}$---------- | | | | | | | | | | |
| Total cells | 0.497$^{a}$ | 0.827$^{ab}$ | 1.663$^{b}$ | 0.998$^{ab}$ | 1.263$^{ab}$ | 1.734$^{b}$ | 2.964$^{c}$ | 0.3575 | 0.58 | <0.001 | 0.19 |
| Lymphocytes | 0.008$^{a}$ | 0.019$^{ab}$ | 0.056$^{b}$ | 0.014$^{ab}$ | 0.026$^{ab}$ | 0.010$^{a}$ | 0.113$^{c}$ | 0.0155 | 0.86 | <0.001 | 0.84 |
| Macrophages | 0.306$^{a}$ | 0.279$^{a}$ | 0.386$^{ab}$ | 0.347$^{ab}$ | 0.337$^{a}$ | 0.472$^{bc}$ | 0.550$^{c}$ | 0.0554 | 0.97 | 0.002 | 0.55 |
| Neutrophils | 0.183$^{a}$ | 0.529$^{ab}$ | 1.221$^{b}$ | 0.612$^{ab}$ | 0.900$^{ab}$ | 1.252$^{b}$ | 2.309$^{c}$ | 0.3252 | 0.55 | <0.001 | 0.19 |

[a-c] Within a row, means without a common superscript letter differ ($P < 0.05$)

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# IMPACT OF SUPPLEMENTED GLUCOGENIC PRECURSORS ON NUTRIENT PARTITIONING IN YOUNG POSTPARTUM RANGE COWS

**J. T. Mulliniks, M. E. Kemp, S. I. Valverde-Saenz, M. B. Horvath, S. H. Cox, A. Cibils, C. A. Löest, and M. K. Petersen**

New Mexico State University, Las Cruces, NM

**ABSTRACT**: Diet and physiological state have been implicated in altering insulin sensitivity in range cows. An increase in diet glucogenic precursors (**GP**) may improve sensitivity to insulin. A study at the Corona Range and Livestock Research Center in 2006 (n = 29) and 2007 (n = 24) evaluated metabolic and lactational responses of postpartum 2 and 3 yr old cows grazing native range and individually fed one of three 36% CP supplements with increasing GP. Supplements were fed at 908 g•cow$^{-1}$•d$^{-1}$ twice weekly providing 1) 328 g CP, 110 g UIP, 44 g GP (**0**), 2) 328 g CP, 157 g UIP + 40 g propionate salt (NutroCal$^{TM}$, Kemin Industries, Inc.), 93 g GP (**40**), 3) 329 g CP, 158 g UIP + 80 g propionate salt, 124 g GP (**80**). In 2006 and 2007, cows were machine milked at 57 d and 69 d postpartum. In 2006, a glucose tolerance test (**GTT**) at 64 d and in 2007, an acetate tolerance test (**ATT**) was conducted at 62 d postpartum. Supplement did not affect milk composition or yield ($P \geq 0.53$; 5736, 6402, and 5797 ± 463 g/ 24h for 0, 40, and 80, respectively). Acetate half-life decreased linearly ($P = 0.08$) with increasing GP (35, 29, 27 ± 3 min for 0, 40, and 80, respectively). Glucose half-life was similar among treatments ($P = 0.88$; 88, 97, 97 ± 15 min for UIP0, UIP40, and UIP80, respectively). As GP increased in supplements, blood β-hydroxybutyrate decreased linearly ($P = 0.01$; 0.38, 0.29, 0.30 ± 0.02 mmol/L for 0, 40, and 80, respectively). Serum insulin concentration ($P = 0.77$; Avg. = 0.42 ng/mL) was not affected by GP. Serum NEFA and glucose concentrations increased linearly with increasing glucogenic potential ($P = 0.10$; 450, 479, and 478 ± 11 mg/100 mL and 54.3, 55.6, and 57.7 ± 0.9 mg/dL for 0, 40, and 80, respectively). Because glucose clearance was similarly extended for all cows, they were considered insulin-resistant. However, cows consuming more GP had reduced serum acetate half-life and blood ketone concentration demonstrating improved glucose status.

Key Words: beef cattle, glucose, insulin, lactation

## INTRODUCTION

Diet quality and physiological state have been implicated in altering insulin sensitivity in beef cows (Bines and Hart, 1982; Endecott et al., 2004). Ruminal fermentation of dormant forage is characterized by predominate acetate production and lower proportion as propionate (Cronje et al., 1991). Metabolizable supply of glucose derived from the propionate, glucogenic precursor, may be inadequate to supply sufficient glucose for oxidative energy metabolism. Therefore, an accumulation of acetate may result from a slow rate of acetate clearance. These events also coincide with an increase in concentrations of ketones and free fatty acids. Elevated ketones and free fatty acids are implicated in insulin resistance (Dresner et al., 1999; Tardif et al., 2001). Waterman et al. (2006) found that cows fed supplements with increasing amounts of GP while grazing dormant forage increased glucose disappearance and altered tissue sensitivity to insulin. Therefore, the objective of this study was to investigate metabolic responses of young postpartum beef cows fed increasing amounts of GP. A secondary objective was to evaluate differences in metabolic response between age groups.

## MATERIALS AND METHODS

This study was conducted in spring of 2006 and 2007 at New Mexico State University's Corona Range and Livestock Research Center in Corona, NM and was part of a 3 yr experiment (Mulliniks et al., 2008). Annual precipitation averages 400 mm, with approximately 30 % occurring from November to April. Precipitation during this study was 122 mm (2006) and 188 mm (2007). Grasses were dormant during the study and were primarily blue grama (*Bouteloua gracilis*) and wolftail (*Lycurus phleoides*) (Forbes and Allred, 2001). All animal handling and experimental procedures were in accordance with guidelines set by the New Mexico State University Institutional Animal Care and Use Committee. Cows (n = 161) were 2 (n = 83) and 3 (n = 78) yr old and were predominately Angus with some Hereford influence. Cows were randomly assigned treatments by calving date. Supplements were fed at 908 g•cow$^{-1}$•d$^{-1}$ twice weekly providing 1) 328 g CP, 110 g UIP, 44 g GP (**0**), 2) 328 g CP, 157 g UIP + 40 g propionate salt (NutroCal$^{TM}$, Kemin Industries, Inc.), 93 g GP (**40**), 3) 329 g CP, 158 g UIP + 80 g propionate salt, 124 g GP (**80**). Glucogenic potential was calculated by the equation described by Preston and Leng (1987), where 40% of the UIP is considered to be glucogenic (Overton et al., 1999). NutroCal$^{TM}$ contains 80% propionate, which is assumed to be 95% glucogenic (Steinhour and Bauman, 1988).

A glucose tolerance test (**GTT**) was conducted in 2006 at approximately 64 d (Figure 1) postpartum on a subsample of cows (n = 29) on a day after supplementation to evaluate glucose half-life and sensitivity to endogenous insulin. A 12- gauge hypodermic needle (Ideal Instruments, Schiller Park, IL) was used to puncture the jugular vein. One-half of a 2.5m 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 portion was secured to the cow's neck and down the middle of the back, where it was secured with tape. A blunt 18-gauge needle (Salvin Dental Specialties, Charlotte, NC) was inserted in the end of the catheter and a 10-mL syringe was used as the tubing end cap. Catheters were inserted in the morning of the GTT. A 50% dextrose solution was infused at 0.5 mL/kg BW via the indwelling jugular catheter. Blood was collected at -1, 0, 3, 6, 9, 12, 15, 20, 40, 60, 80, 100, 120, 140, 160, and 180 min relative to the infusion time. Catheters were flushed with 10 mL of a 9% saline immediately before and after each collection time and after infusion of glucose. Sample collection time -1 was collected before infusion of glucose and 0 immediately after infusion. Ten-milliliter blood samples were collected at each collection time and placed in Corvac serum separator tubes. Serum samples were centrifuged at 2000 × g at 4ºC for 20 min. Serum was stored in plastic vials at -20ºC for later analysis of glucose and insulin. Glucose was analyzed with a commercial kit (enzymatic endpoint, Thermo Electron Corp., Waltham, MA). Insulin was analyzed by solid-phase RIA (DCP kit, Diagnostic Products Corp., Los Angeles, CA) as reported by Reimers et al. (1982). Intra- and inter-assay CV for both glucose and insulin were < 10%. Serum glucose and insulin areas under the curve (**AUC**) were calculated using the trapezoidal summation method. Glucose half-life was estimated by determining the time required for a 50% decrease in peak serum glucose concentration.

In 2007, an acetate tolerance test (**ATT**) was conducted at approximately 64 d (Figure 1) postpartum on a subsample of cows (n = 24) on a day after supplementation to assess acetate clearance as affected by the glucose potential of the experimental supplements. Catheter procedures were the same as reported above. A 20% acetic acid solution was infused at 1.25 mL/kg BW via the indwelling jugular catheter. Serum collection times were -1, 0, 1, 3, 5, 7, 10, 15, 30, 60, and 90 min relative to infusion. Infusion of acetate occurred after -1 and before 0. Serum samples were collected (10 mL) at each collection time and were placed in Corvac serum separator tubes. Serum samples were centrifuged at 2000 × g at 4ºC for 20 min. After centrifugation, samples were stored in plastic vials at -20ºC for later analysis of acetate, insulin, and glucose concentrations. Glucose and insulin were analyzed as described above. Serum was filtered by centrifugation with centrifugal filter device for 60 min at 5000 × g for deproteinization (Millipore Amicon Ultra-4 centrifugal device, Millipore Corp., Burlington, MA). Filtered serum was mixed at a 5:1 ratio with 25% metaphoshoric acid containing 2 g/L of 2-ehtyl butyric acid as an internal standard. Samples, 1 μL in size, were analyzed for acetate concentration using gas chromatography. An internal standard was used to calculate final acetate concentrations and acetate half-life was calculated as the time required for 50% decease in peak serum acetate concentration. Serum acetate, insulin, and glucose AUC were calculated using the trapezoidal summation method.

A subsample of cows in 2006 (n = 29) and in 2007 (n = 24) were randomly selected to be an equal representation of age and treatment and were milked by machine approximately 57d postpartum in 2006 and 69 d postpartum in 2007 (Figure 1). Milk procedures were a modified weigh-suckle-weigh technique described by Appeddu et al. (1997). Milk weights were recorded to calculate 24hr milk production. Milk samples were analyzed for lactose, butterfat, solids non-fat, and protein by Pioneer Dairy Labs, DHIA (Artesia, NM).

Blood samples were collected twice weekly (Monday and Friday) after morning grazing via coccygeal venipuncture beginning approximately 35 d and ending 140 d postpartum for the analysis of insulin, glucose, non-esterified fatty acid (**NEFA**), and serum urea nitrogen (**SUN**) to evaluate nutrient status. Serum metabolites were composited by cow within 3 productive periods: 1) pre-breeding; 2) breeding-supplementation end; and 3) supplementation end-breeding end. Composite samples were analyzed using commercial kits for NEFA (Wako Chemicals, Richmond, VA) and SUN (Thermo Electron Corp., Waltham, MA). Insulin and glucose concentrations were analyzed as previously described.

As a chute-side measure of nutrient status, whole-blood β-hydroxybutyrate levels were measured with a handheld ketone sensor (MediSense/Abbott Laboratories, Abingdon, UK, validated by Bryne et al. (2000)) in early-May during both years. In 2006, the same subsample (n = 29) of cows in the GTT were used and was taken on subsequent milking days. However, in 2007, the whole cow herd (n = 80) was used for the ketone measurements on May 4.

Data was analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) with cow as the experimental unit using the Kenward-Roger degrees of freedom method. The model included fixed effects of supplement, cow age, year, and their interactions. Covariates were calving date, sex of calf, days supplemented and were used when appropriate. Serum metabolite concentrations were analyzed with period as the repeated factor and cow as the subject with unstructured covariance structure. Three contrast statements were used to test for linear, quadratic, or 0 vs. 40 + 80 effects of increasing amounts of glucogenic precursors. Significance was determined at $P \leq 0.10$.

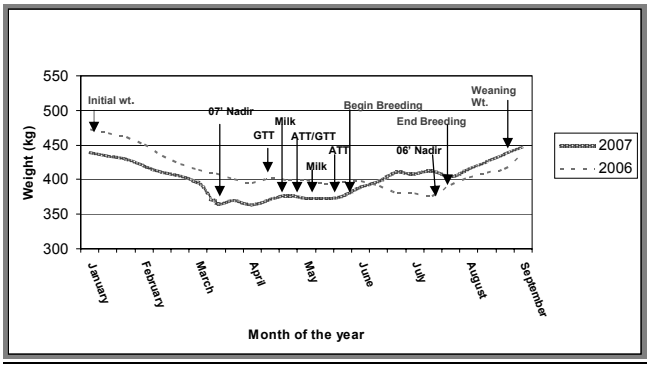


Figure 1. Average weight change and timeline of challenges. GTT = glucose tolerance test, ATT = acetate tolerance test.

## RESULTS AND DISCUSSION

In 2006, cows used in the GTT were found to be insulin resistant. Supplement did not affect ($P \geq 0.40$) glucose, insulin AUC or glucose half-lives (Table 1). All glucose half-lives were nearly 3 times the normal half-life of 35 min as described by Kaneko (1997). Thus, all cows were considered insulin resistant and the increasing concentration of glucose supplied by supplements was likely used for milk production (Figure 1). Waterman et al. (2006) and Endecott et al. (2007) suggest that improvements in insulin sensitivity may decrease milk and milk fat yield which we did not find.

Acetate clearance can be used as an indication of glucogenic potential of the diet and reveal metabolism efficiency. If glucose is limiting, then as diet glucogenic potential increases, acetate clearance rate should be more rapid. This would result from an improved availability of glucose, since an insufficient supply of glucose relative to acetate may reduce the efficiency with which acetate is utilized (Preston and Leng, 1987). Acetate half-life decreased linearly ($P = 0.08$) with increasing GP (35, 29, 27 ± 3 min for 0, 40, and 80, respectively; Table 1). In 2007, acetate half-life has been reported to be as rapid as 10 min (Preston and Leng, 1987). Fonseca et al. (2001) found similar results when higher availability of glucose or glucose substrates where provided in the diet to lambs. Acetate and insulin AUCs were similar ($P \geq 0.70$) among our treatment groups. However, glucose AUC decreased ($P = 0.06$) linearly with increasing amounts of GP in the diet (9105, 8902, 7654 ± 515 for 0, 40, and 80, respectively). These data suggest that the smaller glucose AUC for higher GP supplemented cows facilitated faster acetate clearance.

Due to suspected high concentration of ruminal acetate and an inadequate supply of glucose precursors as indicated by the ATT, acetate derived from body lipolysis (common during lactation) may exacerbate the imbalance leading to a conversion of acetate to β-hydroxybutyrate. Beta-hydroxybutyrate concentrations greater than 1.2 mmol/L are an indication of clinical ketosis in dairy cows (Akers, 2002). Levels of β-hydroxybutyrate did not approach 1.2 mmol/L for any treatment group. However, β-hydroxybutyrate concentrations decreased ($P = 0.01$) with increasing amounts of GP (0.38, 0.29, and 0.30 ± 0.02 mmol/L for 0, 40, and 80, respectively; Table 1). The increasing amount of GP in 40 and 80 appears to have improved utilization of metabolizable acetate, thus decreasing ketone concentration, which would be an outcome of an increased acetate clearance rate.

In the GTT, glucose half-life was shorter ($P = 0.03$) and glucose AUC was smaller ($P = 0.02$) for 3-yr-old cows compared to its 2-yr-old counterparts at the same concentration of serum insulin ($P = 0.97$; Table 2). Consequently, 2-yr-old cows were more insulin resistant than 3-yr-old cows. Acetate half-life, acetate AUC, glucose AUC, and insulin AUC were similar ($P \geq 0.32$) among age groups. Since both 2- and 3-yr-old cows were able to clear infused acetate in similar amounts of time, age didn't affect the efficiency of utilization of acetate.

Twenty-four hour milk production was similar ($P = 0.53$) among treatments groups (Table 1). Butterfat, protein, lactose, and solid non-fat all followed the same trend ($P \geq 0.29$) as the 24 hour milk production. In contrast, Waterman et al. (2006) found a 9% decrease in milk production and a 25% reduction in butterfat secretion for cows fed UIP + 100g/d propionate salt with a 54 min glucose half-life. Rigout et al. (2003) found similar results with a decrease in milk fat, however milk production increased when glucogenic precursors either infused in the rumen or duodenum in dairy cows. In contrast, our cows remained insulin resistant and did not partition nutrients away from milk production.

Cows produced similar ($P = 0.24$) amounts of milk in 2006 and 2007. However, butterfat, protein, and solid non-fat were higher in 2007 than in 2006 ($P \leq 0.10$). Three- and two-yr-old cows produced similar ($P = 0.24$) amounts of milk, with a similar response to age for butterfat, lactose, and solid non-fat ($P \geq 0.18$). Although protein secretion was higher for 3-yr-old than their 2-yr-old counterparts ($P = 0.08$).

From the twice weekly blood samples collected and composited by period, we found serum urea nitrogen and insulin were similar among treatments ($P \geq 0.75$; Table 1). However, both serum glucose and NEFA increased ($P \leq 0.10$) as glucogenic precursors increased in the diet. The increase in serum NEFA might have been contributed by a small increase in dietary fat (3.8% vs. 2.1%) in the 40 and 80 supplements. This small difference in NEFA was not indicative of productivity differences since BW loss or gain was similar among all treatments (Mulliniks et al., 2008).

Serum insulin concentrations were similar during all three composited periods ($P = 0.24$) and for both age groups ($P = 0.22$). However, insulin concentrations were lower in 2007 than in 2006 ($P < 0.01$). Thus, cows in 2006 may have been more insulin resistant as seen in the 2006 GTT contributing to higher serum insulin concentrations due to overall poorer environmental conditions. Serum glucose concentrations were the highest in the 2nd period and lowest in the 3rd ($P < 0.01$). This may indicate increased glucose uptake into tissues as diet quality may have improved with advancing season. Age or yr did not influence serum glucose concentrations ($P \geq 0.84$). Serum urea nitrogen also increased as days postpartum lapsed and as diet quality probably improved with the advancing season ($P < 0.01$). However, NEFA levels decreased over time ($P < 0.01$). The decrease in NEFA concentrations may have been caused by a decreased mobilization of adipose tissue as lactation passed its peak.

## IMPLICATIONS

Results suggest that diets that supply additional glucogenic precursors may decrease serum ketone concentration and increase acetate disappearance rate indicating that energy metabolism was more efficient. These changes in energy metabolism may influence overall energy utilization, animal well-being, and reproduction. However, during drought conditions a greater amount of glucogenic precursors than provided in this study might be needed to partition nutrients away from milk production.

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**Table 1**. Effects of supplements containing increasing amounts of glucogenic precursors (0, 40, or 80 g/d propionate salt) on metabolic and lactational responses.

| | Supplement | | | | | Contrast | |
|---|---|---|---|---|---|---|---|
| Response | 0 | 40 | 80 | SEM | *P*-value | Linear | Quadratic |
| Acetate Tolerance Test | | | | | | | |
| Acetate half-life, min | 35 | 29 | 27 | 3 | 0.19 | 0.08 | 0.08 |
| Acetate AUC | 247 | 277 | 247 | 28 | 0.7 | 0.99 | 0.41 |
| Glucose AUC | 9105 | 8902 | 7654 | 515 | 0.13 | 0.06 | 0.42 |
| Insulin AUC | 36 | 34 | 41 | 7 | 0.76 | 0.63 | 0.57 |
| Glucose Tolerance Test | | | | | | | |
| Glucose half-life, min | 88 | 97 | 97 | 15 | 0.88 | 0.66 | 0.83 |
| Glucose AUC | 10295 | 10890 | 13232 | 1615 | 0.40 | 0.19 | 0.66 |
| Insulin AUC | 183 | 169 | 188 | 22 | 0.82 | 0.88 | 0.54 |
| Insulin:glucose ratio | 0.022 | 0.023 | 0.019 | 0.004 | 0.77 | 0.60 | 0.59 |
| Milk, g/d | | | | | | | |
| 24-h production | 5736 | 6402 | 5797 | 463 | 0.53 | 0.93 | 0.26 |
| Butterfat | 179 | 204 | 169 | 20 | 0.44 | 0.72 | 0.22 |
| Protein | 145 | 172 | 155 | 12 | 0.29 | 0.57 | 0.14 |
| Lactose | 279 | 315 | 281 | 22 | 0.41 | 0.96 | 0.19 |
| Solid non-fat | 475 | 546 | 489 | 38 | 0.38 | 0.79 | 0.18 |
| Serum Metabolites | | | | | | | |
| Beta-hydroxybutyrate | 0.38 | 0.29 | 0.3 | 0.02 | 0.01 | 0.01 | 0.09 |
| SUN, mg/100mL | 8.5 | 8.4 | 8.5 | 0.1 | 0.75 | 0.84 | 0.46 |
| Glucose, mg/dL | 54.3 | 55.6 | 57.7 | 0.9 | 0.03 | 0.01 | 0.74 |
| Insulin, ng/mL | 0.42 | 0.42 | 0.43 | 0.02 | 0.77 | 0.48 | 0.94 |
| NEFA, µmol/L | 450 | 479 | 480 | 11 | 0.10 | 0.06 | 0.30 |

**Table 2**. Effect of cow age on metabolic and lactational responses for 2- and 3-yr-old postpartum cows fed supplements containing increasing amounts of glucogenic precursors (0, 40, or 80 g/d propionate salt).

| | Cow Age | | | | |
|---|---|---|---|---|---|
| Response | 2 | SEM | 3 | SEM | P-value |
| Acetate Tolerance Test | | | | | |
| Acetate half-life, min | 30 | 2 | 30 | 2 | 0.98 |
| Acetate AUC | 238 | 23 | 276 | 23 | 0.32 |
| Glucose AUC | 8800 | 420 | 8307 | 420 | 0.42 |
| Insulin AUC | 39 | 5 | 34 | 5 | 0.51 |
| Glucose Tolerance Test | | | | | |
| Glucose half-life, min | 113 | 11 | 75 | 12 | 0.03 |
| Glucose AUC | 13679 | 1226 | 9266 | 1269 | 0.02 |
| Insulin AUC | 179 | 16 | 180 | 17 | 0.97 |
| Insulin:glucose ratio | 0.017 | 0.003 | 0.026 | 0.003 | 0.06 |
| Milk, g/d | | | | | |
| 24-h production | 5592 | 376 | 6365 | 372 | 0.24 |
| Butterfat | 184 | 17 | 184 | 16 | 0.98 |
| Protein | 145 | 10 | 170 | 10 | 0.09 |
| Lactose | 275 | 18 | 308 | 18 | 0.20 |
| Solid non-fat | 472 | 31 | 534 | 31 | 0.18 |
| Serum Metabolites | | | | | |
| Beta-hydroxybutyrate | 0.29 | 0.02 | 0.36 | 0.02 | 0.01 |
| SUN, mg/100mL | 8.69 | 0.12 | 8.25 | 0.12 | 0.01 |
| Glucose, mg/dL | 55.7 | 0.75 | 55.9 | 0.77 | 0.86 |
| Insulin, ng/mL | 0.44 | 0.01 | 0.41 | 0.01 | 0.22 |
| NEFA, µmol/L | 460.5 | 9 | 478.2 | 10 | 0.18 |

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# 60% DRIED DISTILLERS GRAINS IN LAMB RATIONS RESULTS IN ACCEPTABLE PERFORMANCE AND CARCASS QUALITY[1]

**C. S. Schauer[*], M. M. Stamm[*], T. Maddock[†], and P. B. Berg[†]**

[*]Hettinger Research Extension Center; North Dakota State University, Hettinger, ND 58639;
[†]Department of Animal Sciences; North Dakota State University, Fargo, ND 58105.

**ABSTRACT:** Little research is available evaluating the maximum level of dried distillers grain (DDG) inclusion in lamb rations. Our objectives were to evaluate increasing levels of DDG on performance and carcass characteristics of lambs. Two-hundred forty Rambouillet wether and ewe lambs (31.7 ± 0.6 kg BW) were stratified by weight and sex, randomly allotted to one of 16 pens, and assigned to treatment (n = 4). Diets were balanced to meet CP, energy, and Cu requirements; however, treatments were not kept isocaloric or isonitrogenous. The basal diet consisted of alfalfa, soybean meal, barley, and a trace mineral supplement. Dried distillers grains replaced barley and soybean meal at 0, 20, 40, and 60% of the diet (0%, 20%, 40%, and 60%, respectively; DM basis). Sulfur concentrations of diets were 0.22, 0.32, 0.47, and 0.55% for 0%, 20%, 40%, and 60%, respectively. Thiamin was included at 142 $mg \cdot hd^{-1} \cdot d^{-1}$ (DM basis) in all rations for the prevention of polioencephalomalacia. Rations were mixed, ground, and provided ad-libitum. Lambs were weighed on day 0, 32, 56, 83, and 111. Lambs were harvested and carcass data collected. Performance and carcass data were analyzed as a randomized complete design. The model included treatment. Contrast statements included 1) 0% vs DDG inclusion; 2) linear effect of DDG inclusion; and 3) quadratic effect of DDG inclusion. Final weight, ADG, G:F, mortality, hot carcass weight, leg score, conformation score, fat depth, body wall thickness, ribeye area, quality grade, yield grade, and % boneless closely trimmed retail cuts were not affected by treatment ($P \geq 0.15$). Feed intake increased in a linear manner ($P < 0.001$) as level of DDG inclusion increased. Additionally, flank streaking increased quadratically ($P = 0.09$) as level of DDG inclusion increased. Dried distillers grains maintained lamb performance and had no negative effect on lamb carcass traits. Maximizing the use of DDG may become economically feasible for lamb feeders when prices become favorable compared to conventional grains. However, the level of use of supplemental thiamin for the prevention of potential S toxicity in lambs needs to be evaluated.

Key Words: DDG, Lamb, S

## Introduction

Coproducts from the ethanol industry are increasingly available in the northern Great Plains as the ethanol industry continues to expand. Dried distillers grain (**DDG**), one such coproduct, is an excellent source of energy and protein for beef cattle and sheep (Lardy, 2003). Historically, research conducted in beef cattle diets report that DDG can be fed as a source of both supplemental protein and energy to cattle during backgrounding and finishing, with optimum inclusion levels at approximately 20% of the diet dry matter (Lardy, 2003). However, DDG are high in potassium, phosphorus, and sulfur; therefore care must be used when feeding DDG at the upper limits of the feeding recommendations because of health problems. Additionally, as commodity grain prices remain high, the interest in feeding maximum levels of DDG in ruminant finishing rations increases. To prevent polioencephalomalacia in sheep, current recommendations are to keep dietary concentrations of S below 0.3% DM when animals are fed concentrate diets or below 0.5% DM when fed high-forage diets (NRC, 2007). Recent research results in cattle indicate that as much as 50% of the ration (DM basis) may contain DDG when 150 $mg \cdot hd^{-1} \cdot d^{-1}$ supplemental thiamin is provided (Huls et al., 2008). Little research has evaluated the inclusion of DDG as a replacement for concentrate in lamb finishing rations, especially as inclusion rates rise to the point where S becomes potentially toxic. Schauer et al. (2005, 2006) and Huls et al. (2006) reported that DDG can be included at levels up to 22.5% of a finishing ration with no negative affect on lamb performance or carcass traits. Thus, our objectives were to evaluate the influence of increasing levels of DDG in lamb finishing rations on performance and carcass characteristics, specifically when S concentrations become potentially toxic.

## Materials and Methods

All procedures were approved by the North Dakota State University Institute for Animal Care and Use Committee. A randomized complete design was used to evaluate the influence of DDG in lamb finishing diets. Two-hundred forty western white-faced Rambouillet wether and ewe lambs (31.8 ± 0.6 kg initial BW) were stratified by weight and sex and assigned randomly to 16 pens (15 lambs/pen). Pens were then assigned to one of four treatments (n = 4). Lambs were fed a finishing diet for 111 days. Diets were balanced to at least meet crude

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protein, energy, and copper requirements (NRC, 2007); however, they were not kept isocaloric or isonitrogenous as level of DDG inclusion increased (Table 1). The basal diet consisted of alfalfa, soybean meal, barley, and a trace mineral supplement (Table 1). Dried distillers grains replaced barley and soybean meal at 0, 20, 40, and 60% of the diet (**0%**, **20%**, **40%**, and **60%**, respectively; DM basis). Thiamin was included at 142 $mg \cdot hd^{-1} \cdot d^{-1}$ (DM basis) in all rations for the prevention of polioencephalomalacia. Rations were mixed and ground through a grinder-mixer and provided ad-libitum via bulk feeders. Grab samples of the ration were collected on d 0, 56, and 111, dried at 55°C for 48 h, and analyzed by a commercial laboratory (Midwest Laboratories Inc., Omaha, NE) for DM, OM, NDF, ADF, TDN, Crude Fat, N, S, mineral concentrations, and thiamin (Table 1). Sulfur concentrations of diets were 0.22, 0.32, 0.47, and 0.55% for 0%, 20%, 40%, and 60%, respectively. Lambs were weighed on d 0, 32, 56, 83, and 111. Initial and final weights were an average of two-day un-shrunk weights. Following the 111 d finishing period, lambs were harvested and carcass data collected at Iowa Lamb Corp, Hawarden, IA. Feedlot performance and carcass trait data were analyzed as a randomized complete design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NY). The model included treatment. Contrast statements included 1) 0% vs DDG inclusion; 2) linear effect of DDG inclusion; and 3) quadratic effect of DDG inclusion.

## Results and Discussion

The effects of treatments on feedlot performance and carcass traits are shown in Table 2. Final weight, ADG, G:F, mortality, hot carcass weight (**HCW**), leg score, conformation score, fat depth, body wall thickness, ribeye area, quality grade, yield grade, and % boneless closely trimmed retail cuts (**%BCTRC**) were not affected by treatment ($P \geq 0.15$). Intake increased in a linear manner ($P < 0.001$) as level of DDG inclusion increased. Additionally, flank streaking increased ($P = 0.09$) in a quadratic relationship to the control as level of DDG inclusion increased, with all DDG treatments having greater ($P = 0.02$) flank streaking than 0%.

Dried distillers grains replacing up to 60% of the ration in a barley and alfalfa based finishing ration had no affect on lamb performance. However, intake did increase linearly as level of DDG inclusion increased. One possibility for the increase in intake was increased ration palatability, possibly due to the increased fat concentration of the ration. Although intake increased, a significant increase in ADG was not observed. However, a numerical increase in ADG of approximately 6% was observed for all DDG treatments when compared to the control diet. Other researchers suggest that DDG can be an effective replacement of concentrate with no affect of livestock performance compared to control rations. Erickson et al. (1989) provided up to 28% of a finishing ration as DDG and observed no negative affects on lamb performance. Similarly, Schauer et al. (2005) incorporated DDG up to 15% of the total ration and Huls et al. (2006) substituted up to 22.9% of the finishing rations with DDG and found no difference in lamb performance or carcass traits. However, Schauer et al. (2006) reported an increase in performance from increasing DDG levels up to 22.5% of the ration. In both Schauer et al. (2006) and the current trial, CP levels of the DDG rations are in excess of the requirements for lambs (NRC, 2007). In the control rations, CP may be limiting as corn and barley CP concentrations are substantially lower than DDG crude protein concentrations. Additionally, supplemental fat from the DDG may have affected intake and performance in both trials. Future research is needed to determine if adequate lamb performance can be maintained while utilizing lower quality forages than alfalfa with DDG replacing a portion of the concentrate in the diet.

The majority of carcass traits were not affected by increasing levels of DDG in the ration. These results are supported in research conducted by Schauer et al. (2005, 2006) and by Huls et al. (2006). In the current trial only marginal increases in flank streaking were observed. This response could potentially be the result of the increased energy density in the rations with higher levels of DDG inclusion.

In our trial supplemental thiamin was provided to aid in the prevention of S toxicity, potentially preventing the incidence of polioencephalomalacia. Current research suggests that S toxicity in concentrate rations fed to lambs is 0.3% DM, and 0.5% DM in lamb fed high-forage diets (NRC, 2007). As concentrate levels increase in lamb diets, ruminal pH decreases and excessive production of rumen sulfide can result (Gould, 1998). While decreases in ruminal pH have not been found to decrease the microbial production of thiamin (Alves de Oliveira et al., 1996), the ruminants main source of thiamin, the decreases in pH have been found to increase the bacteria that produce thiaminase – a compound that in turn destroys the thiamin that is already present, inducing a thiamin deficiency and subsequently polioencephalomalacia (Morgan and Lawson, 1974; Boyd and Walton, 1977, Thomas et al., 1987). In rations containing greater than 0.3% sulfur, the combination of increased dietary S concentration, increased ruminal sulfide production, and increased thiaminase production can result in an increase in polioencephalomalacia (Gould, 1998). Sulfur toxicity may additionally result in decreased intake and performance as well as health problems associated with S binding to copper, resulting in secondary copper deficiencies. One potential remedy for excessive dietary S is to include supplemental thiamin in the ration (NRC, 2007). Recent beef cattle research has reported mixed results using supplemental thiamin. Huls et al. (2008) successfully fed 50% of the diet as modified distillers grains plus solubles while supplementing with 150 mg/hd/d thiamin, noting no change in performance when compared to control diets. However, a 50% DDG with solubles treatment had to discontinued by Buckner et al. (2007) when multiple steers exhibited signs of polioencephalomalacia, even though they were providing 150 mg/hd/d supplemental thiamin. In our trial, no increases in mortality or morbidity were observed, indicating that the lambs on increasing levels of DDG had no deleterious effects from increasing dietary S concentrations. Additional research is needed to further

quantify the supplemental thiamin needs of lambs fed high DDG rations.

## Implications

The expansion of the ethanol industry in the U.S. may result in an increase in the availability of dried distillers grains for cattle and sheep feeders. Maximizing the use of dried distillers grains may become economically feasible for lamb feeders when prices become favorable, especially in relation to the current grain prices. When appropriately priced relative to corn and barley, dried distillers grains and supplemental thiamin can effectively replace up to 60% of a lamb finishing ration with no negative effects on feedlot performance or carcass traits.

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**Table 1.** Dietary ingredient and nutrient composition of control and dried distillers grain (DDG) diets

| | Diets[1] | | | |
|---|---|---|---|---|
| Item | 0% | 20% | 40% | 60% |
| Ingredient | %, DM basis | | | |
| Barley | 76.5 | 61.48 | 41.48 | 21.48 |
| DDG | --- | 20.0 | 40.0 | 60.0 |
| Alfalfa | 12.5 | 12.5 | 12.5 | 12.5 |
| Soybean Meal | 5.0 | --- | --- | --- |
| Ammonium Chloride | 0.50 | 0.5 | 0.50 | 0.50 |
| Trace Mineral[2] | 5.0 | 5.0 | 5.0 | 5.0 |
| CTC[3] | 0.50 | 0.5 | 0.50 | 0.50 |
| Nutrient Concentration | | | | |
| CP, % | 19.8 | 20.1 | 25.1 | 27.2 |
| $NE_{maintenance}$, Mcal/kg[4] | 1.87 | 1.94 | 2.02 | 2.02 |
| $NE_{gain}$, Mcal/kg[4] | 1.25 | 1.30 | 1.34 | 1.34 |
| Crude Fat, % | 2.50 | 4.03 | 6.69 | 8.34 |
| ADF, % | 10.2 | 9.72 | 10.9 | 12.5 |
| Sulfur, %[5] | 0.22 | 0.32 | 0.47 | 0.55 |
| Calcium, % | 2.14 | 1.77 | 1.17 | 1.38 |
| Phosphorus, % | 0.48 | 0.55 | 0.66 | 0.67 |
| Copper, ppm | 12 | 10 | 11 | 10 |
| Zinc, ppm | 73 | 75 | 86 | 63 |
| Thiamin, $mg \cdot hd^{-1} \cdot d^{-1}$ | 142 | 142 | 142 | 142 |

[1]Control = 0% replacement of barley with dried distillers grains; 20% = 20% dried distillers grain in ration replacing barley; 40% = 40% dried distillers grain in ration replacing barley; 60% = 60% dried distillers grain in ration replacing barley.

[2]Trace mineral: 0.12 % S, 0.31% P, 1.2% K, 1.45% Mg, 17.47% Ca, 2.82% Na, 509 ppm Fe, 375 ppm Mn, 50 ppm Cu, 715 ppm Zn, 5 ppm Se, 1960 mg/kg Thiamine, 95.15 KIU/kg Vitamin A, 9.46 KIU/kg vitamin D3, 9504 IU/kg Vitamin E, 946 mg/kg lasalocid.

[3]CTC (chlorotetracycline - 4G) was formulated to provide 48 g/ton chlortetracycline.

[4]Calculated analysis.

[5]Sulfur may be toxic at levels of 0.30% of diet (DM basis).

**Table 2.** The influence of dried distillers grains (DDG) on feedlot lamb performance and carcass characteristics

| | Treatment[1] | | | | | | *P*-value[3] | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 0% | 20% | 40% | 60% | SEM[2] | *P*-value | Linear | Quadratic | 0% Vs. DDG |
| Initial Weight, kg | 31 | 32 | 32 | 32 | 0.6 | 0.55 | 0.47 | 0.22 | 0.22 |
| Final Weight, kg | 60 | 62 | 62 | 62 | 0.9 | 0.27 | 0.15 | 0.25 | 0.06 |
| ADG, kg/d | 0.26 | 0.28 | 0.28 | 0.28 | 0.01 | 0.21 | 0.11 | 0.41 | 0.05 |
| Intake, $kg \cdot hd^{-1} \cdot d^{-1}$ | 1.68 | 1.78 | 1.83 | 1.91 | 0.03 | 0.001 | < 0.001 | 0.71 | < 0.001 |
| G:F | 0.16 | 0.16 | 0.15 | 0.15 | 0.005 | 0.53 | 0.20 | 1.00 | 0.39 |
| Mortality, % | 0.75 | 0.25 | 0.25 | 0 | 0.30 | 0.38 | 0.12 | 0.68 | 0.12 |
| Hot Carcass Weight, kg | 30 | 32 | 31 | 31 | 0.45 | 0.27 | 0.19 | 0.16 | 0.06 |
| Leg score | 10.3 | 10.5 | 10.5 | 10.5 | 0.3 | 0.89 | 0.56 | 0.66 | 0.45 |
| Conformation score | 10.3 | 10.3 | 10.5 | 10.5 | 0.27 | 0.83 | 0.42 | 1.0 | 0.60 |
| Fat Depth, cm | 0.74 | 0.81 | 0.76 | 0.81 | 0.05 | 0.57 | 0.36 | 0.69 | 0.24 |
| Body Wall Thickness, cm | 2.44 | 2.16 | 2.57 | 2.59 | 0.08 | 0.47 | 0.13 | 0.87 | 0.22 |
| Ribeye Area, $cm^2$ | 14.96 | 15.35 | 15.16 | 15.42 | 0.32 | 0.72 | 0.43 | 0.83 | 0.35 |
| Flank Streaking | 324 | 357 | 342 | 345 | 8 | 0.08 | 0.19 | 0.09 | 0.02 |
| Quality Grade | 10.3 | 10.8 | 10.8 | 11 | 0.2 | 0.15 | 0.04 | 0.57 | 0.04 |
| Yield Grade[4] | 3.26 | 3.57 | 3.42 | 3.55 | 0.18 | 0.63 | 0.39 | 0.65 | 0.26 |
| %BCTRC[5] | 45.1 | 44.9 | 44.9 | 44.8 | 0.21 | 0.76 | 0.35 | 0.70 | 0.31 |

[1]0% = 0% replacement of barley and SBM with dried distillers grains; 20% = 20% dried distillers grain in ration replacing barley and SBM; 40% = 40% dried distillers grain in ration replacing barley and SBM; 60% = 60% dried distillers grain in ration replacing barley and SBM.

[2]Standard Error of Mean; n = 4.

[3]*P*-value for 0% vs DDG treatments and linear and quadratic affect of dried distillers grains inclusion.

[4]Yield Grade = 0.4 + (10 x adjusted fat depth).

[5]% boneless closely trimmed retail cuts (49.936 - (0.0848 x Hot Carcass Weight) - (4.376 x Fat Depth)-(3.53 x BW) + (2.456 x Ribeye Area)).

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# EFFECT OF FLAXSEED INCLUSION ON RUMINAL FERMENTATION, DIGESTION, AND MICROBIAL PROTEIN SYNTHESIS IN GROWING AND FINISHING DIETS FOR BEEF CATTLE

**T. C. Gilbery, G. P. Lardy, D. S. Hagberg, and M. L. Bauer**

Department of Animal & Range Sciences North Dakota State University, Fargo, ND

**ABSTRACT:** Four Holstein steers (339 ± 10kg initial BW) fitted with ruminal and duodenal cannulae were used in a 4 x 4 Latin square design to evaluate the effects of flax inclusion in growing and finishing diets on intake, ruminal fermentation, and site of digestion. Flax replaced linseed meal and part of corn at 8% of dietary DM in growing (40% concentrate) and finishing (80% concentrate) diets. Data was analyzed as a 2 x 2 factorial; the main effects were ration type (growing vs. finishing) and flax inclusion (with or without flax) and their interaction. Diets were formulated to contain 13% and 15% CP for growing and finishing, respectively. All diets were formulated to provide a 2:1 Ca: P ratio. No differences ($P = 0.24$) were observed for DM intake (10.2 ± 0.54 kg/d; 2.4 ± 0.09% BW). Flax decreased microbial OM flow at the duodenum ($P = 0.02$). Total tract OM digestion was greater for steers fed finishing diets ($P = 0.02$) and apparent ruminal OM digestibility tended to increase for steers fed finishing diets ($P = 0.09$). Steers consuming finishing diets had greater ($P = 0.001$) total tract CP digestion. Microbial efficiency increased ($P = 0.04$) for steers on growing diet. Steers fed growing diets had increased ($P \leq 0.004$) ruminal NDF and ADF digestion. Steers consuming flax had lower ($P = 0.02$) ruminal ammonia. There was no effect ($P \geq 0.19$) of flax on crude protein, NDF, ADF, and OM ruminal and total tract digestion. Results indicate flax inclusion did not alter OM and CP digestion when replacing part of corn and linseed meal in growing and finishing diets for beef cattle.
Key Words: Ammonia, Digestion, Flax, Ruminal

## Introduction

Flax (*Linum usitatissimum*), is an oilseed grown primarily for its oil rich seed that contains 41% oil, 20% CP, and 20% NDF (Canadian Grain Commission, 2001). North Dakota flaxseed production accounted for 90% of the U.S. flax crop in 2007 (NASS, 2008). Flax in receiving diets has improved calf health, performance, and potentially nutritional aspects of beef for consumers (Drouillard et al., 2001; Maddock et. al, 2006). Average daily gain and G:F were superior in diets containing flax compared to tallow and death loss was lowest for flax-fed calves (Drouillard et al., 2002; 2004). Beef from cattle fed flax contains greater levels of omega-3 fatty acids, potentially resulting in niche marketing opportunities for beef producers (Maddock et al., 2006). Drouillard et al. (2002) reported increased DMI with the inclusion of flax at 10% of diet DM in finishing diets for cattle. Furthermore, Maddock et al. (2006) reported increased HCW and lower USDA yield grades when measuring carcass composition of heifers fed finishing diets containing flax. Drouillard et al. (2002, 2004) observed an increase in the percentage of carcasses grading USDA Choice or greater following a finishing phase in which cattle consumed flax at 5% of dietary DM. Our objective was to evaluate the effects of flax inclusion in growing and finishing diets on intake, ruminal fermentation, and site of digestion.

## Materials and Methods

**Animal diets and treatments.** All animal care, handling techniques, and surgical procedures were approved by the North Dakota State University Animal Care and Use Committee before the initiation of research. Four ruminally and duodenally cannulated Holstein Steers (339 ± 10 kg initial BW) were used in a 4 x 4 Latin square design to evaluate the effects of flax inclusion on ruminal fermentation and digestion. Steers consumed growing and finishing diets in which flax replaced linseed meal and a portion of the corn at 8% of dietary DM in growing (40% concentrate) and finishing (80% concentrate) diets. Steers were housed in an enclosed barn in individual stanchions (1.2 x 2.2 m). Steers were fed the diets in the form of a totally mixed ration at 0700 and 1900 daily and were allowed free access to water. Diets were offered to ensure ad libitum intakes and 10% feed refusal daily. Feed ingredients were alfalfa hay, corn silage, dry rolled corn, linseed meal, or rolled flax, and supplement (Table 1). Treatments were arranged in a 2 x 2 factorial with the main effects being diet concentrate level (growing diet at 40% concentrate or finishing diet at 80% concentrate) and flax inclusion (0 vs. 8% flax). The resulting treatments included: 1) growing diet without flax**,** 2) growing diet with 8% flax, 3) finishing diet without flax, and 4) finishing diet with 8% flax (Table 1). Diets were formulated to provide 13 and 15% CP (growing and finishing diets respectively; DM basis) while all diets were formulated to provide Ca: P ratio of 2:1 (DM basis). Corn and flax were processed by dry rolling (roller mill model K, Roskamp Mfg, Inc., Cedar Falls, IA)

***Sample Collection.*** Each experimental period was 14 d in length, allowing 9 d for adaptation to diet and 5 d for sample collection. Chromic oxide was mixed into the supplement and fed at 0.25% of the diet (DM basis) for use as an external marker to determine duodenal DM flow. Samples of duodenal fluid (200 g) were collected on d 11 to 14 in a manner to achieve a sampling point every other hour between feedings (0700 and 1900). Samples were

composited within steer for each period. From d10 through 14 fecal output was weighed, sampled (10% of wet weight), and composited across days within steer for each period.

On d 12 of each period, ruminal fluid samples were collected at 0, 2, 4, 6, 8, 10, 12, and 24 h after feeding. Following the 0 h collection the rumen was doused with 200 mL of CoEDTA solution (20 g/L Co) to determine ruminal liquid dilution rate (Uden et al., 1980). Ruminal fluid samples were stored frozen (-20ºC) until analysis for $NH_3$ and VFA. On d 14 a 4-kg ruminal sample was taken, and 2 L of 3.7% formaldehyde/0.9% NaCl (wt/vol) was added (Zinn and Owens, 1986) for isolation of bacterial cells and analysis for DM, ash, N, and purines. Samples were stored (-20ºC) until analysis.

***Laboratory Analysis.*** Dietary ort, fecal, and duodenal samples were analyzed for DM, ash, N (methods 4.1.06, 4.1.10, 4.2.10, respectively; AOAC 1997), ADF, and NDF (ANKOM, Fairport, NY). Ruminal content samples were analyzed for DM. Duodenal samples were analyzed for Cr by the spectrophotometer method of Fenton and Fenton (1979). Bacterial cells were isolated from formalized ruminal contents. Ruminal contents were blended (Model 37b119, Waring, New Hartford, CT) and the mixture was strained through 2 layers of cheesecloth. Feed particles and protozoa in the ruminal samples were removed through centrifugation at 500 x *g* for 20 min. The sample was then centrifuged at 30,000 x *g* for 20 min to collect the bacteria from the supernatant. Isolated bacteria were frozen, lyophilized, and analyzed for DM, ash, N (Methods 4.1.06, 4.1.10, 4.2.10, respectively; AOAC, 1997) and purines (Zinn and Owens, 1986).

***Calculations.*** Microbial organic matter and N leaving the abomasum were calculated using purines as microbial markers (Zinn and Owens, 1986). Organic matter fermented in the rumen was OM intake minus the difference between the amount of total OM reaching the duodenum and microbial OM reaching the duodenum. Feed N escape to the small intestine was calculated by subtracting microbial N from total N and thus includes any endogenous and $NH_3$-N contribution.

***Statistical analysis.*** Data were analyzed as a 4 x 4 Latin square with treatments arranged as a 2 x 2 factorial using Mixed procedures of SAS (version 9.1). The model for ruminal fermentation included treatment and period as fixed effects and steer as the subject. Ruminal data over time were analyzed as repeated measures using the variance components covariance structure in the Mixed procedures of SAS.

## Results and Discussion

Dry matter intake was not affected by flax inclusion or ration type (growing vs. finishing) when expressed as kg/d ($P = 0.24$) or percentage of BW ($P = 0.26$; Table 3). Organic matter intake was not altered by flax inclusion or ration type ($P \geq 0.24$; Table 3). There were no interactions between diet type and flax inclusion for OM intake and digestion ($P \geq 0.12$). Microbial OM flow decreased ($P \leq 0.02$) with flax inclusion and in steers consuming finishing diets. The decreases in microbial OM flow in flax-fed steers may in part be attributed to the supplemental fat from the rolled flaxseed. Dietary inclusion of polyunsaturated fatty acids inhibits (Immig et al. 1991; Jenkins 1993) ruminal fermentation by impeding ruminal cellulase activity Total tract OM digestion increased ($P = 0.02$) and apparent ruminal OM digestion tended ($P = 0.09$) to increase for steers fed finishing diets compared to growing diets. The higher levels of digestion we observed for the finishing diets were not unexpected as due to the reduced amount of roughage supplied by high grain finishing diet compared to growing diets.

Crude protein intake tended to be greater ($P = 0.09$) for steers fed finishing diets. Finishing diets in the current study contained 10% more CP and had similar DM intakes which resulted in 10% greater intake of CP for steers. Flax inclusion decreased ($P = 0.02$) total and microbial CP flow to the small intestine and tended ($P = 0.09$) to decrease feed CP flow to the small intestine. When expressing CP digestion as a percent of intake we observed no effect ($P \geq 0.19$) of flax inclusion on ruminal, intestinal and total tract CP digestion. Steers fed finishing diets displayed decreased ($P = 0.02$) microbial CP flow. Apparent ruminal and total tract CP digestion was greater ($P \leq 0.03$) and true ruminal CP tended to be greater ($P = 0.08$) for steers fed finishing diets which reflect the more readily digestible nutrient sources available in the high concentrate diets. Growing fed steers had greater ($P = 0.04$) microbial efficiency, which followed the same trends as microbial CP.

Ruminal and post-ruminal flow (0.71 ± 0.06 kg/d) and digestion (72.4 ± 3.4) of NDF in growing and finishing diets were not altered ($P \geq 0.13$) by flax inclusion. Total tract NDF digestion tended to increase ($P = 0.10$) for steers fed finishing diets compared to growing diets (74 vs. 67%, respectively). The observed increase ($P \leq 0.03$) in NDF and ADF intake and increased intestinal flow of NDF and ADF for steers fed the growing diets reflect the higher level of forage present in the growing diet.

Ruminal data for pH, $NH_3$, and VFA are mean values reported for samples taken over 24 h post feeding. Ruminal pH and total VFA were not different ($P \geq 0.29$) for diet type or flax inclusion, with pH averaging 6.40 ± 0.12 for all treatments. There were no time x diet type x flax interactions ($P \geq 0.29$) for pH, $NH_3$-N and VFA. Flax-fed steers had reduced ($P = 0.02$; 4.60 vs. 6.40 m*M*) ruminal ammonia; no differences were observed in ruminal $NH_{3\text{-}N}$ for diet type ($P = 0.43$).

Flax inclusion in growing and finishing diets fed to steers did not affect ($P \geq 0.13$) total VFA concentration. Steers consuming growing diets had greater ($P = 0.003$) ruminal acetate concentrations compared to steers fed finishing diets. Conversely, molar proportion of propionate increased ($P = 0.04$) for steers fed the finishing diet. Our results are consistent with those of Zinn and Plascencia (1996), in which increased dietary fiber levels correlated with increased acetate to propionate ratios while supplemental fat in the diet had no effect on acetate to propionate ratios.

## Implications

The results from this experiment indicate feeding flax at 8% of dietary DM in either growing or finishing diets did

not change ruminal fermentation characteristics. In addition, flax inclusion did not alter OM and CP digestion when replacing part of corn and linseed meal in growing and finishing diets for beef cattle.

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Table 1. Formulation of dietary treatments (% of DM)

| | Treatment | | | |
|---|---|---|---|---|
| | No Flax | | With Flax | |
| Item | Growing | Finishing | Growing | Finishing |
| Corn Silage | 40.00 | 10.00 | 40.00 | 10.00 |
| Corn | 26.09 | 66.11 | 23.88 | 63.11 |
| Alfalfa | 20.00 | 10.00 | 20.00 | 10.00 |
| Flax | - | - | 8.00 | 8.00 |
| Concentrated separator by-product[1] | 5.00 | 5.00 | 5.00 | 5.00 |
| Linseed meal | 5.80 | 5.00 | - | - |
| SBM | 1.00 | 1.00 | 1.00 | 1.00 |
| Trace mineral premix[2] | 0.60 | 0.60 | 0.60 | 0.60 |
| Limestone | 0.90 | 1.40 | 0.80 | 1.40 |
| Dicalcium phosphate | 0.10 | - | 0.10 | - |
| Chromic oxide | 0.25 | 0.25 | 0.25 | 0.25 |
| Urea | 0.24 | 0.60 | 0.38 | 0.60 |
| Monensin premix[3] | 0.02 | 0.02 | 0.02 | 0.02 |
| Vitamin A and D premix[4] | 0.02 | 0.02 | 0.02 | 0.02 |

[1]De-sugared sugar beet molasses.
[2]Contained (per kg) a minimum of 32.9 g Ca, 25.6 g Cu, 160 g Zn, 65.0 g Fe, 40.0 g Mn, 1.05 g of I, 0.250 g Co.
[3]Contained 176 g/kg of monensin; formulated to contain 200 mg of monensin/kg of diet.
[4] Contained vitamin A and D concentrations of 22,000 and 2,100 IU/ kg, respectively.

Table 2. Analyzed nutrient composition of feed ingredients in growing and finishing diets (% of DM)

| Item | OM | NDF | ADF | CP | Fat |
|---|---|---|---|---|---|
| Corn Silage | 93.2 | 50.3 | 27.2 | 7.6 | 2.8 |
| Alfalfa | 89.4 | 57.0 | 41.8 | 16.5 | 0.7 |
| Corn | 98.2 | 18.7 | 3.8 | 10.2 | 5.3 |
| Flax | 95.7 | 37.0 | 23.8 | 23.4 | 24.4 |
| Linseed meal | 93.2 | 26.1 | 14.6 | 40.4 | 4.8 |
| Growing | | | | | |
| No flax | 89.4 | 37.4 | 20.2 | 13.6 | 2.6 |
| With flax | 89.9 | 38.8 | 21.7 | 13.4 | 5.0 |
| Finishing | | | | | |
| No flax | 90.5 | 30.0 | 12.9 | 15.0 | 4.0 |
| With flax | 90.9 | 30.3 | 13.0 | 14.5 | 7.3 |

Table 3. Effect of flax inclusion on dry matter intake, OM digestion, and CP digestion in steers consuming growing and finishing diets

| | No flax | | With flax | | | Contrast[a] | | |
|---|---|---|---|---|---|---|---|---|
| Item | Grower | Finisher | Grower | Finisher | SEM[b] | Ration | Flax | Ration x Flax |
| DMI | | | | | | | | |
| kg/d | 10.00 | 11.02 | 10.16 | 9.72 | 0.54 | 0.53 | 0.24 | 0.14 |
| % of BW | 2.36 | 2.58 | 2.43 | 2.30 | 0.09 | 0.64 | 0.26 | 0.08 |
| OMI, kg/d | 8.94 | 9.99 | 9.12 | 8.85 | 0.47 | 0.33 | 0.24 | 0.12 |
| Duodenal OM flow | | | | | | | | |
| Microbial, kg/d | 1.32 | 1.01 | 1.16 | 0.74 | 0.07 | 0.001 | 0.02 | 0.46 |
| Digestion, % of intake | | | | | | | | |
| Apparent ruminal | 53.3 | 61.6 | 55.9 | 61.3 | 4.4 | 0.09 | 0.74 | 0.68 |
| True ruminal | 68.1 | 71. 8 | 68.7 | 69.9 | 4.1 | 0.46 | 0.83 | 0.69 |
| Small intestine | 14.3 | 14. 5 | 15.6 | 15.8 | 3.1 | 0.95 | 0.62 | 0.98 |
| Large intestine | 5.6 | 3.2 | 1.8 | 0.4 | 1.8 | 0.19 | 0.04 | 0.70 |
| Total tract | 73.3 | 79.3 | 73.4 | 77.6 | 1.8 | 0.02 | 0.66 | 0.64 |
| CP Intake kg/d | 1.37 | 1.63 | 1.38 | 1.398 | 0.077 | 0.09 | 0.16 | 0.14 |
| Duodenal CP flow | | | | | | | | |
| Microbial, kg/d | 0.71 | 0.59 | 0.60 | 0.46 | 0.049 | 0.02 | 0.02 | 0.82 |
| CP digestion, % of intake | | | | | | | | |
| Apparent ruminal | -6.1 | 20.8 | 11.6 | 23.8 | 8.6 | 0.03 | 0.19 | 0.34 |
| True ruminal | 46.2 | 57.1 | 50.0 | 57.5 | 6.3 | 0.08 | 0.64 | 0.71 |
| Small intestine | 67.2 | 54.4 | 54.4 | 53.0 | 7.5 | 0.29 | 0.29 | 0.39 |
| Large intestine | 2.4 | -0.5 | -0.1 | -3.1 | 2.1 | 0.18 | 0.25 | 0.96 |
| Total tract | 63.6 | 74.8 | 65.9 | 73.6 | 2.1 | 0.001 | 0.78 | 0.42 |
| Microbial efficiency[c] | 18.8 | 13.4 | 17.1 | 12.0 | 2.3 | 0.04 | 0.45 | 0.93 |

[a]Probabilities for contrast *F*-test.

[b]n = 4.

[c]Grams duodenal microbial N per kg ruminal OM truly fermented.

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# SUPPLEMENTATION PRACTICES OF BEEF HEIFERS IN THE CENTRAL PLAINS OF VENEZUELA

**L. Depablos[1], J. Ordoñez[2], S. Godoy[3]*, and C.F. Chicco[1]**
Universidad Central de Venezuela (UCV)[1], Universidad Nacional Experimental de Los Llanos Ezequiel Zamora (UNELLEZ)[2], Instituto Nacional de Investigaciones Agrícolas (INIA-CENIAP)[3]. Venezuela.

**ABSTRACT**: To evaluate supplementation practices of beef heifers under grazing conditions an experiment was carried out in the central plains of Venezuela, corresponding to the dry tropical forest ecological system. Brahman heifers (n= 166) and their crossbred types with native cattle, with body weight average (BW) of 219±15.6 kg were assigned by BW, reproductive condition and racial predominance to three treatments (S=common salt, MS= mineral supplement, PMS= protein-mineral supplement, with 42.5 % CP), during the transition rainy-dry season and dry seasons (242 days long). Body weight changes, body condition scores (BC), reproductive tract conditions, pregnancy and calving percentage were measured. Concentration of minerals, urea and non esterified fatty acids (NEFA) in blood were also measured. Animals grazed in pastures of *Cynodon sp*. and *Brachiaria sp*. Mineral supplement and PMS had no significant effects on growth and BC of the animals when compared with S. The average growth rate (0.483 kg/animal/day) was adequate, mainly due to pasture management. No differences among treatments were found for uterine involution, ovarian structure and heifers' pregnancy, though the latter in the PMS treatment was 8% units higher than the other supplements. Calving percentage was influenced ($P < 0.03$) by the type of supplementation, being the values of 70, 64 and 85 % for S, MS and PMS, respectively. Calcium (8.9 mg/dl) and P (4.4 mg/dl) in blood serum were normal. The values in serum of Mg, Na, K, Cu and Zn were higher the critical values. Blood urea was affected ($P < 0.05$) by supplementation, with values of 23.1, 23.4 and 23.9 mg/dl for S, MS and PMS, respectively, being within the normal ranges. The NEFA did not present differences due to supplementation being higher than reference values (0.884 mEq/l). It is concluded that under this experimental conditions, mineral, and mineral-protein supplementation did not improve biological response of the animals.
Key words: Heifers, Supplementation, Minerals, Protein, Reproduction

## Introduction

Beef cattle production in the northern central plains of Venezuela is limited by environmental and nutritional conditions related mainly to poor quality forages, with low protein and mineral content, particularly P. In addition, there are high levels of Fe that may reach toxic levels, impairing the utilization of other minerals (Morillo et al., 1989; Mc Dowel et al., 1989; Chicco and Godoy, 1996). Under these conditions, retarded growth and poor reproduction performance characterize cattle industry of the region.

Among nutritional and other applicable technologies, mineral supplementation plays an important role, increasing reproductive performance of the herd up to 20-25% units (Chicco and Godoy, 1996). Improvement of the reproduction rate from 58.6 to 86.9%, in Brahman cattle through supplementation, pasture fertilization and improved management was reported by Arriaga et al. (2001). In consequence, the objective of this research was to evaluate the effect of mineral and a mineral-protein supplementation on growth, reproduction performance and blood chemistry of a Brahman and crossbred Brahman herd in the central plains of Venezuela.

## Materials and Methods

The experiment was carried out in the northern central plains of Venezuela, characterized by heavy-clay and poorly drained soils, partially floated during the rainy season, corresponding to the dry tropical forest ecological system (Ramia, 1967). One hundred and sixty-six Brahman heifers and their crossbred types with native cattle, with initial body weight average (BW) of 219±15.6 kg were assigned by BW, reproductive condition and racial predominance to three treatments: S=common salt; MS= mineral supplement; and PMS= protein-mineral supplement with 42.5 % CP of which 17.4 % was bypass protein The trial was conducted during 242 days, corresponding to the transition rainy-dry season and dry season. The breeding period lasted 103 days and the heifers were naturally mated with bulls, previously evaluated for semen quality

The animals were rotated in six pastures, every 24 days, with a stocking rate of 0.66 animals/ha. The pastures contained mainly improved grasses (*Cynodon sp. and Brachiaria sp*), including some native grasses (*Axonopus sp., Sporobolus sp., Paspalum sp*.) and native legumes (*Desmodium sp., Centrosema sp*.). Forage samples for chemical analysis (AOAC, 1984) were taken by hand-plucking method (Wallis de Vries, 1995) and available forage dry matter was determined by clipped harvesting. Both measurements were taken at 24 days intervals. Salt and MS were offered ad-lib, while PMS was restricted to one kg/animal/day. The composition of MS and PMS is presented in Table 1.

Body weight changes, body condition scores (BC) and reproductive tract conditions were measured at 24 days intervals. At the same time blood samples were

taken for minerals, urea and non esterified fatty acids (NEFA) analyses by conventional methods (AOAC, 1984). Pregnancy was determined by rectal palpation four times equally spaced during the mating period.

**Table 1. Ingredients of supplements for beef heifers**

| Ingredients | MS[1] (%) | PMS[2] (%) |
|---|---|---|
| Tricalcium phosphate | 80.0 | 34.0 |
| Calcium carbonate | 0 | 10.0 |
| Magnesium oxide | 7.96 | 2.68 |
| Copper sulfate | 1.92 | 1.4 |
| Zinc oxide | 1.32 | 0.9 |
| Cobalt sulfate | 0.01 | 0.01 |
| Sodium selenite | 0.01 | 0.009 |
| Sulfur | 0.78 | 0 |
| Common salt | 8.00 | 51.0 |
| Hydrolyzed feather meal | --- | 30 |
| Wheat bran | --- | 23.5 |
| Rice polishing | --- | 31 |
| Molasses | --- | 7 |
| Urea | --- | 2 |
| Minerals (MS) | --- | 1.5 |
| Ammonium sulfate | --- | 5 |
| **Nutrient content** | | |
| Crude protein, % | --- | 42.5 |
| ME, Mcal/Kg | --- | 2.65 |

[1]Mineral content (%): Ca: 24.2; P: 13.6; Na: 3.11; Mg: 2.63; S: 1.01; Cu: 0.65; Zn: 1.54; Co: 0.0024; Se: 0.0049.
[2]Mineral content (%): Ca: 14.8; P: 6.12; Na: 19.8; Mg: 1.45; S: 0.18; Cu: 0.35; Zn: 0.72; Co: 0.0021; Se: 0.0041.

Descriptive and inferential statistic was used by mean of a variance-covariance analysis. Chi-square analysis was applied for discrete variables.

## Results and Discussion

At the beginning of the experiment (transition rainy-dry season) available forage dry matter was 155 kg/animal/day and diminished to 34 kg/animal/day, during the dry season. Appreciable drop of CP content was registered in the dry season (10.7 vs. 5.25 %). Mineral concentration of forage showed differences between seasons in Ca, K and Na concentrations (Table 2). Similar trends were reported by other authors (Morillo et al., 1989; McDowell et al., 1989; Tejos, 1998)

Heifer's body weight gains were greater at the beginning of the experiment (Table 3) and declined approximately 100 days after the trial was started. This was mainly due to a decrease of the quantity and quality of available forage. A significant ($P < 0.05$) seasonal effect was registered, with lower body weight gains in the dry season. Mineral and mineral-protein supplements were unable to prevent this change. The daily consumption of supplements was 32, 30 and 700 g, respectively for S, MS and PMS. The overall body gain during the experimental period was 0.483 kg/day. This value is considered satisfactory under tropical conditions for mating at 20-22 months of age (González-Stagnaro, 1992). At breeding time, heifers had an overall weight of 288±20 kg, value that is considered adequate for mating (Plasse et al., 1989).

**Table 2.** Concentration of nutrients in forage samples during the transition rainy-dry season and dry season

| | CP | NDF | ADF | Ca | P | Mg | K | Na | Fe | Cu | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ----------%---------- | | | | | | | | ----------ppm---------- | | | |
| Transition rainy-dry season | 10.7 ±2.1 | 76.8 ±3.05 | --- | 0.67 ±0.05 | 0.2 ±0.06 | 0.15 ±0.05 | 1.91 ±0.37 | 0.31 ±0.11 | 245.1 ±221 | 12.9 ±4.3 | 63.4 ±52.4 | 214 ±116 |
| Dry season | 5.25 ±1.3 | 81.3 ±2.6 | 50.7 ±4.8 | 0.22 ±0.02 | 0.25± 0.07 | 0.18 ±0.06 | 0.72 ±0.4 | 0.17 ±0.12 | 205.4 ±93.1 | 11.4 ±2.1 | 75.8 ±55 | 57.5 ±21 |

CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber Ca: calcium; P: phosphorus; Mg: magnesium: potassium; Na: sodium; Fe: iron; Co: cobalt; Cu: copper; Mn: manganese, Zn: zinc

Brahman heifers with 289 kg at the beginning of the breeding season had 73% pregnancy, as reported by Cárdenas et al. (2001). BC (kg BW/cm wither height) were 2.40 ± 0.15, 2.39 ± 0.15 and 2.38 ± 0.14 respectively for S, MS and PMS.

No differences among treatments were found for uterine involution, ovarian structure and heifers' pregnancy; though the latter in the PMS treatment was 8% units higher than the other supplements (Table 4). No response to protein supplementation was also reported (Citto and Ramos, 1993) when cows were grazing on *Panicum maximum*, with nutritive value similar of the forage grazed in this study. This suggests that response to supplementation is conditioned by the quality and quantity of available forage.

Calcium (8.9 mg/dl) and P (4.4 mg/dl) in blood serum were normal (Table 5). Serum concentrations of Mg, K, Cu and Zn were higher than critical values. Sodium concentration was near the inferior adequate level (McDowell et al., 1989). Blood urea was higher in PMS group with values of 23.05, 23.38 and 23.88 respectively for S, MS and PMS (Table 5). A linear relationship between protein content of the diet and corresponding values of blood urea has being reported (Huntington et al., 2001). In all treatments, no differences were found for NEFA.

**Table 3.** Adjusted average weight (kg) and daily BW gain (g/animal/d) of beef heifers fed different supplements

| Day | Adjusted weight mean | | | Daily body gain | | |
|---|---|---|---|---|---|---|
| | Treatment | | | Treatment | | |
| | S | MS | PMS | S | MS | PMS |
| 0 | 223.6 | 223.6 | 222.5 | --- | --- | --- |
| 33 | 254.2 | 252.7 | 253.5 | 0.928 | 0.882 | 0.940 |
| 56 | 268.9 | 266.8 | 269.0 | 0.640 | 0.613 | 0.673 |
| 81 | 280.4 | 280.5 | 281.3 | 0.461 | 0.548 | 0.492 |
| 104 | 281.4 | 284.4 | 283.0 | 0.040 | 0.170 | 0.076 |
| 128 | 285.2 | 280.8 | 277.1 | 0.162 | -0.151 | -0.245 |
| 152 | 301.6 | 301.7 | 302.4 | 0.683 | 0.872 | 1.053 |
| 176 | 315.6 | 314.9 | 316.0 | 0.582 | 0.548 | 0.565 |
| 201 | 318.6 | 317.6 | 322.0 | 0.119 | 0.108 | 0.243 |
| 224 | 322.4 | 324.5 | 327.2 | 0.165 | 0.302 | 0.225 |
| 242 | 320.2 | 320.3 | 322.5 | -0.123 | -0.237 | -0.264 |

**Table 4.** Percent pregnant heifers during breeding season ( BS)

| | Treatments | | | |
|---|---|---|---|---|
| Days | S | MS | PMS | *P* < |
| 37 | 1.8 | 0 | 0 | 0.37 |
| 62 | 47.3 | 44.4 | 50.9 | 0.80 |
| 85 | 76.4 | 59.3 | 72.7 | 0.13 |
| 103 | 78.2 | 68.5 | 78.2 | 0.52 |
| 48 d after BS | 81.8 | 81.5 | 90.9 | 0.26 |

**Table 5.** Blood Urea (mg/dl) and NEFA (mEq/l) of beef heifers fed different dietary supplements[1]

| | Days of supplementation | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 30 | 140 | 185 | 245 | 315 |
| Urea | 22.4 ± 0.84$^{de}$ | 24.6 ± 1.95$^{c}$ | 23.1 ± 0.89$^{d}$ | 23.1 ± 1.08$^{d}$ | 21.7 ± 0.79$^{e}$ | 24.9 ± 1.8$^{c}$ |
| NEFA | 0.95 ± 0.07$^{a}$ | 0.97 ± 0.06$^{a}$ | 0.79 ± 0.04$^{c}$ | 0.89 ± 0.05$^{b}$ | 0.86 ± 0.07$^{b}$ | 0.79 ± 0.09$^{c}$ |

[1]Pool data for treatment effect.

a,b,c,d,e Means with different superscript letters in the same row differ ($P < 0.05$)

## Implications

Brahman heifers and their crosses with native cattle under grazing conditions on tropical grassland supplemented with common salt or a complete mineral supplement and this plus a protein concentrate did not present significant improvement in growth, conception rate and blood chemistry values, suggesting that when animals are kept on good quality and well managed forage, even in the tropics, no additional benefits are achieved trough supplementation.

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# EFFECT OF FEEDING LEVELS ON BODY WEIGHT CHANGES AND MILK PRODUCTION OF COWS UNDER TROPICAL CONDITIONS

**C. F. Chicco*[1] and S. Godoy[2]**

[1]Universidad Central de Venezuela and [2]Centro Nacional de Investigaciones Agropecuarias, Instituto Nacional de Investigaciones Agrícolas, Maracay, Venezuela

**ABSTRACT**: To determine the effect of feeding levels on body weight changes and milk production, 108 dairy cows (C), divided in uniform groups of Brown Swiss (BS) and Holstein cows (HF) and first calf heifers (FCH) were assigned to three experimental diets. Diets consisted of green chopped forage (*Sorghum vulgare*) and a supplement (18 % CP and 75% TDN), to obtain approximately 80 (L), 100 (M) and 120 (H) % of the 1989 NRC nutrient requirements. The experiment was carried out during 8 weeks prior calving and 22 weeks post-partum using a 3x3 factorial arrangement in a completely randomized design. The trial was conducted in Venezuela, located at 452 m.a.s.l., with an average yearly temperature of 24.5 °C, relative humidity of 77 % and rainfalls of 976 mm. The rations were adjusted weekly according to body weights and milk production, with daily records of forage and concentrate intake. Crude protein was determined chemically and TDN was estimated by IVDMD. Data were analyzed by least squares including in the model breed, age, pre- and post-calving feeding effects and the corresponding interactions. Prior calving, C and FCH body weights were 539 ± 18.3 and 489 ± 15.2 kg respectively. During post-partum period C and FCH lost 3.8 ± 0.48 and 4.9 ± 0.66 % body weight ($P$<0.05). Cows produced more milk ($P$<0.05) than FCH (11.3 ± 1.4 vs. 10.5 ± 1.4) and HF more ($P$<0.05) than BS (12.2 ± 1.5 vs. 9.6 ± 0.59 kg). Pre-partum and post-partum feeding regimes influenced (P<0.05) milk production, averaging 10.5 and 9.8, 11.0 and 11.2 and 11.2 and 11.7 kg/day, respectively for feeding periods and L, M and H feeding levels. Cows had a daily intake of 5.2 ± 1.4 kg concentrate and FCH 4.9 ± 1.6 kg. Significant regressions ($P$<0.01) of concentrate intake vs. milk production were: y= 6.735 + 0.878x and y= 8.125 + 0.484x, respectively for C an FCH. It is concluded that milk production and body weights are influenced by pre- and post- calving nutrition as well as by breed type and animal age.

Key word**s:** Holstein, Brown Swiss, Feeding levels, Age, Milk, Weights changes

## Introduction

Dairy cattle production in tropical areas is impaired by environmental, technological and nutritional conditions, as well as diseases, that have a negative effect on milk production, health and reproductive performance of the herd. As a consequence, dairy activity is characterized by low milk production and low economical profits. This is particularly true for European breeds that are more sensible to the adverse tropical environment, showing poor adaptability, production and procreation and, as a consequence, present low capital investment returns (Bodisco et al., 1971; Pérez Quintero et al., 2005). Nevertheless, most tropical countries have imported Holstein and Brown Swiss animals in an attempt to palliate milk deficit for human consumption. Failure of this policy is largely described in the international literature (Holmann, 1998). Within the adverse effects of the tropical environment for milk production, nutrition plays an important role due to poor quality forages, seasonal effects of rainfalls, high cost of feed concentrates, and high temperature that negatively influence feed intake and other physiological processes. Therefore, the objective of this research was to provide additional information on the effect of the nutritive density of the diet on production performance of Holstein and Brown Swiss cattle, under tropical conditions.

## Materials and Methods

To determine the effect of feeding levels on body weight and milk production an experiment was conducted in the central northern region of Venezuela, located at 452 m.a.s.l., with an average yearly temperature of 24.5 ºC, relative humidity of 77 % and rainfalls of 976 mm. A total of 108 dairy cows, divided in uniform groups of Brown Swiss (BS) and Holstein (HF) cows and first calf heifers of both breeds, using individual feeders, were assigned to three experimental diets. Rations consisted of a combination of green chopped forage (*Sorghum vulgare*) and a supplement (18 % CP and 75% TDN), to obtain diets approximately similar to 80 (L), 100 (M) and 120 (H) % of the 1989 NRC nutrient requirements (Table 1).

**Table 1**. Composition of concentrate fed to dairy cows and fist calf heifers[1] [2]

| Ingredients | % |
|---|---|
| Cotton cake meal | 14.0 |
| Ground corn meal | 73.0 |
| Urea | 2.0 |
| Dicalcium phosphate | 1.0 |
| Molasses | 9.0 |
| Calcium carbonate | 0.5 |
| TOTAL | 100.0 |

[1]Supplement contained (%): CP, 18.5; estimated TDN: 74.8; Ca: 0.5; P 0.40; [2] a complete mineral supplement vas offered ad-lib.

The experiment was carried out during 8 weeks prior calving and 22 weeks post-partum, using a 3x3 factorial arrangement in a completely randomized design. Prior calving, body weights of cows and first calf heifers were 539±18.3 and 489±15.2 kg respectively. The rations were adjusted weekly according to body weights and milk production, with daily records of milk yield (corrected to 3.5% fat), forage and concentrate intake. Crude protein was determined by Kjeldahl method (AOAC, 1989) and TDN was estimated by IVDMD. Data were analyzed by least squares including in the model breed, age, pre- and post-calving feeding effects and the corresponding interactions (Harvey, 1982).

## Results and Discussion

During 22 weeks after calving, adjusted mean values (Table 2) indicate that Holstein cows produced more milk (12.21±0.73 kg/day) than Brown Swiss cows (9.59±0.07 kg/day), and the former lost more weight (4.92±0.64%) than the latter (3.78±0.45%) when data were expressed as percent of initial body weight.

**Table 2.** Milk production (kg/day) and body weight losses (%)[1] during 22 weeks after calving[2]

| Breed | First calf heifers | | Cows | | Average | |
|---|---|---|---|---|---|---|
| | Milk | BW | Milk | BW | Milk | BW |
| Holstein | 11.48 | 5.56 | 12.94 | 4.28 | 12.21±0.73[a] | 4.92± 0.64 [A] |
| Brawn Swiss | 9.52 | 4.23 | 9.66 | 3.33 | 9.59±0.07[b] | 3.78± 0.45 [B] |
| Average | 10.50 ±0.98[B] | 4.90 ±0.66[b] | 11.30±1.64[A] | 3.81± 0.48[a] | 10.90±1.40[3] | 4.35 ± 0.79[3] |

[1]As % initial BW; [2]Adjusted means; [3] Overall means.
[a,b] Means within the same row or column with different superscript letters differ *(P* < 0.05).
[A,B] Means within the same row or column with different superscript letters differ (*P* < 0.05*)*.

When cows were compared with first calf heifers, cows showed greater milk yield (11.30±1.64 vs. 10.50±0.98 kg/day) and lower body losses (3.81±0.48 vs. 4.90±0.66%). In all cases the differences were significant (*P* < 0.05). Similar tendencies were reported by Brandt et al. (1974). This is a common finding for European breeds in the tropics where animal are subject to high temperature and other environmental stresses. Genetic-environmental effects in relation to temperature on milk production are not clear yet (Nogara et al., 2000), though other factors such as comfort, diseases, chromosome maternal effect, besides nutrition, may play an important role ( Bodisco et al., 1971*;* Santoro et al., 1992).

Milk production and body weight (Table 3) varied during the 22 weeks of the recorded data. Cows showed noticeable body weight losses during the first 10 weeks of lactation, averaging 222 and 290 g/day respectively for mature animals and first calf heifers, with a milk production of 12.18 and 11.41 kg/day for the same animal type order.

**Table 3.** Milk production (kg/day) and body weight changes (g/day) of cows and first calf heifers during 22 weeks after calving

| Weeks | First calf heifers | | Cows | |
|---|---|---|---|---|
| lactation | Milk | BW | Milk | BW |
| 01-10 | 11.41 | -290 | 12.18 | -222 |
| 11-22 | 9.75 | 74 | 10.57 | 73 |
| 01-22 | 10.58 [b]± 0.98 | -108 [B]± 182 | 11.3[a]± 0.81 | -74 [A]± 147 |

[a,b] Means within the same row with different superscript letters differ (*P* < 0.05).
[A,B] Means within the same row with different superscript letters differ (*P* < 0.05).

By the end of the 22 week period, Holstein and Brown Swiss animals were unable to recover body losses, being these (*P* < 0.05) lower than initial body weights. Body losses at peak lactation are a physiological condition in lactating animals that are unable to have a nutrient intake and efficient utilization of absorbed nutrients at tissue level to compensate nutrient losses through milk (Nepham, 1987)

Pre-partum and post-partum feeding regimes influenced milk production (*P* < 0.05) averaging 10.50 and 9.79, 11.17, and 11.18 and 11.74 kg/day, respectively for feeding periods (pre- and post-calving) and L, M and H feeding levels (Table 4). Corresponding body weight losses (%) were 5.42 and 4.87, 4.54 and 4.34, and 3.10 and 3.87 as percent of initial body weights, being the differences significant (*P* < 0.05).

**Table 4.** Influence of pre- and post-partum feeding levels on milk production (kg/day) and boy weight losses (%)[1] of cows and first calf heifers

| Levels | Low | | Medium | | High | | Average | |
|---|---|---|---|---|---|---|---|---|
| | Milk | BW | Milk | BW | Milk | BW | Milk | BW |
| Low | 9.47 | 5.82 | 11.05 | 6.42 | 10.98 | 4.02 | $10.50^{a}$ ±0.73 | $5.42^{C}$ ±1.01 |
| Medium | 9.46 | 5.78 | 12.02 | 4.08 | 11.58 | 3.78 | $11.02^{a}$ ±1.11 | $4.54^{B}$ ±0.88 |
| High | 10.44 | 3.02 | 10.44 | 2.52 | 12.66 | 3.82 | $11.18^{a}$ ±0.29 | $3.10^{A}$ ±0.95 |
| Average | $9.79^{a}$ ±0.46 | $4.87^{B}$ ±1.31 | $11.17^{b}$ ±0.65 | $4.34^{AB}$ ±1.60 | $11.74^{a}$ ±0.69 | $3.87^{A}$ ±0.11 | 10.90[2] ±1.02 | 4.37[2] ±1.26 |

[1]As percent initial body weight; [2]Averall means.
[a,b] Means in the same row or column whit different superscripts differ ($P < 0.05$).
[A,B] Means in the same row or column with different superscripts differ ($P < 0.05$ )

Adjusted means suggest that feeding regimes during pre-calving period had a major effect in diminishing body weight losses when animals were lactating, while post-partum feeding had a greater effect on milk production.

These findings were reported earlier by Wiltbank et al. (1967). Cows had a daily intake of 5.2±1.4 kg concentrate and first calf heifers of 4.9±1.6 kg (Table 5).

**Table 5.** Intake of forage and concentrate supplement (DM) of cows and first calf heifers at different feeding levels during lactation

| Feeding levels[1] | Dry matter intake (kg/day) | | |
|---|---|---|---|
| | Forage | Concentrate | Total |
| Low | 7.358 ± 1.4 | 2.773 ± 1.2 (27.4) | $10.131^{a}$ ± 2.1 |
| Medium | 7.194 ± 1.3 | 5.163 ± 1.7 (41.8) | $12.357^{b}$ ± 2.2 |
| High | 6.958 ± 1.1 | 7.214 ± 1.8 (50.9) | $14.172^{c}$ ± 1.8 |
| Fist calf heifers | 6.831 ± 1.9 | 4.903 ± 1.6 (41.8) | $11.734^{a}$ ± 2.0 |
| Cows | 7.509 ± 1.8 | 5.197 ± 1.4 (40.9) | $12.706^{b}$ ± 1.9 |

[a,b,c] Means in the same column with different superscript letters differ ($P < 0.05$).
( ) Values in parenthesis are percent of total intake.

Significant regressions ($P < 0.05$) of concentrate intake vs. milk production were: y= 6.735 + 0.878x and y= 8.125 + 0.484x, respectively for cows and first calf heifers. For the same order, intake vs. body weight losses, the equations were: y= 221.3 - 3.591x and y= 130.4 – 5.762 - $10^{-1}$

The estimated values for body weight and milk production (Table 6) indicate that, with 80% concentrate in the diet, milk production can reach 17 kg/day, while 60% concentrate in the diet maintains negative values for body weight changes after the first 22 weeks of lactation. These calculated data suggest the importance of the quality of the diet for higher milk production, and that body reserves are mobilized to sustain milk production during the first 10 weeks after calving, and by the 22$^{th}$ week body losses were not totally recuperated.

**Table 6.** Estimated milk production (kg/day) and body weight losses (g/day) of cows and first calf heifers in dependence of feeding levels[1]

| Weeks of lactation | Milk production | | | BW losses | | | Significance |
|---|---|---|---|---|---|---|---|
| | Concentrate intake as % of dry matter intake | | | | | | |
| | 0 | 40 | 80 | 0 | 30 | 60 | |
| Cows | 4.92 | 11.48 | 17.10 | 222 | 105 | 10 | $P<0.05$ |
| FCH[2] | 6.82 | 10.95 | 12.18 | 130 | 113 | 97 | $P<0.05$ |

[1]Values calculated by regression ($P < 0.05$).
[2]First calf heifers.

## Implications

It is concluded that milk production and body weights are influenced by pre- and post-calving nutrition as well as by breed type and animal age. Milk production is low with European breeds, being milk yield of Holstein higher than Brown Swiss either in cows as in first calf heifers.

Feeding levels appear to have higher effect on body weight prior calving, while post-partum feeding is more

effective on milk production. Peak of milk yield corresponds to major body losses that were still in negative values after the first 22 weeks of lactation.

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# EFFECTS OF FEEDING DRIED DISTILLERS GRAINS AND SOLUBLES TO GROWING AND FINISHING BEEF STEERS ON PRODUCTION, DIGESTIBILITY, RUMINAL FERMENTATION AND CARCASS CHARACTERISTICS

**D. R. ZoBell[1,*], C. K. Chapman[1], R. D. Wiedmeier[1] and M. Stuart[1]**
[1]Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT 84322-4815

**ABSTRACT:** Byproducts from the ethanol industry are providing nutritious feeds that can provide supplemental energy and/or protein for beef cattle The objective of these studies was to evaluate growing and finishing beef cattle using a concentrate source that was either barley or barley and DDGS. In both studies, forty-five predominantly British-based crossbred steer calves were placed in pens with five head per pen and received a corn silage/alfalfa hay diet where the concentrate source was either barley or barley and DDGS. The concentrate portion of the diets in the growing trial (84d) for control calves (C) were 35% barley; T1 calves received 24.5% barley and 10.5% DDGS and T2 calves, 17.5% barley and 17.5% DDGS (DMB). Calf finishing (112d) concentrate amounts for C were 83% barley; T1 calves received 70.7% barley and 11.4% DDGS and T2 calves, 63.8% barley and 18.3% DDGS (DMB). The finishing study was terminated when it was determined that the majority of the calves had reached the Choice quality grade. A single crossover designed digestibility study was also conducted utilizing four cannulated yearling heifers fed either the growing studies C or T2 diets. Results showed that in the growing study overall ADG was 1.29, 1.40 and 1.45 $kg.d^{-1}$ ($P>0.05$); DMI 8.50, 7.82 and 7.59 $kg.d^{-1}$ ($P<0.05$), and FE 6.67, 5.57 and 5.32 ($P>0.05$) for C, T1 and T2 respectively. Finishing calf results for ADG were 1.30, 1.36 and 1.33 $kg.d^{-1}$ ($P<0.05$); DMI 10.8, 10.0, and 9.77 $kg.d^{-1}$ ($<0.05$); and FE 7.88, 6.85 and 6.59 ($P>0.05$) for C, T1 and T2, respectively. There were no differences for any of the carcass traits measured ($P>0.05$). DM and NDF digestibility and VFA levels were not affected by treatment ($P>0.05$) although acetate was reduced from 58.1 to 52.5 $mMol.100mMol^{-1}$ ($P<0.05$). These studies show that beef cattle on growing diets, where the concentrate source is barley, can benefit from the inclusion of DDGS but there appear to be no significant effects in finishing diets for the levels used.

## Introduction

The type and amount of concentrate included in beef cattle diets has significantly changed due to the emergence of the ethanol industry in recent years. Traditional cereal gains have become increasingly more expensive and cost of gain has soared. However, byproducts from the ethanol industry are providing nutritious feeds that can provide supplemental energy and/or protein for growing and finishing beef cattle (Firkins, et al., 1985; Ham et al., 1994; Lodge et al., 1997; Lardy, 2003). Distillers dried grains and solubles (DDGS) is one of these by-products of the ethanol industry and for the western states has more relevance at the present time than those that are wet-milled due to transportation expenses and other considerations. Additionally, barley is the predominant cereal grain used in growing and finishing diets in the intermountain west based on feedlot cost of gain. The objective of these studies was to evaluate growing and finishing cattle using a concentrate source that was either barley or barley and DDGS.

## Materials and Methods:

*Trial 1: Growing Beef Steer Study*

Forty five predominantly British-based crossbred steer calves (initial wt.=258 kg) were used in this trial. All calves had been processed similarly prior to trial initiation by receiving a Brucellosis vaccination, parasite treatment (Dectomax, Pfizer Animal health, Exton, PA), 8-Way Clostridial vaccine (Pfizer Animal health, Exton, PA) and intranasal respiratory product (BoviShield, Pfizer Animal Health, Exton, PA). Calves were placed in pens with five head per pen and received a corn silage/alfalfa hay based growing diets and concentrate. The concentrate portion of the diet for control calves (C) was 35% dry rolled barley grain; T1 calves received 24.5% barley and 10.5% DDGS and T2 calves, 17.5% barley and 17.5% DDGS (DMB; Tables 1, 2 and 3). The treatment diets were largely isocaloric and isonitrogenous. There were three pens per treatment. Calves were fed at 0800 h such that there were no refusals. Individual calf weights were recorded on days 0, 28, 56 and 84 and feed intake was recorded daily. No animals required health treatment throughout the study.

*Trial 2: Finishing Beef Steer Study*

The forty five steer calves used in the growing study continued on in the same pens through finishing. At the conclusion of the 84d growing study the concentrate portion of the diet for all pens was increased over a 21 day period to a suitable finishing ration. The concentrate portion of the diet for control calves (C) was 83% dry rolled barley grain; T1 calves received 70.7% barley and 11.4% DDGS and T2 calves, 63.8% barley and 18.3% DDGS (DMB; Tables 1, 2 and 3). The treatment diets were again largely isocaloric and isonitrogenous. As in the growing trial, there were three pens per treatment with calves fed at 0800 h such that there were no refusals. Individual calf weights were recorded on days 0 and every 28 days for 140 days with feed intake recorded daily. No animals required health treatment throughout the study.

The study was terminated when it was determined visibly that the majority of the calves had reached the Choice quality grade. Steers were slaughtered at the JBS

facility (Hyrum, UT) and carcasses were graded after a 24 h chill.

*Trial3: Digestibility Study*

The C and T2 diets that were used in study 1 were fed to four ruminally cannulated beef cows in a digestibility trial using a replicated 2 × 2 Latin square design. Cows were individually housed in open front 4 m x 10 m pens with concrete floors. All feedstuffs were fed once daily at 08:00 h for a 21 d adaptation period followed by a 6-d collection period. Diets were fed at 1.5% of body weight and were totally consumed daily. During the collection periods, fecal grab samples (300 g) were obtained at 08:00 h from each cow. Samples of the total mixed diet (TMD), feces and individual feedstuff samples were also obtained daily throughout the collection period. Feed samples were weighed and dried at 60EC for 72 h and ground in a Wiley mill to pass a 1- mm screen and the ground material analyzed for DM (AOAC 2000; 934.01). Neutral detergent fiber of feeds and fecal samples were determined using an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY). Acid insoluble ash (AIA) (Van Keulen and Young, 1977) was used as an internal marker to estimate apparent nutrient digestibility of DM and NDF. These procedures were described in detail by Zobell et al. (2003).

On day 6, ruminal fluid was obtained from the ventral sac of the rumen via the rumen cannula of each cow at 0,1, 2, 4, 6, 8, 10 and 12 h after feeding and immediately analyzed for pH using a combination electrode. Rumen fluid was strained through eight layers of cheese-cloth and 2 ml of the fluid was acidified with 18 ml of 6 *N* HCL. Volatile fatty acid (VFA) concentrations were measured in acidified samples using gas chromatography (Hewlett Packard 5890, Avondale, PA) with a 1.83 m X 2 mm ID glass column packed with GP 10% SP-1200/1% $H_3PO_4$ on 80/100 mesh Chromosorb W-AW.

The study was approved and conducted according to the protocol established by the Institutional Animal Care and use Committee at Utah State University.

All data were analyzed using the ANOVA procedure of Statistix 8 (Tallahassee, Fl). Least squares analysis of variance was employed to detect treatment differences and derive least squares means and standard errors for variables that were measured.

## Results and Discussion

*Growing Study*

Overall treatment means for ADG did not differ (P>0.05; Table 4). However, DMI for the treated calves tended to decrease resulting in greater FE for these treatments (P<0.05). All rations were accepted readily but even though the diets were isocaloric and isonitrogenous, T1 and T2 calves were more efficient in their use. Although an economic assessment has not been performed it would follow through that the increased FE for T1 and T2 diets would have resulted in a lower cost per unit of gain.

*Finishing Study*

Finishing data (Table 5) shows that all calves, regardless of treatment, performed the same for ADG, DMI and FE (P>0.05). Carcass data (Table 6) also showed no difference for any of the variables measured between treatments (P>0.05). The DDGS replaced 12.3% and 19.2% of the barley in the T1 and T2 respective finishing diets, relative to C. If an economic assessment were conducted there would have been no economic advantage under these conditions in using DDGS in the finishing diet as barley cost is presently below that for DDGS.

*Digestibility Study*

Table 7 shows the effect of treatment on in vivo ruminal fermentation characteristics. It is apparent that there were no differences in any of the variables measured with the exception of acetate levels which were decreased with the inclusion of DDGS. NDF digestibility tended to be improved when DDGS was added to the diet. This may have been the reason that FE values were more favorable for T1 and T2 calves in the growing trial.

## Conclusions

These studies suggest that DDGS may be included in growing and finishing diets at up to 18.3% of the diet DM without adverse affects on production, carcass or ruminal fermentation characteristics. An economic assessment would provide additional information relative to cost of gain although it appears this was decreased in the growing diet with the inclusion of DDGS but no advantage in the finishing trial.


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Table 1. Ration ingredients (DMB) for growing and finishing studies.

| | Feedstuff[1,2] | | | | |
|---|---|---|---|---|---|
| Study and Treatment | AH | CS | BG | DDGS | SUPP[3] |
| | % | % | % | % | % |
| Growing Steers | | | | | |
| Control | 25.0 | 35.0 | 35.0 | 0 | 5.00 |
| T1 | 15.0 | 45.0 | 24.5 | 10.5 | 5.00 |
| T2 | 10.0 | 50.0 | 17.5 | 17.5 | 5.00 |
| Finishing Steers | | | | | |
| Control | 8.00 | 5.00 | 83.0 | 0 | 4.0 |
| T1 | 0 | 12.4 | 70.7 | 11.4 | 4.80 |
| T2 | 0 | 12.5 | 63.8 | 18.3 | 4.50 |

[1]AH=Alfalfa Hay; CS=Corn Silage; BG=Barley Grain; DDGS=Dried Distiller's Grains and Solubles; SUPP=Supplement.
[2]Finishing steers also received limestone – C: 0, T1: .60% and T2: .90%.
[3]Consisted of 5.0% NaCl, .24% Mg, .76% K, 200 ppm Cu, 400 ppm Mn, 650 ppm Zn, 2 ppm Se, 22 ppm I, 9 ppm Co, 121000 IU.kg$^{-1}$ Vit. A, 37400 IU.kg$^{-1}$ Vit. D, 55 IU.kg$^{-1}$ Vit. E and 360 ppm Rumensin.

Table 2. Nutrient specifications of feedstuffs used in growing and finishing studies.

| | Nutrient | | | | | |
|---|---|---|---|---|---|---|
| Feedstuff[1] | DM (%) | $NE_m$ (Mcal/kg) | $NE_g$ (Mcal/kg) | CP (%) | Ca (%) | P (%) |
| AH | 85.3 | 1.63 | 1.01 | 21.2 | 1.38 | .23 |
| CS | 38.3 | 1.76 | 1.14 | 7.50 | .26 | .25 |
| BG | 92.6 | 2.00 | 1.34 | 14.5 | .08 | .38 |
| DDGS | 89.6 | 2.13 | 1.47 | 31.6 | .04 | .84 |
| SUPP | 95 | 1.61 | 1.01 | 11.0 | 8.4 | .88 |

[1]AH=Alfalfa Hay; CS=Corn Silage; DDGS= Dried Distillers Grains and Solubles; BG=Barley Grain; SUPP=Supplement

Table 3. Nutrient composition (DMB) of diets fed to growing and finishing steers.

| | Nutrient | | | | | |
|---|---|---|---|---|---|---|
| Study and Treatment | DM (%) | $NE_m$ (Mcal/kg) | $NE_g$ (Mcal/kg) | CP (%) | Ca (%) | P (%) |
| Growing Steers | | | | | | |
| Control | 61.1 | 2.00 | 1.17 | 13.6 | .86 | .30 |
| T1 | 56.0 | 2.05 | 1.19 | 14.0 | .75 | .35 |
| T2 | 53.8 | 2.05 | 1.21 | 14.5 | .69 | .39 |
| Finishing Steers | | | | | | |
| Control | 86.1 | 2.16 | 1.28 | 14.5 | .54 | .37 |
| T1 | 78.6 | 2.18 | 1.30 | 15.3 | .72 | .42 |
| T2 | 78.4 | 2.18 | 1.30 | 16.5 | .82 | .45 |

Table 4. The effect of including DDGS in growing steer rations on performance[1].

| Period | Variable | Control | T1 | T2 | SEM |
|---|---|---|---|---|---|
| 0-28d | | | | | |
| | ADG (kg) | 1.25 | 1.48 | 1.60 | .136 |
| | DMI (kg) | 8.52[a] | 7.84[a,b] | 7.59[b] | .027 |
| | FE | 6.92[a] | 5.42[a] | 4.78[b] | .493 |
| 28-56d | | | | | |
| | ADG (kg) | 1.35 | 1.51 | 1.53 | .088 |
| | DMI (kg) | 8.60[a] | 7.91[b] | 7.64[c] | .008 |
| | FE | 6.39[a] | 5.26[b] | 5.00[b] | .210 |
| 56-84d | | | | | |
| | ADG (kg) | 1.28 | 1.22 | 1.22 | .090 |
| | DMI (kg) | 8.36[a] | 7.77[b] | 7.50[c] | .055 |
| | FE | 6.72 | 6.05 | 6.19 | .542 |
| 0-84d | | | | | |
| | ADG (kg) | 1.29 | 1.40 | 1.45 | .061 |
| | DMI (kg) | 8.50[a] | 7.82[a,b] | 7.59[b] | .027 |
| | FE | 6.67[a] | 5.57[b] | 5.32[b] | .271 |

[1]Treatment means within rows with different superscripts differ (P<.05).

Table 5. The effect of varying levels of WM on finishing steer productivity[1].

| | | Treatment | | | |
|---|---|---|---|---|---|
| Period | Variable | Control | T1 | T2 | SEM |
| 0-28d | | | | | |
| | ADG (kg) | .91 | .88 | .94 | .093 |
| | DMI (kg) | 8.41[a] | 7.86[b] | 7.41[c] | .032 |
| | FE | 9.51 | 9.51 | 8.22 | 1.31 |
| 28-56d | | | | | |
| | ADG (kg) | 1.93 | 2.14 | 2.13 | .096 |
| | DMI (kg) | 9.68[a] | 9.50[b] | 9.18[c] | .059 |
| | FE | 5.02 | 4.51 | 4.32 | .251 |
| 56-84d | | | | | |
| | ADG (kg) | 2.17 | 2.07 | 1.91 | .114 |
| | DMI (kg) | 11.8[a] | 10.8[b] | 10.8[b] | .045 |
| | FE | 5.45 | 5.21 | 5.67 | .223 |
| 84-112 | | | | | |
| | ADG (kg) | 1.15 | 1.45 | 1.46 | .107 |
| | DMI (kg) | 11.9[a] | 11.0[b] | 10.8[b] | .014 |
| | FE | 10.8 | 7.77 | 7.60 | 1.11 |
| 112-140d | | | | | |
| | ADG (kg) | 1.24 | 1.15 | 1.15 | .107 |
| | DMI (kg) | 12.1[a] | 10.8[b] | 10.6[b] | .100 |
| | FE | 8.60[a] | 7.27[b] | 7.14[b] | .253 |
| Overall | | | | | |
| | ADG (kg) | 1.30 | 1.36 | 1.33 | .038 |
| | DMI (kg) | 10.8[a] | 10.0[a,b] | 9.77[b] | .038 |
| | FE | 7.88[a] | 6.85[b] | 6.59[b] | .427 |

[1]Treatment means within rows with different superscripts differ (P<.05). Overall FE differed at P<0.06.

Table 6. The effect of varying levels of treatments on carcass characteristics of finishing steers[1].

| | Treatment | | |
|---|---|---|---|
| Variable[2] | Control | T1 | T2 |
| SW (kg) | 573.8 | 592.9 | 591.7 |
| HW (kg) | 347.1 | 359.5 | 359.8 |
| Carcass Yield (%) | 60.5 | 60.7 | 60.8 |
| QG (% Choice) | 93.3 | 100.0 | 73.3 |

[1]Treatment means did not differ (P>.05) for any variable.
[2]SW=Slaughter Weight; HW=Hot Weight; MS=Marbling Score; YG=Yield Grade; QG=Quality Grade.

Table 7. The effect of treatment on in vivo ruminal fermentation characteristics.

| | Treatment | | |
|---|---|---|---|
| Item | Control | Treated | P |
| pH | 5.86 | 5.93 | .55 |
| Volatile Fatty Acids, mMol/100 mMol | | | |
| Acetate | 58.1 | 52.5 | .018 |
| Propionate | 27.5 | 26.1 | .31 |
| Butyrate | 14.1 | 15.5 | .12 |
| DM Digestibility (%) | 58.7 | 60.0 | .48 |
| NDF Digestibility (%) | 42.6 | 47.2 | .07 |

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# EFFECT OF A FIBROLYTIC ENZYME SUPPLEMENTATION ON GROWING BEEF STEERS

**J.-S. Eun*, D. R. ZoBell, C. M. Dschaak, and D. E. Diaz**
Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT

**ABSTRACT:** The objective of this study was to determine growth performance of growing beef steers when fed with a fibrolytic feed enzyme product in a completely randomized design. This growing beef study consisted of 60 group-penned Angus crossbred steers randomly assigned to treatments: control (**C**; no enzyme), low enzyme (**LE**), and high enzyme (**HE**). Five animals were placed in each pen, and 4 pens allocated to each treatment. All steers were adapted to C diet for a 2-week period prior to start of the trial. The growing ration contained 15% alfalfa hay, 43% corn silage, 30% barley grain, 7% soybean meal, and 5% feedlot supplement (DM basis), and was fed as a TMR. For the enzyme treatments, an experimental enzyme product from Alltech Inc. (Nicholasville, KY) was added to the C diet at dose rate of 1 or 2 g of the enzyme/kg DM TMR to the LE or HE treatment, respectively. The enzyme product was in powder form and contained endoglucanase and xylanase activities. All steers were fed once per day, and feed bunks read each afternoon and prior to morning feeding which was used to determine the amount of feed to deliver to each pen the following day. The experiment lasted for 84 d, and all steers were weighed on days 0, 28, 56, and 84. Intake of DM averaged 8.12 kg/d across the treatments, but supplementing the enzyme to the growing diet did not affect DMI regardless of dose rate ($P = 0.83$). Body weight gain numerically increased with supplementing enzyme (101 and 99 kg for LE and HE, respectively) compared to C (96 kg), although supplementing the enzyme product failed to detect a significant effect ($P = 0.67$). Average daily gain throughout the experiment was 1.13 kg/d on average for all treatments, and enzyme supplementation did not affect ADG ($P = 0.95$). In addition, enzyme did not influence feed-to-gain ratio ($P > 0.05$). Supplementing the fibrolytic feed enzyme in a beef growing diet at 1 and 2 g/kg DM TMR resulted in no effects on growing performance of beef steers.

**Key Words:** Growing beef steers, Fibrolytic feed enzyme, Feed-to-gain ratio.

## Introduction

The use of feed enzyme additives in ruminant diets is gaining acceptance as a means of improving feed utilization and performance of domestic ruminants. The feed enzyme additives help bridge the gap between actual digestibility of the feed that occurs in vivo and the potential digestibility of the feed that would be possible if the conditions were ideal (Beauchemin et al., 2003). To ensure consistent results of feed enzyme products, however, product formulation and dose rate must be considered, as these are main factors that affect the key enzymatic activities supplied (Beauchemin et al., 2003; Eun and Beauchemin, 2007b).

Identifying the key enzymatic activities needed for feed enzyme additives to be consistently effective in ruminants is challenging because the mechanisms whereby feed enzymes improve microbial digestion of feed are not well understood (Beauchemin et al., 2004). The key activities needed to improve forage fiber degradation for ruminants likely differ from those needed in industrial applications in which fibrolytic enzymes are commonly used (e.g., textile and food industries). With ruminants, the enzymes must act synergistically with the endogenous enzyme activities of the rumen microbes (Morgavi et al., 2000). In addition, for enzymes to improve forage degradation, the array of enzyme activities supplemented must be specific to the chemical composition of the targeted forage, due to the specificity of enzymes for their substrate (White et al., 1993). Thus, key enzymatic activities may differ among forages.

The objective of the current study was to evaluate the effects of a fibrolytic feed enzyme (**FFE**) product on growth performance of growing beef steers fed a corn silage-based TMR diet. The FFE product used in the current feeding study contained similar enzymatic activities to the enzyme product assessed by Ranilla et al. (2007), in which they reported its beneficial effect on in vitro degradation of grass hay. We expected a similar, positive effect of the FFE on corn silage-based diet of growing beef steers due to the fact that grass hay and corn silage have a similar cell wall structure.

## Materials and Methods

*Enzyme product and its dose rate.* A developmental FFE product from Alltech Inc. (Nicholasville, KY) was used in this study. The FFE product was in powder form, and contained endoglucanase (EC 3.2.1.4) and xylanase (EC 3.2.1.8) activities, but no amylase (EC 3.2.1.1) and exoglucanase (EC 3.2.1.91) activities were detected. The FFE was applied at 0, 1, or 2 g of the enzyme/kg DM TMR to the control (**C**), low enzyme (**LE**), or high enzyme (**HE**) treatment, respectively. The FFE product was applied onto the corn silage followed by mixing with other ingredients of diet.

*Growing beef steer study.* The cattle used in this study were cared for according to the Live Animal Use in Research guidelines of Institutional Animal Care and Use Committee at Utah State University. The study was conducted at the Utah State University beef research farm for 84 days, and consisted of 60 group-penned Angus crossbred steers randomly assigned to treatments: C, LE, or HE treatment.

Five animals were placed in each pen. All steers were allowed to adapt to C diet for a 2-week period prior to start of the trial. Steers were fed a corn silage-based TMR diet (Table 1), and had free access to fresh water. All feedstuffs were analyzed initially for DM and nutrient concentrations according to AOAC (1995), and corn silage DM obtained weekly. Feed was mixed and delivered to the steers in a Rissler feed cart (Rissler Mfg, Mohnton, PA) which recorded amounts fed daily. All steers were fed once per day (0700) to appetite and feed bunks were read each afternoon and prior to morning feeding which was used to determine the amount of feed to deliver to each pen the following day. Feed samples were obtained weekly and composited by month for each treatment. The DM content of feed was determined by oven drying at 55°C. Mean DMI was calculated for each pen as the total amount of DM allocated daily divided by the number of cattle per pen on that particular day. Thus, intake accounted for any sick cattle removed from the pen during treatment. The assumption was that DMI was the same for all cattle within the pen. All steers were weighed on d 0, 28, 56, and 84. Body weight gain was determined by comparing the initial and final BW for individual animals. Feed-to-gain ratio (**FGR**) was calculated as kilograms of DMI divided by kilograms of BW gain.

**Table 1.** Diet composition and nutrient concentration

| Item | % of DM |
|---|---|
| Ingredient | |
| Corn silage | 43.0 |
| Alfalfa hay | 15.0 |
| Barley grain, rolled | 30.0 |
| Soybean meal | 7.0 |
| Feedlot supplement[1] | 5.0 |
| Nutrient | |
| CP | 12.2 |
| Ca | 0.81 |
| P | 0.29 |

[1]Composition (% of supplement DM): 5.0% NaCl, 0.24% Mg, 0.76% K, 200 ppm Cu, 400 ppm Mn, 650 ppm Zn, 2 ppm Se, 22 ppm I, 9 ppm Co, 121,000 IU/kg Vitamin A, 37,400 IU/kg Vitamin D, 55 IU/kg vitamin E, and 360 ppm Rumensin® (Elanco Animal Health, Indianapolis, IN).

*Statistical analysis.* All the data in this study were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC) with a repeated measures treatment structure. Pen was the experimental unit with monthly data collection periods as repeated measures of treatments. Treatment and period were fixed effects and pen a random effect. The Kenward-Roger option was used to estimate denominator degrees of freedom. Least squares means are reported, and differences were considered significant at $P < 0.05$.

## Results

The BW at the start and end of the experiment were not affected by FFE supplementation (Table 2). Intake of DM averaged 8.12 kg/d across the treatments, but supplementing the FFE to the growing diet did not affect DMI regardless of dose rate ($P = 0.83$). Body weight gain numerically increased with supplementing FFE (101 and 99 kg for LE and HE, respectively) compared to C (96 kg), although supplementing the FFE product failed to detect a significant effect ($P = 0.67$). Average daily gain throughout the experiment was 1.13 kg/d on average for all treatments, and FFE supplementation did not affect ADG ($P = 0.95$). In addition, enzyme did not influence FGR ($P = 0.98$).

**Table 2.** Performance of growing beef steers fed a corn silage-based TMR diet without or with a fibrolytic feed enzyme product

| | Dietary treatment[1] | | | | |
|---|---|---|---|---|---|
| Item | C | LE | HE | SE | *P* |
| Steers, n | 20 | 20 | 20 | | |
| Initial BW, kg | 290 | 289 | 282 | 7.3 | 0.33 |
| Final BW, kg | 385 | 386 | 382 | 5.3 | 0.69 |
| BW gain, kg | 96 | 101 | 99 | 4.7 | 0.67 |
| ADG, kg | 1.10 | 1.16 | 1.13 | 0.115 | 0.95 |
| DMI, kg/d | 8.12 | 8.09 | 8.16 | 0.064 | 0.83 |
| Feed-to-gain ratio, kg/kg | 8.42 | 8.50 | 8.25 | 0.906 | 0.98 |

[1]C = control diet without enzyme supplementation; LE = diet with low level of enzyme supplementation (1 g/kg TMR); HE = diet with high level of enzyme supplementation (2 g/kg TMR).

## Discussion

A similar feed enzyme product to the FFE used in this study showed some positive effects on in vitro degradation of grass hay, but not alfalfa hay (Ranilla et al., 2007). Due to the small proportion of alfalfa hay used in this study (15% of DM), the alfalfa hay may not affect the efficacy of the FFE used. The positive effect of the FFE on grass hay in the previous study may be caused by endoglucanase activity in the FFE, which has been suggested as a key enzymatic activity to improve corn and grass silage degradation (Wallace et al., 2001; Eun and Beauchemin, 2007a,b). Providing key enzymatic activities in feed enzyme products is one of the most important requirements for feed enzyme additives to have positive effects on feed digestion and resultant animal performance. Additionally, the array of feed enzyme activities supplemented must be specific to the chemical composition of the targeted forage, due to the specificity of enzymes for their substrate (White et al., 1993). In addition to the activity of endoglucanase, exoglucanse activity has been suggested as another key enzymatic activity to improve corn silage degradation (Eun and Beauchemin, 2007a,b). One of the main characteristics of exoglucanases is that they act on cellulose chains in a progressive manner. They progress along the polymer chain while releasing cellobiose in a recurrent fashion (Tomme et al., 1996; Reverbel-Leroy et al., 1997), resulting in thinning of crystalline cellulose (Boisset et al., 2000). Although the FFE used in this study contained endoglucanase activity, it had no exoglucanase activity. Therefore, no positive effects of the FFE supplementation on animal performance in this feeding study may be resulted from the lack of the

exoglucanase activity in the FFE product used, which may be important for the degradation of more recalcitrant fiber, such as the corn silage used in this study.

## Implications

In vitro bioassays that reflect the conditions of the rumen are a good alternative to feeding studies to identify ideal feed enzyme candidates for use in ruminant diets, but the results must be confirmed in vivo. Designing feed enzyme additives that deliver the key enzymatic activities is challenging in order for feed enzymes to have consistently positive effects on feed digestion by ruminants, and it may be better to provide all the key enzyme activities in feed enzyme products rather than providing a single, key enzyme activity.

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