Proceedings

Western Section
American Society of Animal Science

Volume 62

Miles City Montana

June 21–23, 2011

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Executive

1. D. H. Crews, Jr., President (12, CSU)**
2. A. J. Roberts, President-Elect (13, USDA-LARRL)
3. G. C. Duff, Secretary-Treasurer (14, MSU)
4. G. E. Moss, Past-President (11, UW)
5. J. C. Whittier, ASAS Board Director (13, CSU)
6. C. K. Larson, Industry Director (12, Zinpro Corp.)
7. J. B. Taylor, A&C Chair (11, USDA-USSES)

Awards

1. A. J. Roberts, President-Elect (11, USDA-LARRL)**
2. T. E. Engle (11, CSU)
3. J. M. Thompson (11, OSU)
4. M. D. MacNeil (11, USDA-LARRL)
5. A. L. Van Eenennaam, (13, UC-Davis)
6. A. Ahmadzadeh (13, UI)

Symposium

1. S. I. Paisley (11, UW)**
2. J. B. Hall (11, UI)
3. A. M. Encinias (12, NMSU)
4. R. N. Funston (12, UNL)
5. J. R. Jaeger (12, KSU)
6. Brett Kirch (13, CSU)

Advising and Coordinating

1. J. B. Taylor (11, USDA-USSES)**
2. J. K. Ahola (11, CSU)
3. B. J. May (11, Angelo State)
4. M. P. Shipka (12, Alaska)
5. J. P. Bruemmer (12, CSU)
6. D. M. Hallford (13, NMSU)
7. J. B. Glaze (13, UI)
8. K. M. Cammack (13, UW)
9. R. Cockrum (13, UW) – Graduate Student Director

Paper Competition

1. D. L. Boss (11, MSU-Havre)**
2. H. L. Neibergs (12, WSU)
3. S. L. Ivey (12, NMSU)
4. R. L. Endecott (12, MSU)
5. R. K. Peel (12, CSU)
6. S. L. Lake (13, UW)
7. E. J. Scholljegerdes (13, NMSU)
8. C. Mueller (13, OSU)

Academic Quadrathlon

1. D. C. Rule (UW)**
2. J. B. Lamb (BYU-Idaho)
3. S. A. Sotto-Navarro (NMSU)
4. R. D. Weidemeier (USU)
5. H. Han (CSU)

Extension

1. R. L. Kott (11, MSU)**
2. J. B. Glaze (11, UI)
3. S. L. Lake (11, UW)
4. T. R. Whitney (12, TAMU)
5. C. P. Mathis (12, NMSU)
6. D. R. Zobell (13, USU)

Necrology

1. G. E. Moss, Past-President (11, UW)**

Nominating

1. G. E. Moss, Past-President (13, UW)**
2. R. A. Battaglia (12, UI)
3. K. C. Olson (11, SDSU)

BUSINESS MEETING MINUTES
Western Section, American Society of Animal Science
July 14, 2010
Denver Convention Center, Denver, CO

President Gary Moss called the meeting to order at 7:30 AM.

Acceptance of 2009 WSASAS Minutes.
After a call for additions or amendments, minutes of the 2009 WSASAS Business Meeting were accepted as printed in the 2010 Western Section ASAS Proceedings.

2009 Financial and 2010 Meeting Reports.
Andy Roberts, Secretary-Treasurer, USDA, ARS, Fort Keogh LARRL, Miles City, MT

The WSASAS financial report as of December 31, 2009 was summarized. In the 2009 calendar year, the Section total revenue was $67,023.07 and total expense was $67,799.92, leaving a balance of $55,119.98. The detailed report is included in these minutes as an appendix.

The 2010 Western Section ASAS meeting was held in conjunction with the Joint Annual Meeting (JAM) of ASAS, ADSA, AMPA, CSAS, and PSA. Total registration for the JAM meeting was 3963 (4638 including spouses). There were 69 papers accepted for publication in the Proceedings of the WSASAS: 21 Graduate Student Competition Papers, 26 other oral presentations and 22 posters. The awards luncheon was attended by 92 individuals.

Necrology Committee Report.
Dick Battaglia, Past-President, University of Idaho (Presented by Andy Roberts, Secretary-Treasurer)

Two WSASAS members passed away during 2008-2010:
1. David Porter Price, Las Cruces, New Mexico
2. Dr. Venerand (Vennie) Nayigihugu,University of Wyoming

The report was followed by a moment of silence in memory of our deceased members.

Nominating Committee Report.
Dick Battaglia, Past-President, University of Idaho (Presented by Andy Roberts, Secretary-Treasurer)

Committee Members
1. R. Battaglia, Past President, University of Idaho
2. K.C. Olson, South. Dakota State University
3. Tim Ross, New Mexico State University

Nominees for 2010 WSASAS elections were:

President-Elect:	Andy Roberts, USDA-ARS-LARRL, Miles City, MT
Secretary-Treasurer:	Glenn, Duff, University of Arizona
ASAS Western Section Director:	Jack Whittier, Colorado State University
Graduate Student Representative:	Rebecca Cockrum, University of Wyoming
	Megan Van Emon, North Dakota State University

Election results were:

President-Elect:	Andy Roberts, USDA-ARS-LARRL, Miles City, MT

Secretary-Treasurer:	Glenn, Duff, University of Arizona
ASAS Western Section Director:	Jack Whittier, Colorado State University
Graduate Student Representative:	Rebecca Cockrum, University of Wyoming

Extension Symposium Committee Report.
Not applicable this year

Beef Symposium Committee Report.
Not applicable this year

Academic Quadrathlon Committee Report.
Dan Rule, University of Wyoming

Committee Members:
1. Dan Rule, University of Wyoming, Chair
2. Sergio Soto-Navarro, New Mexico State University
3. Hyungchul Han, Colorado State University
4. Randy Weidemeier, Utah State University
5. Jim Lamb, BYU – Idaho

The 2010 Western Section Academic Quadrathlon contest was held on the Utah State University campus. Dr. Brett Bowman with the assistance of Ms. Tami Spackman organized this year's event. Participating schools included Utah State University (Brett Bowman, Advisor), New Mexico State University (Kali Benson, advisor), Colorado State University (Scott Howard, Advisor), Oregon State University (Matt Kennedy, advisor), BYU-Idaho (Dr. Jim Lamb and Willy Twitchell, Advisors). Order of placement in the written exam was USU, CSU, OSU, NMSU, and BYU-I. Order of placement in the oral presentations was CSU, OSU, USU, BYU-I, and NMSU. Placement in the laboratory practicum was USU, NMSU, BYU-I, OSU, and CSU. Order of placement in the quiz bowl was CSU, OSU, USU, BYU-I, and NMSU. Order of overall placement was USU, CSU, OSU, NMSU, and BYU-I. Utah State University will be invited to the National AQ contest. Participants from each school were:

Utah State University: Jaci Fasselin, Scott Heins, Tara Roche, Kristin Sittner.
New Mexico State University: Jim Armendariz, Jovani Armendariz, Scott Gutierrez, Patrick Pachta.
Oregon State University: Ruben Mendoza, Kirby Flynn, Sam Moxley, Jaime Senthirajah.
BYU-Idaho: Benjamin Baird, Jill Searle, Cari Berrett, Shaun Harris.
Colorado State University: Blake Aiton, Will Callis, Mike Ochs, Dani Shubert.

The 2011 WS AQ contest will be held in conjunction with the WS ASAS meetings at Miles City, MT.

Awards Committee Report.
Denny Crews, President-Elect, Colorado State University.

Committee Members:
1. D. H. Crews, Jr., Colorado State University
2. M. Du, University of Wyoming
3. M. E. Doumit,, University of Idaho
4. T. E. Engle, Colorado State University
5. J. M. Thompson, Oregon State University
6. M. D. MacNeil, USDA-ARS-LARRL, Miles City, MT

Distinguished Service Award
Recipient: Dr. Charles T. Gaskins, Washington State University
Sponsor: DSM Nutritional Products, Inc.
c/o Scot Williams and Yvonne Towns
45 Water View Blvd.
Parsippany, NJ 07054-1298
Nominator: Dr. Denny Crews, Colorado State University

Distinguished Teaching Award
Recipient: Dr. Amin Ahmadzadeh, University of Idaho
Sponsor: Elanco Animal Health
c/o Dr. Todd Armstrong
2001 W. Main Street
PO Box 708
Greenfield, IN 46140-2714
Nominator: Drs. Matt Doumit and J. Benton Glaze, University of Idaho

Extension Award
Recipient: Dr. Alison Van Enennaam, University of California, Davis
Sponsor: Western Section ASAS
Nominator: Dr. Anita Oberbauer, University of California, Davis

Young Scientist Award
Recipient: Dr. Clint Loest, New Mexico State University
Sponsor: Western Section ASAS
Nominator: Dr. Dennis Hallford, New Mexico State University

Drs. Gary Moss and Denny Crews presented awards at the banquet on July 13. Denny Crews thanked all who submitted nominations and encouraged nominators to get to work early and nominate our deserving colleagues in 2011.

Applied Paper Awards.
Connie Larson, Chair

Results of the Applied Paper Competition were presented at the banquet on July 13 . The Applied Animal Science Paper Awards were presented to:

1st place Summers, A. F., K. H. Ramsay, and R. N. Funston. University of Nebraska West Central Research and Extension Center. Influencing steer performance through maternal nutrition.

2nd place Funston, R. N., J. L. Martin, A. F. Summers, D. C. Adams, and D. M. Larson. University of Nebraska West Central Research and Extension Center. Paper title: Winter grazing system and supplementation of beef cows during late gestation influence heifer progeny.

3rd place Martinez-Pérez, M. F., D. Calderón, F. Loya-Holguin, A. Soto-Gaspar De Alba, C. Murdock, A. M. Encinias, and S. A. Soto-Navarro. New Mexico State University. Paper title: Supplemental corn dry distillers grains plus solubles on performance of steers grazing native range.

Graduate Student Competition Committee Report .
Kristi Cammack, University of Wyoming

Committee Members:
1. Kristi Cammack, University of Wyoming, Chair
2. Darrin Boss, Montana State University
3. Cory Parsons, Oregon State University
4. Shanna Ivey, New Mexico State University
5. Rachel Endecott, Montana State University
6. Holly Neibergs, Washington State University

The committee nominated Darrin Boss (Montana State University) as committee chair for 2011. Cory Parsons had not participated in this committee to-date. Therefore, the committee recommends Chad Mueller of Oregon State University as a replacement for Parsons. Kristi Cammack completed her 3-year term. The committee recommends Scott Lake (University of Wyoming) as the replacement committee member.

For the first time, abstracts were submitted to the respective discipline committee for acceptance or rejection prior to being seen by the GSPC Committee. The committee received 24 abstracts; 3 abstracts were not considered for the competition due to failure to meet proceedings deadlines or scheduling conflicts with the student. Therefore, 21 abstracts were considered for competition:

Eckerman (North Dakota State University)
Saevre (North Dakota State University)
Tracey (New Mexico State University)
Sproul (Kansas State University)
McDonald (Montana State University)
Macek (Kansas State University)
Eckerle (Kansas State University)
Nichols (Montana State University)
Sharman (Oklahoma State University)
Russell (New Mexico State University)
Carter (New Mexico State University)
Schutz (Colorado State University)
Sanchez (New Mexico State University)
Keithley (Montana State University)
Cooprider (University of California-Davis)
McCosh (Montana State University)
May (Oregon State University)
Moriel (University of Wyoming)
Allen (University of Arizona)
McMurphy (Oklahoma State University)
Cruz (University of California-Davis)

Conflicts of interest were identified; those with conflicts of interest did not participate in judging and discussion of the respective paper/presentation. Results of the GSPC were tabulated and awards were presented at the banquet on July 13. Individual awards for the Graduate Student Competition were:

1st place	Philip Moriel	University of Wyoming
2nd place	Casey McMurphy	Oklahoma State University
3rd place	Morgan Russell	New Mexico State University

Institutional Award. The institutional award for highest average score with 2 or more contestants was presented to New Mexico State University. This award is made possible by the generous support of Zinpro by Connie Larson from Zinpro Corporation. WSASAS expresses it's gratitude to Zinpro and Connie Larson for their continued support of the Graduate Student Competition and Institutional Awards.

Advisory and Coordinating Committee Report.
Glenn C. Duff, University of Arizona

Committee Members:
1. G. C. Duff, University of Arizona, Chair
2. P. A. Ludden, University of Wyoming
3. J. G. P. Bowman, Montana State University
4. C. J. Mueller, Oregon State University
5. R. Mark Enns, Colorado State University
6. J. R. Carpenter, University of Hawaii
7. A. J. Roberts, USDA-ARS, Miles City
8. J. K. Ahola, University of Idaho
9. J. B. Taylor, USDA-ARS-USSES, Dubois, ID
10. B. J. May, Angelo State University
11. M. P. Shipka, U. Alaska
12. J. E. Bruemmer, CSU

The only item brought before the Advising and Coordinating Committee was to consider changing the bylaws to add the Graduate Student Representatives to the Executive board. Members that responded were in favor of the consideration. The Committee recommended that actions be implemented to allow changing of the by-laws next year so that The Graduate Student Representatives could serve on the Executive Committee, similar to ASAS and some other sections. The chair requested input from the committee on any additional items and none were brought forth.

Report from the ASAS President.
Jim Oltjen, ASAS President
Meghan Wulster-Radcliffe, ASAS Executive Director

Dr. Oltjen, ASAS President, and Dr. Meghan Wulster-Radcliffe, ASAS Executive Director, summarized progress on the 5 Year Strategic Plan and reported on activities and plans of the American Society of Animal Science at the national level. Some items discussed included: 5% increase in membership over last 4 years; over 5000 individual memberships, of which 17 % are from outside the US; and a large increase in graduate student memberships. Items associated with the Journal of Animal Science included: currently has the highest impact factor ever; a decrease of 30 days for time of submission to publication; ½ of submissions being from outside the US; a decrease of 10%/yr for institutional subscriptions, yet the Journal is making money. Efforts to improve services and thereby increase membership included providing numerous webinars, development of new ways to recognize extension and teaching, looking into establishing bundled memberships and expanding international opportunities. President Oltjen reminded everyone of future Joint Annual Meetings in New Orleans (2011), Phoenix (2012), and Indianapolis (2013).

Transfer of the Gavel.
Gary Moss transferred the WSASAS Presidency to Denny Crews and Past-President Gary Moss was presented with the Presidential plaque.
The 2010 Western Section Business Meeting was adjourned by President Crews at 9 AM.

APPENDIX

WSASAS Detailed Financial Report: December 31, 2009.

Andy Roberts, Secretary-Treasurer

	Actual @ 12/31/09
Balance as of January 1	***$55,896.83***
Revenue and Support	
Dues-ASAS	3,390.10
Registrations	31,210.00
Donations-Awards	4,250.00
Donations-General	250.00
Donations-Symposium	8,245.00
Symposium Support-ASAS	3,000.00
Proceedings	11,420.85
Miscellaneous	
Investment Earnings Gain(Loss)	5,257.12
Total Revenue and Support	***67,023.07***
Expenses	
Programs/ Registration	236.11
Awards/Plaques	6,352.00
Quadrathalon	3,600.00
Convention Center	32,115.36
Travel-Speaker	1,266.85
Travel-Staff	1,523.88
Proceedings	2,034.71
Postage, Shipping & Supplies	200.02
Writer's Workshop	1,425.99
Symposium	2,277.13
Miscellaneous	5,839.20
Insurance	212.17
Telephone	96.86
General Printing	42.24
Staff Support	10,577.40
Total Expenses	***67,799.92***
Net Revenue over Expense	***(776.85)***
Balance as of December 31	***$55,119.98***

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ADMINISTRATION OF GNRH ON DAY 9 OF A 14-DAY CIDR WITH CO-SYNCH 72 H IN LACTATING BEEF COWS

R. L. Giles[1], J. T. French[1], P. E. Repenning[1], J. K. Ahola[1], J. C. Whittier[1], G. E. Seidel Jr.[2], and R. K. Peel[1]
[1]Department of Animal Sciences, Colorado State University, Fort Collins, 80523
[2]Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, Colorado 80523

ABSTRACT: Most progestin-based estrous synchronization protocols focus on inducing one new follicular wave before progestin removal. Our objective was to determine the effectiveness of an extended controlled internal drug release (**CIDR**) protocol with 2 induced follicular waves. Lactating beef cows at 3 locations (n = 247, n = 395; n = 137) were randomly assigned to 3 treatment groups. Cows in the 14-d 50 PG group received a CIDR (1.38 g progesterone) insert and 100 µg GnRH im on d 0, 100 µg GnRH im on d 9, CIDR removal and 50 mg $PGF_{2\alpha}$ im on d 14, and 100 µg GnRH im with Timed-AI (**TAI**) 72 ± 3 h later. Cows in the 14-d 6 h PG group were assigned to the same protocol as the first group except that 25 mg $PGF_{2\alpha}$ im was given at CIDR removal on d 14, plus 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later. Cows in the control group (5-d CO-Synch + CIDR) received a CIDR insert and 100 µg GnRH im on d 0, CIDR removal and 25 mg $PGF_{2\alpha}$ im on d 5, 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later, and 100 µg GnRH im with TAI 72 ± 3 h after CIDR removal. Body condition scores for Tx 1, 2, and 3 averaged 4.9 ± 0.73, 4.9± 0.65, and 4.9 ± 0.72 (SD). Post partum interval at TAI ranged from 38 to 115 d. Pregnancy status was determined on d 37 to 40 by ultrasonography. Averaged over all locations, pregnancy rates for 14-d 50 PG, 14-d 6 h PG, and 5-d CO-Synch + CIDR protocols were 58.2, 46.8, and 41.9% respectively. Pregnancy rates were higher in 14-d 50 PG group than the 14-d 6 h PG and 5-d CO-Synch + CIDR group ($P < 0.05$, GLIMMIX). Cycling status at 2 locations (n = 243; n = 391) was determined from blood collected on d -7 and d 0; progesterone levels >1 ng/mL at either bleed (or both) were considered cycling. Averaged over the 2 locations, pregnancy rates by cycling status did not interact with treatments ($P > 0.05$) (14-d 50 PG: cycling-79/117 (67.5%), not cycling-58/111 (52.3%); 14-d 6 h PG: cycling-59/92 (64.1%), not cycling-54/99 (54.6%); 5-d CO-Synch: cycling-53/99 (53.5%), not cycling-58/106 (54.7%). Overall conception rates were higher for 14-d 50 PG group than the 14-d 6h PG and 5-d CO-Synch protocols.

Key Words: CIDR, Timed-AI, PGF2α

Introduction

Recent estrous synchronization protocols for Timed-AI (TAI) such as 5-d CO-Synch + CIDR have achieved conception rates up to 70% in beef cows (Bridges et al., 2008; Gunn et al., 2009). These conception rates to TAI are higher than previous estrous synchronization protocols used on beef cows, but a second $PGF_{2\alpha}$ injection 8 ± 2 h after controlled internal drug release (CIDR) removal is required. This and similar protocols only attempt to induce one new follicular wave with GnRH at the initiation of the protocol, which does not adequately address the stage of the estrous cycle when they aren't responsive to GnRH. Approximately 66% of cows are in a stage where GnRH will ovulate, luteinize, or regress the follicle and induce a new follicular wave, likely resulting in a drop in pregnancies to TAI in animals that did not respond to the GnRH (Geary et al., 2000).

Presynchronization with progestins has added benefits in inducing normal cyclicity in anestrous cows plus added synchrony of estrous cycles, but these protocols tend to be extensive in labor and time (Perry et al., 2004; Smith et al., 1987). The need to set up a new follicular wave after progestin removal lengthens these protocols because of the poor fertility of oocytes in persistent follicles resulting from long term progestin influence (Patterson et al., 1989; Kojima et al., 1992).

The first objective was to assess the ability to increase conception rates to TAI by setting up 2 follicular waves within a 14 d progestin program by giving GnRH at the beginning of the CIDR protocol, and again 9 d later. With this protocol, we hypothesized that a higher number of cows will be responsive to the GnRH injection prior to progestin removal. This protocol also mimics the dynamics of the 5-CO-Synch + CIDR by giving the second GnRH injection 5 d prior to CIDR removal. The second objective was to evaluate the ability of the 14 d CIDR protocol to induce cyclicity in anestrous cows without a prolonged presynchronization period. The final objective was to assess the efficacy of giving a single 50 mg dose of $PGF_{2\alpha}$ at CIDR removal compared to a 25 mg dose with another 25 mg dose 6 h later when using a 14 d CIDR protocol.

Materials and Methods

Animals. All experimental procedures with animals were approved by the Colorado State Animal Care and Use Committee.

Multiparous and primiparous Angus, Angus cross, and Hereford cross beef cows at 3 ranches in Wyoming and Colorado (RC; n = 247, BIC; n = 395, Max; n = 137) were randomly assigned to 3 treatments (Fig 1). At BIC, treatment groups were blocked by age and post partum interval (**PPI**). At RC and Max locations, treatments were randomly assigned by ear tag ID. All animals were body condition scored on d 9 of protocols. The day of initial GnRH injection and CIDR insertion was considered d 0.

Cows in the 14-d 50 PG group received a CIDR (1.38 g progesterone, EAZI-BREED CIDR®, Pfizer Animal Health, NewYork, NY,) insert and 100 µg GnRH im (Factrel®, Fort Dodge Animal Health, Fort Dodge, IA) on d 0, 100 µg GnRH im on d 9, CIDR removal and 50 mg $PGF_{2\alpha}$ im (Lutalyse®, Pfizer Animal Health) on d 14, and 100 µg GnRH im with TAI 72 ± 3 h after CIDR removal. Cows in 14-d 6 h group received a CIDR insert and 100 µg GnRH im d 0, 100 µg GnRH im on d 9, CIDR removal and 25 mg $PGF_{2\alpha}$ im on d 14, 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later, and 100 µg GnRH im with TAI 72 ± 3 h after CIDR removal. Cows in the control group (5-d CO-Synch + CIDR) received a CIDR insert and 100 µg GnRH im on d 0, CIDR removal and 25 mg $PGF_{2\alpha}$ im on d 5, 25 mg $PGF_{2\alpha}$ 6 ± 1 h later, and 100 µg GnRH im with TAI 72 ± 3 h after CIDR removal (Fig 1). For all treatments, estrus detection patches (Estrotect®, Estrotect, Inc., Spring Valley, WI) were placed on the tail head of cows upon CIDR removal for detection of estrus starting 36 h after CIDR removal at 12 h intervals and continuing until TAI at RC and BIC locations; this was not done at the Max location. BCS for treatment groups 14-d 50 PG, 14-d 6 h PG, and 5-d CO-synch + CIDR averaged 4.8 ± 0.05, 4.9 ± 0.04, and 5.0 ± 0.05 respectively. PPI at TAI for 14-d 50 PG, 14-d 6 h PG, and 5-d CO-Synch + CIDR treatments groups averaged 76 ± 1.1, 76 ± 1.2, and 76 ± 1.2, respectively, with a range of 38 to 115 days.

Blood Collection. Reproductive cycling status was determined from blood obtained by coccygeal venipuncture on d -7 and 0 for serum concentrations of progesterone. Blood was collected in 10 mL serum vacutainer tubes (BD Vacutainer™, Becton, Dickinson and Company, Franklin Lakes, NJ) and placed directly on ice within 10 min after collection. Samples were centrifuged at 2800 x *g* for 10 min within 8 h after collection, and stored at -20 °C until analysis for progesterone concentration could be completed. Concentrations of progesterone were determined using the RIA procedure as previously described (Niswender et al., 1973). Cows with progesterone levels greater than 1 ng/mL at either bleeding date (or both) were identified as cycling at the initiation of protocols, and cows with progesterone levels less than 1 ng/mL for both bleeding dates were identified as anestrus at initiation of protocols. Inter-assay and intra-assay CV were 9.3% and 3.1%, respectively. Average sensitivity of assays was 0.03 ng/mL. Blood collection was only feasible at RC and BIC locations.

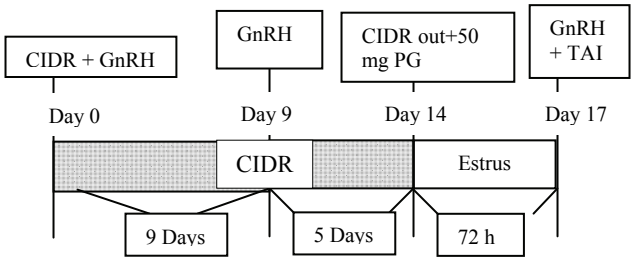
Pregnancy Diagnosis. TAI Pregnancy was status was diagnosed between d 37 and 40 by transrectal ultrasonography (3.5 MHz linear transducer GP-DV, E.I. Medical, Loveland, CO). Cows were exposed to intact bulls 10 days after TAI at all locations.

Statistical Analysis. Data were analyzed using the GLIMMIX procedure in SAS (SAS Inst., Inc., Cary, NC). The final model included location, treatment, location x treatment, age (primiparous and multiparous), BCS, and PPI at TAI. TAI pregnancy rates (Table 2) are presented as least squares means.

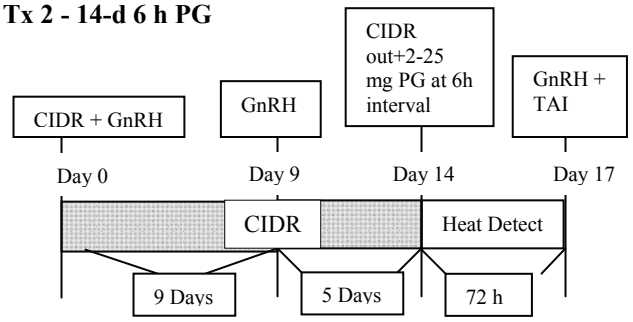
Results

Numbers of cows, PPI at TAI, BCS, and age are presented in Table 1. PPI did not differ amongst treatments within location ($P > 0.1$), but differed by location ($P < 0.01$). Similarly, BCS did not differ amongst treatments within location ($P > 0.1$), but differed by location ($P < 0.01$). Pregnancy rates to TAI by treatment and location are presented in Table 2. The pregnancy rates did not differ between treatments ($P > 0.1$) at RC and BIC loctions (Table 2). At the Max location, pregnancy to TAI was higher ($P < 0.01$) in the 14-d 50 PG group (60.7%) than 14-d 6h PG (32.0%) and 5- d CO-Synch + CIDR (26.5%). Averaged over all 3 locations, pregnancy rates to TAI for 14-d 50 PG, 14-d 6h PG, and 5-d CO-Synch + CIDR protocols were 58.2, 46.8, and 41.9% respectively (Table 2). Pregnancy rates to TAI for the 14-d 50 PG were higher than both the 14-d 6 h PG and 5-d CO-Synch + CIDR groups ($P < 0.05$).

Tx 1 - 14-d 50 PG



Tx 2 - 14-d 6 h PG



Tx 3 - 5-d CO-Synch + CIDR

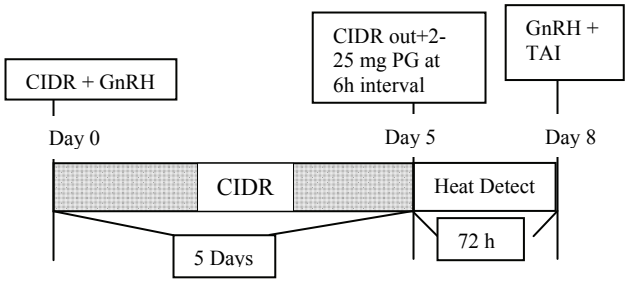


Figure 1. Design for treatment groups; the 14-d 50 PG and 14-d 6 h PG vary in that 14-d 50 PG get a double dose of $PGF_{2\alpha}$ (PG; 50 mg Lutalyse im) at CIDR removal and the 14-d 6 h PG cows receive 25 mg $PGF_{2\alpha}$ at CIDR removal and another 25 mg $PGF_{2\alpha}$ 6 ± 1 h later. Cows in the control group were enrolled into the 5- CO-Synch + CIDR protocol with GnRH + TAI at 72 h after CIDR removal.

Table 1. Post partum interval (PPI), BCS, and age (parity) of cows

Location and Tx	No.	PPI	BCS	Age	
				Primiparous (2 yr)	Multiparous (>3 yr)
RC					
14-d 50 PG	103	80 ± 1.7	5.0 ± .07	21	82
14-d 6h PG	70	82 ± 2.03	4.9 ± .08	18	52
5-d CO-Synch	74	82 ± 2.0	5.1 ± .08	23	51
Total	247	81 ± 1.1[a]	5.0 ± .05[a]	62	185
BIC					
14-d 50 PG	128	75 ± 1.9	4.3 ± .04	23	105
14-d 6h PG	133	75 ± 2.0	4.6 ± .04	18	115
5-d CO-Synch	134	74 ± 1.7	4.7 ± .05	18	116
Total	395	75 ± 1.1[b]	4.5 ± .03[b]	59	336
Max					
14-d 50 PG	47	71 ± 2.3	5.6 ± .07	4	43
14-d 6h PG	46	73 ± 2.04	5.6 ± .07	6	40
5-d CO-Synch	44	72 ± 2.5	5.6 ± .08	6	38
Total	137	72 ± 1.3[c]	5.6 ± .04[c]	16	121
Overall					
14-d 50 PG	278	76 ± 1.1	4.8 ± 0.05	48	230
14-d 6h PG	249	76 ±1.2	4.9 ± 0.04	42	207
5-d CO-Synch	252	76 ± 1.2	5.0 ± 0.05	47	205
Total	779	76 ± 0.7	4.9 ± 0.003	137	779

[abc] Mean without common superscript letter differ within a column ($p<0.01$).

For RC and BIC, averaged over the 2 locations, pregnancy rates by cycling status did not interact with treatments ($P > 0.1$) (14-d 50 PG: cycling-79/117 (67.5%), not cycling-58/111 (52.3%); 14-d 6 h PG: cycling-59/92 (64.1%), not cycling-54/99 (54.6%); 5-d CO-Synch: cycling-53/99 (53.5%), not cycling-58/106 (54.7%)). Early post partum cows (PPI < 45) had reasonable pregnancy rates (14-d 50 PG: 13/24 (54.2%), 14-d 6 h PG: 10/19 (52.6%), 5-d CO-Synch + CIDR: 17/20 (85.0%)). At RC and BIC locations, few cows showed signs of estrus at 36 and 48 h after CIDR removal before TAI. No cows at either location came into estrus at 36 h after CIDR removal. Those that initiated estrus at 48h still had adequate pregnancy rates to TAI. (14-d 50 PG: 6/11 (54.5%), 14-d 6 h: 8/12 (66.7%), 5-d CO-Synch + CIDR: 5/8 (62.5%)).

Discussion

As previously mentioned, response to the initial GnRH injection of most short term CIDR-based estrous synchronization protocols is a key factor in setting up a follicle to ovulate a fertile oocyte upon CIDR removal for maximizing conception rates to TAI. The ability to increase likelihood of cows responding to the GnRH has been well documented with presynchronization of estrous cycles (Perry et al., 2004; Shafer et al., 2006; Leitman et al., 2009) and has been proven to increase conception rates in beef cows and heifers. That concept was used in the current experiment with a different approach by inclusion of 2 GnRH injections within 14 d of progestin influence. The idea was that cows having a follicle responsive to GnRH at day 0 would ovulate that follicle and start a new follicular wave. They would then have a new responsive follicle to GnRH at d 9 as well, and ovulating this follicle under progestin influence would set up another wave until CIDR removal on d 14. We expected cows that did not respond to the initial GnRH at d 0 to then have a responsive follicle to GnRH by d 9, and thus set up another wave to ovulate after CIDR removal. In the present study, the potential to induce 2 follicular waves within a 14 d CIDR protocol resulted in promising pregnancy rates to TAI when compared to a recommended estrous synchronization protocol, the 5-d CO-Synch + CIDR. In pooled data from all 3 locations, there were higher pregnancy rates to TAI in the 14-d 50 PG (58.2%) than the 5-d CO-Synch + CIDR group (41.9%). However, the high differences in treatment means at Max location had significant effects on the overall data analysis. This location also had the lowest number of animals.

Postpartum anestrus in beef cows results in infertility and poor responses to certain estrous synchronization protocols (Perry et al., 2004; Short et al., 1990). However, at the 2 locations that cycling status was determined, pregnancy to TAI in previously anestrus cows were similar amongst treatments. The final comparison evaluated the efficacy of a single 50 mg dose of $PGF_{2\alpha}$ in the 14-d 50 PG group compared to 2-25 mg doses at 6 h intervals in the 14-d 6 h group. The analysis showed that at RC and BIC locations there were no significant differences between

Table 2. TAI pregnancy least squares means by treatment

Location and Tx	n	TAI PR[a] (%)
RC		
14-d 50 PG	103	54.7
14-d 6h PG	70	60.5
5-d CO-Synch	74	47.0
BIC		
14-d 50 PG	128	59.1
14-d 6h PG	133	48.6
5-d CO-Synch	134	54.0
Max		
14-d 50 PG	47	60.7[x]
14-d 6h PG	46	32.0[y]
5-d CO-Synch	44	26.5[y]
Overall		
14-d 50 PG	278	58.2[x]
14-d 6h PG	249	46.8[y]
5- CO-Synch	252	41.9[y]

[a] PR = Pregnancy rate to TAI determined at 37 to 40 d
[x,y,z] Percentages within location and overall with different superscripts differ ($P < 0.05$).

these 2 groups in ensuring pregnancy rates to TAI ($P > 0.1$); at the Max location pregnancy rates to TAI were higher in the 14-d 50 PG group than the 14-d 6 h group ($P < 0.05$).

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CAMELINA MEAL SUPPLEMENTATION TO BEEF CATTLE: I. EFFECTS ON PERFORMANCE, DMI, AND ACUTE-PHASE PROTEIN RESPONSE OF FEEDER STEERS FOLLOWING TRANSPORT

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ABSTRACT: Sixty Angus x Hereford steers were ranked by BW on d -28 of the study and allocated to 20 drylot pens, which were randomly assigned to receive: 1) supplement containing (as-fed basis) 84 % corn, 14 % soybean meal, and 2 % mineral mix (CO) offered during preconditioning (PC; d -28 to 0) and feedlot receiving (FR; d 1 to 29), 2) supplement containing (as-fed basis) 70 % corn, 28 % camelina meal, and 2 % mineral mix (CAM) offered during PC and FR, 3) CAM offered during PC and CO offered during FR, 4) CO offered during PC and CAM offered during FR. Treatments were offered daily at a rate of 2.20 and 2.04 kg of DM/steer for CO and CAM, respectively. Alfalfa-grass hay was offered ad libitum during the study. On d 0, steers were loaded into a commercial livestock trailer, transported for 24 h, and returned to the research facility (d 1). Total DMI was evaluated daily, and shrunk BW was collected on d -31, 1, and 30 for ADG calculation. Blood samples were collected on d 0 (prior to loading), 1 (immediately upon arrival), 4, 7, 10, 14, 21, and 29 for determination of plasma cortisol and haptoglobin. Rectal temperatures were recorded concurrently with blood sampling on d 0, 1, 4, and 7. During PC, CAM steers tended to have reduced (P = 0.10) ADG compared to CO (0.26 vs. 0.37 kg/d, respectively). No treatment effects were detected (P > 0.16) for ADG during FR and total ADG. Steers receiving CAM during PC had reduced total DMI during PC and FR compared to CO cohorts (3.07 vs. 3.35 % of BW during PC, and 3.20 vs. 3.35 % of BW during FR, respectively). Steers receiving CAM during PC had reduced mean haptoglobin concentrations vs. CO cohorts on d 0 and 1 (1.64 vs. 1.79 absorbance at 450 nm × 100, respectively). Steers receiving CAM during FR had reduced (P = 0.02) mean haptoglobin and rectal temperatures during FR compared to CO cohorts (1.69 vs. 2.02 absorbance @ 450 nm × 100 of haptoglobin, and 39.05 vs. 39.14 ºC for temperature, respectively). In conclusion, camelina meal supplementation alleviated the acute-phase protein response stimulated by transport, but did not benefit performance of feeder steers.

Introduction

Three of the most stressful events encountered by a feeder calf are weaning, transportation, and feedlot entry. These events, which may occur together or in a short period of time, lead to physiological, nutritional, and immunological changes that highly affect subsequent calf health and feedlot performance (Loerch and Fluharty, 1999). One example is the acute-phase response, an important component of the innate immune system that can be detrimental to growth rates in cattle (Qiu et al., 2007). Consequently, management strategies that prevent and/or alleviate the acute-phase response have been shown to benefit cattle productivity and overall efficiency of beef operations (Arthington et al., 2008).

Supplementation of a commercial source of polyunsaturated fatty acids (PUFA) to feeder calves prior to (Cooke et al. 2010) and after transportation (Araujo et al., 2010) reduced the acute-phase response during the initial days following transport, and benefited feedlot performance and carcass parameters (Cooke et al., 2010). Camelina meal, a byproduct from the mechanical processing of the camelina seeds for oil extraction, may contain up to 20% oil with the majority of the fatty acid content as PUFA (Moriel et al., 2010). Therefore, we theorized that camelina meal also serves as a sustainable nutritional alternative to modulate the acute-phase response in cattle subjected to stress of management. Based on this rationale, the objectives of the present study were to evaluate performance, physiological, and health parameters of feeder steers supplemented with camelina meal prior to and/or after transport to the feedyard.

Materials and Methods

This experiment was conducted in accordance with an approved Oregon State University Animal Care and Use protocol, and was divided into a preconditioning (PC; d -28 to 0) and a feedlot receiving phase (FR; d 1 to 29). Both phases were conducted at the Eastern Oregon Agricultural Research Center, Burns. Sixty Angus x Hereford steers weaned at 7 mo of age (d -55) were ranked by initial BW (221 ± 28.51 kg) on d -28 of the study, and randomly allocated to 20 dry lot pens (3 steers/pen). Pens were assigned to 1 of 4 treatments (5 pens/treatment): 1) supplement containing (as-fed basis) 84 % corn, 14 % soybean meal, and 2 % mineral mix (CO) offered during PC (d -28 to 0) and FR (d 1 to 29), 2) supplement containing (as-fed basis) 70 % corn, 28 % camelina meal, and 2 % mineral mix (CAM) offered during PC and FR, 3) CAM offered during PC and CO offered during FR, 4) CO offered during PC and CAM offered during FR. Supplements were offered once a day (0700 h) at a rate of 2.20 and 2.04 kg of DM/steer for CO and CAM, respectively. Composition and nutritional profile of the supplements are described in Table 1. Supplement intakes were formulated to

be iso-caloric and iso-nitrogenous, whereas mixed alfalfa-grass hay was offered in amounts to ensure ad libitum access throughout the experiment. On the morning of d 0, steers were loaded into a commercial livestock trailer, transported for 24 h, and returned to the research facility (d 1). Total and forage DMI were evaluated daily (d -28 to 28), and shrunk BW was assessed on d -31, 1, and 30 for ADG calculation.

Table 1. Composition and nutrient profile of supplements offered during the study.

Item	CO	CAM
Ingredient, DM basis		
Corn, kg	1.82	1.39
Soybean Meal, kg	0.32	--
Camelina, kg	--	0.59
Mineral Salt, kg	0.06	0.06
Nutrient profile, DM basis		
DM, %	87.0	88
TDN, %	94	95
CP, %	14.7	15.6
NDF, %	9.6	14.7
Ether extract, %	4.5	9.8
Ca, %	0.1	0.3
P, %	0.4	0.5

Blood samples were collected on d 0 (prior to loading), 1 (immediately upon arrival), 4, 7, 10, 14, 21, and 29, via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin. Steer rectal temperature (RT) was measured at 30-min intervals with an automatic RT recording device during transport (Reuter et al., 2010), whereas on d 4 and 7 RT was measured with a digital thermometer (GLA M750 digital thermometer; GLA Agricultural Electronics, San Luis Obispo, CA) concurrently with each blood collection. All blood samples were harvested for plasma and stored at −80°C until assayed for concentrations of cortisol (Endocrine Technologies Inc., Newark, CA), and haptoglobin (Makimura and Suzuki, 1982).

Performance and physiological data were analyzed using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for PC performance contained the effects of PC treatment. Data were analyzed using pen(PC treatment) as the random variable. The model statement for FR performance contained the effects of PC treatment, FR treatment, and the resultant interaction. Data were analyzed using pen(PC × FR treatment) as the random variable. The model statement used for RT, cortisol, and haptoglobin data obtained on d 0 and 1 relative to transport contained the effects of PC treatment, day, and the resultant interaction because steers were assigned to their FR treatment after blood sampling on d 1. Data were analyzed using pen(PC treatment) as the random variable. Accordingly, the model statement used for RT, cortisol, and haptoglobin data obtained from d 4 to d 29 contained the effects of PC treatment, FR treatment, day, and all the resultant interactions. Data were analyzed using pen(PC × FR treatment) as the random variable. Results are reported as least square means and separated using LSD or PDIFF. Significance was set at $P \leq 0.05$. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected.

Results & Discussion

During the PC phase (Table 2), CAM steers had reduced (P < 0.01) forage and total DMI compared to CO cohorts. Accordingly, CAM steers tended (P = 0.10) to have reduced ADG during PC compared to CO cohorts. However, no treatment effects (P = 0.24) were detected on preconditioning G:F. These findings support previous studies from our research group indicating that PUFA supplementation reduced DMI in cattle, but did not impair feed efficiency parameters (Araujo et al., 2010; Cooke et al., 2010).

Table 2. Preconditioning performance of beef steers supplemented (CAM) or not (CO) with camelina meal.

Item	CAM	CO	SEM	*P* =
Forage DMI, % of BW	2.23	2.46	0.04	< 0.01
Total DMI, % of BW	3.07	3.35	0.04	< 0.01
ADG, [1] kg/d	0.26	0.37	0.04	0.10
G:F, [2] kg/kg	0.038	0.049	0.006	0.24

[1] Calculated using shrunk values obtained on d -31 and d 1.
[2] Calculating using total DMI and BW gain from d -28 to d 1.

During the FR phase (Table 3), steers that received CAM during PC had reduced (PC treatment effect; P < 0.01) forage and total DMI compared to steers that received CO during the same period (2.46 vs. 2.61 % of BW for forage DMI, and 3.20 vs. 3.35 % of BW for total DMI, respectively; SEM = 0.03). Feed intake during FR was not affected by FR treatment or the PC × FR treatment interaction (P > 0.20). Moreover, ADG during FR was also not affected by PC treatment, FR treatment, or the PC × FR treatment interaction (P > 0.21). However, steers that received CAM during PC tended (PC treatment effect; P = 0.10) to have improved G:F during the FR compared to steers that received CO during the same period (0.231 vs. 0.215 kg/kg of G:F, respectively; SEM = 0.006). No FR treatment or PC × FR treatment interaction were detected for G:F during the FR phase.

Regarding RT and blood samples collected on d 0 and 1, no PC treatment effects were detected (P > 0.56) for plasma cortisol concentrations (41.8 vs. 39.4 ng/mL for CAM and CO steers, respectively; SEM = 5.2) or RT (39.19 vs. 39.16 ºC for CAM and CO steers, respectively; SEM = 0.03). However, CAM steers had reduced (P = 0.04) haptoglobin concentrations compared to CO cohorts (1.65 vs. 1.80 absorbance at 450 nm x 100, respectively; SEM =

0.05). Regarding RT and blood samples collected after d 4, no main treatment effects (P > 0.51) or interactions (P > 0.11) effects were detected for plasma cortisol concentrations (Table 3). During the same period, mean RT and plasma haptoglobin concentrations were reduced (FR treatment effect; P = 0.02) for steers receiving CAM during FR compared to cohorts receiving CO (Figure 1).

These results suggest that, based on similar cortisol concentrations among treatment combinations, all steers experienced a similar stress challenge due to transport and feedlot entry (Crookshank et al., 1979; Sapolsky et al., 2000), whereas CAM supplementation modulated the stress-induced haptoglobin response. More specifically, steers receiving CAM during preconditioning had reduced haptoglobin concentration at the time of transport, whereas steers receiving CAM supplementation after transport had reduced haptoglobin concentrations during FR. Rectal temperature, another key component of the acute-phase response (Carroll and Forsberg, 2007) was also reduced for steers receiving CAM following transportation and feedlot entry. Similar to our previous effort (Cooke et al., 2010), PUFA supplementation during preconditioning improved feedyard performance of beef steers, as reported herein by the PC treatment effects detected on G:F during FR. On the other hand, PUFA supplementation during FR alleviated the concurrent acute-phase protein response, but did not benefit steer FR performance (Araujo et al., 2010).

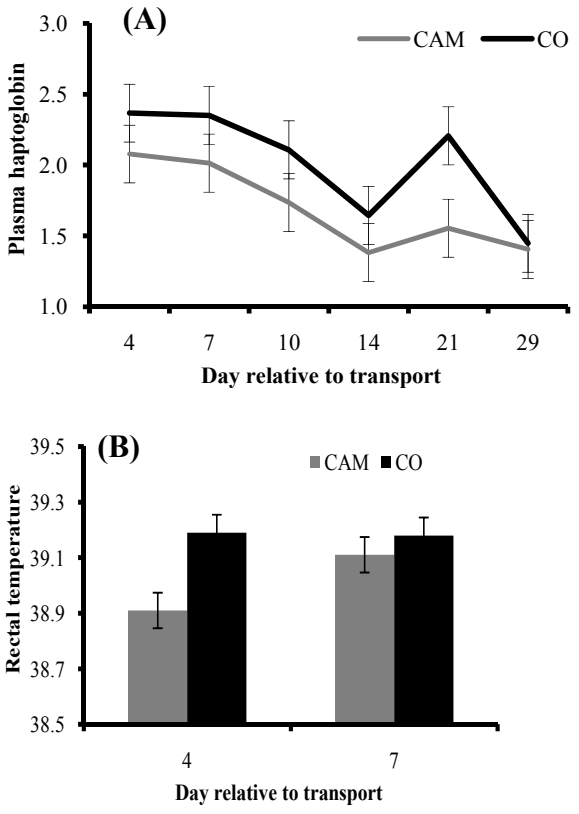

Figure 1. Plasma haptoglobin concentrations (Panel A; absorbance at 450 nm × 100) and rectal temperatures (Panel B; ºC) of steers transported to the feedlot on d 0, and supplemented (CAM) or not (CO) with camelina meal beginning on d 1 of the study. A treatment effect was detected (P = 0.02) for both variables.

Implications

Camelina meal supplementation alleviated the acute-phase protein response stimulated by transport and feedlot entry, but benefited, at least partially, feedlot performance of feeder steers if supplemented during preconditioning only.

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Table 3. Feedlot receiving performance and plasma cortisol concentrations of beef steers supplemented (CAM) or not (CO) with camelina meal during preconditioning and/or feedlot receiving.

Item[1]	**CAM-CAM**	**CO-CO**	**CAM-CO**	**CO-CAM**	**SEM**	***P* =**
Forage DMI, % of BW	2.50	2.63	2.42	2.59	0.05	0.20
Total DMI, % of BW	3.22	3.39	3.18	3.30	0.05	0.20
ADG,[2] kg/d	1.76	1.79	1.78	1.63	0.07	0.31
G:F,[3] kg/kg	0.225	0.221	0.237	0.210	0.009	0.99
Cortisol,[4] ng/mL	29.22	32.42	25.95	29.44	4.68	0.51

[1] Treatment description; first component refers to treatment provided preconditioning phase (CO or CAM), whereas second component refers to treatment provided during feedlot receiving phase (CO or CAM).
[2] Calculating using shrunk values obtained on d 1 and 30.
[3] Calculating using total DMI and BW gain from d 1 to d 28.
[4] Blood samples collected on d 4, 7, 10, 14, 21, and 29 relative to transport (d 0) and feedlot entry (d 1).

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EVALUATION OF RESIDUAL FEED INTAKE IN RAMS USING THE GROWSAFE SYSTEM

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ABSTRACT: The sheep industry has yet to fully investigate the effects of selecting for residual feed intake (RFI). For RFI to be an appropriate measure of feed efficiency, it must not be unfavorably correlated with growth traits, carcass merit, or reproductive efficiency. In cattle, it has been estimated that 63 d are needed to accurately estimate individual RFI. The aims of this study were to 1) determine if a relationship exists between individual RFI values and backfat (BF), loin eye area (LEA), body condition score (BCS), scrotal circumference (SC), and raw fleece weight (RFW), and 2) determine an adequate length of testing period necessary to accurately estimate individual RFI ranking. Rambouillet rams (n = 87) submitted to the Fall 2010 University of Wyoming Ram Test were evaluated for 140 d on the GrowSafe System. Twelve rams were removed from the study due to poor adaptation to the GrowSafe System, health complications, or missing data. The Growsafe System records individual animal intake data that can be used to evaluate individual RFI ranking. Rams were weighed on wk 2, 4, 6, and weekly thereafter, with the last weight recorded on wk 20. Overall and weekly RFI values were generated in SAS using GLM and MIXED procedures (repeated measures with an unstructured model), respectively. Correlation coefficients were estimated between RFI and BF, LEA, BCS, SC, and RFW using the CORR procedure. An alpha of 0.05 was assumed. There was no relationship ($P \geq 0.154$) between RFI values and BF, LEA, BCS, and RFW. However, SC tended ($P = 0.082$) to be positively correlated with RFI values. Weekly variation of RFI estimates was consistent from wk 7 through wk 15, and was lowest at wk 9 (d 63), suggesting that a testing period similar to that used in beef cattle (approximately 60 to 70 d) may be sufficient to estimate RFI in sheep. These preliminary results indicate that RFI ranking does not adversely affect carcass, growth, or fleece traits in sheep, and that performance test periods currently used in the University of Wyoming Ram Test are more than sufficient to estimate RFI.

Key Words: Performance test, Residual feed intake, Sheep

Introduction

Feed costs represent approximately 50-70% of total input costs for sheep producers (Nash, 1991). Furthermore, by 2050 the world population is expected to increase by 50% (U.S. Census Bureau, 2011). Producers face a growing dilemma to reduce current input costs while providing increased outputs (i.e. meat, milk, wool) with reduced resources (land and feedstuffs). A potential solution is to select for feed efficiency traits. In the past, producers have primarily focused on gain:feed; however, animals with similar ratios differ in their feed intake and rate of gain. As an alternative to gain:feed, Koch et al. (1963) proposed selecting for residual feed intake (RFI) as an indicator for feed efficiency. Currently, the swine, poultry, and cattle industries are investigating the effectiveness of selecting for feed efficiency using RFI. The sheep industry, however, has yet to fully investigate the impacts associated with selecting for RFI on carcass merit, growth traits, reproduction, and wool characteristics. For RFI to be an appropriate measure of feed efficiency in the sheep industry, it must not be unfavorably correlated with any of these traits. In addition to RFI's correlation to traits of economic importance, it is unknown how long data must be collected to best determine an individual animal's RFI value. Sainz and Paulino (2004) estimated that upwards of 60 d are required to accurately estimate RFI in cattle, and it is suspected that this will be similar in sheep. Therefore, the objectives of this research were to 1) evaluate if a relationship exists between RFI values and backfat (BF), loin eye area (LEA), body condition score (BCS), scrotal circumference (SC), and raw fleece weight (RFW), and 2) determine the appropriate length of time needed to accurately estimate individual RFI ranking in sheep. Data from the University of Wyoming Ram Test will be used to accomplish these objectives.

Materials and Methods

Animal procedures. All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. Rambouillet rams (n = 87; 5-8 mo age) submitted to the 2010 University of Wyoming Ram Test from area producers were evaluated for 140 d on the GrowSafe System (GrowSafe Systems Ltd., Airdrie, AB, Canada). The GrowSafe System used by the University of Wyoming was constructed specifically for sheep rather than using a modified cattle system. Rams were fitted with an electronic identification device (EID) in their ears. Each time a ram inserted his head into the bunk, the GrowSafe System scanned his EID to determine the amount of feed removed (i.e. consumed) and the time spent for each event. Rams were group-housed in one pen and had equal access

to eight GrowSafe nodes where they were fed *ad libitum*. Twelve rams were removed from the study due to poor adaptation to the GrowSafe System, health complications, or missing data. Feed intake information collected by the GrowSafe System was used to determine individual RFI values. Rams were fed a commercial diet formulated according to NRC recommendations. A forage-based pellet diet (15% CP) consisting of alfalfa, soyhulls, corn glutten, and wheat midds was fed throughout the test. During the first half of the Ram Test, pellets contained Bovatec, and during the second half contained 50 g/ton of Chlortetracycline. Rams achieved an ADG of 0.37 kg and a total average gain of 51.26 kg.

Rams were weighed on wk 2, 4, 6, and weekly thereafter, with the last weigh on wk 20. Upon cessation of the Ram Test, rams were shorn (d 142) for fleece analyses, and carcass and growth trait data (ultrasound BF, ultrasound LEA, BCS, and SC) were collected on d 147. Jugular blood was also collected for future DNA analysis using vacutainer tubes containing K^+ EDTA (Tyco Healthcare Group LP, Mansfield, MA) to prevent clotting.

Data analyses. Prediction equations were calculated using the GLM and MIXED procedures (repeated measures with an unstructured model used) in SAS (SAS Inst. Inc., Cary, NC) for both overall and weekly expected feed intakes (EFI), respectively. The model used to estimate EFI was:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon$$

where y = actual feed intake, β_0 = intercept, β_1 = average daily gain (ADG), β_2 = metabolic mid-weight ($MMWT^{0.75}$), and ε = residuals.

Residual feed intake (RFI) was calculated by the difference between actual feed intake and EFI. Relationships were determined between RFI and BF, LEA, BCS, SC, and RFW using the CORR procedure (Pearson method) in SAS, assuming an alpha of 0.05. Weekly and cumulative variation among RFI values was determined using the MEANS procedure in SAS. Differences in feed intake between the top and bottom 15% RFI ranked rams were calculated using a two-tailed t-test.

Results

Based on RFI ranking, the 15% most efficient rams consumed 22% less ($P < 0.001$) feed than the 15% least efficient rams. **Figure 1** illustrates the actual individual feed intakes compared to the expected feed intake, with RFI being the difference between the actual feed intake and the predicted feed intake. Rams above the predicted feed intake regression line were considered less efficient (higher RFI value), and rams below the predicted feed intake regression line more efficient (lower RFI value). Sequential weekly RFI estimates were highly correlated ($P < 0.001$) starting wk 4, suggesting that rankings are consistent after that time point.

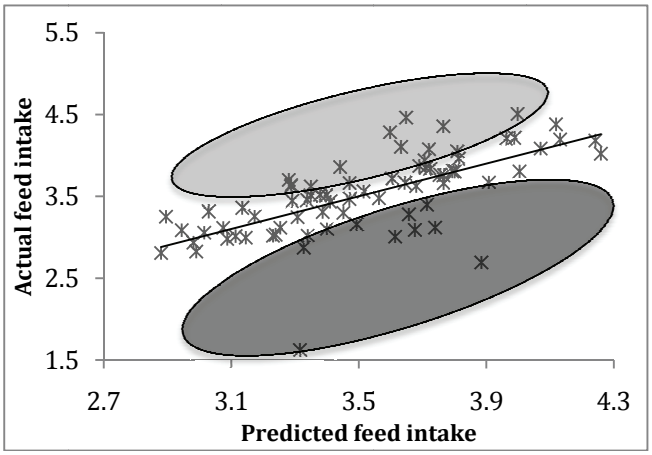

Figure 1. Comparison of actual versus predicted feed intake. Values above the regression line represent positive RFI values, and therefore less efficient animals. Values below the regression line represent negative RFI values, and therefore more efficient animals.

Phenotypic relationships between RFI and carcass traits (BF and LEA), growth traits (SC and BCS), and fleece characteristics (RFW) were determined (**Table 1**). There was no phenotypic correlation ($P \geq 0.154$) between RFI and BF, LEA, BCS, or RFW. Scrotal circumference tended ($P = 0.082$) to be positively correlated with RFI.

Table 1. Correlations between RFI and carcass, growth, and wool traits[1].

Item (units)[2]	Mean (n = 75)	SD	r[3]	*P*-value[4]
RFI (kg/d)	6.965^{-9}	0.37		
BF (cm)	0.688	0.13	-0.17	0.154
LEA (cm^2)	9.517	1.45	0.16	0.172
SC (cm)	87.523	5.16	0.21	0.082
BCS (1-5)	3.289	0.42	-0.04	0.754
RFW (kg)	1.772	0.22	0.10	0.405

[1]Pearson method used for all correlations.
[2]Residual feed intake (RFI), backfat (BF), loin eye area (LEA), scrotal circumference (SC), body condition score (BCS), raw fleece weight (RFW)
[3]Phenotypic correlation (r) of RFI with respective trait (BF, LEA, SC, BCS, RFW).
[4]Assume significance at $P < 0.05$.

To determine the time needed to collect intake data to accurately determine RFI, cumulative variances were generated. Furthermore, weekly variances were generated to determine the appropriate time needed to acclimate rams to the GrowSafe System. **Figure 2** illustrates both cumulative and weekly variances throughout the Ram Test. Both weekly and cumulative variation was consistent in wk 7 – 15, with the lowest variance seen in wk 9. However, variation increased after wk 9. Prediction of RFI using cumulative data through wk 9 also produced the highest R^2 (0.515), suggesting that the addition of data beyond this time point does not add accuracy to the RFI estimates. Both the variance and the R^2 results provide support that 60-70 d are needed to accurately determine individual RFI values in sheep, similar to beef cattle estimates. The reason for the greater variation after wk 15 is unknown, but

additional performance tests are expected to further deduce the optimal test duration for estimating RFI in sheep.

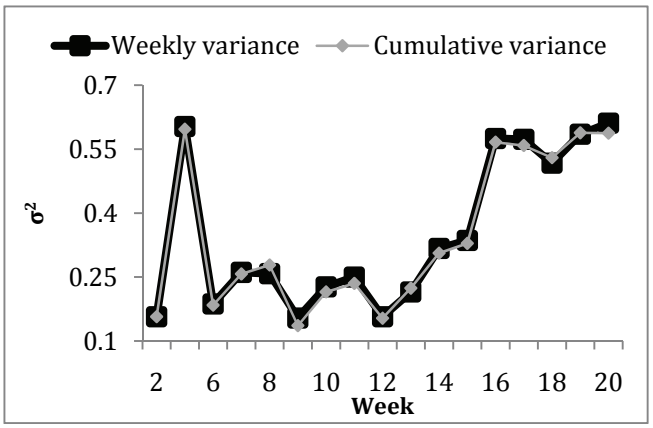

Figure 2. Weekly and cumulative variances of RFI. Week 9 (d 63) had the lowest weekly and cumulative variance.

Discussion

Residual feed intake is determined by the difference between actual feed intake and expected feed intake. When actual intakes are lower than the expected intake values (low RFI), the animal is considered more efficient. Conversely, when actual intakes are greater than the expected intake values (high RFI), the animal is considered less efficient.

Residual feed intake ranking. In this study, RFI variance was 0.37 kg^2/d^2. There was a difference of 2.84 kg/d between the least efficient ram and the most efficient ram. In cattle, 73% of RFI variation could be explained by heat production from metabolic processes, body composition, and physical activity (Herd and Arthur, 2009). Many of these processes have been further identified by microarray performed in low and high RFI cattle (Chen et al., 2008). Functional categories included cellular growth, protein synthesis, lipid metabolism, carbohydrate metabolism, cancer, drug metabolism, and small molecular biochemistry. Similar explanations for the variation in RFI observed among sheep would be expected.

Correlations with economic traits. For RFI to be an appropriate measure of feed efficiency in the sheep industry it must not be unfavorably correlated with any traits of economic importance (i.e. carcass merit, growth traits, reproductive efficiency, and wool characteristics). Carcass traits such as rib fat thickness, loin mass area, and marbling score are not correlated with RFI in beef cattle (Ribeiro et al., 2006; Cai et al., 2008). However, Herd and Bishop (2000) and Cai et al. (2008) demonstrated that selection for RFI might slightly increase carcass leanness in bull calves and swine, respectively.

Moore et al. (2009) found that there was no relationship (r = 0.16-0.17) between BF and RFI in cattle. In pigs, Gilbert et al. (2007) also found that there was no phenotypic relationship (r = 0.13) between BF and RFI; however, there was a positive genetic correlation between BF and RFI (r = 0.44 ± 0.16). Johnson et al. (1999) and Hoque et al. (2009) reported that there was a positive phenotypic correlation (r = 0.33, 0.46) and a positive genetic correlation (r = 0.67, 0.77 ± 0.04) between RFI and BF in pigs, respectively. Differences in correlations between BF and RFI may be attributed to models used. Both Johnson et al. (1999) and Hoque et al. (2009) adjusted for weight, age, and test ADG. Results from this study reflect those found by Moore et al. (2009) and Gilbert et al. (2007).

Johnson et al. (1999) reported no phenotypic correlation (r = -0.10) and a negative genetic correlation (r = -0.51) between RFI and LEA in pigs. Furthermore, Hoque et al. (2009) confirmed that there was no phenotypic correlation (r = -0.16), and a negative genetic correlation (r = -0.60 ± 0.06) between RFI and LEA in pigs. There is little to no information available on those effects in cattle or sheep. Species may differ on their response to RFI selection on LEA.

Arthur et al. (2001) found no phenotypic (r = 0.10) or genetic correlation (r = -0.03 ± 11) between SC and RFI in cattle. Schenkel et al. (2004) also confirmed that there was no phenotypic (r = 0.02) or genetic (r = 0.15) correlation between SC and RFI. The possible difference detected in our study may be due to a lack of adjustment for age in the statistical model (although there was only three months difference in ram age).

There has been no known reported research involving the effect of selecting for RFI on BCS or any fleece characteristics in sheep. There was no relationship between RFI on BCS or RFW in this study.

Testing period. The approximate time period needed to determine RFI in sheep may be similar to beef cattle at 60 to 70 d. Sainz and Paulino (2004) estimated that approximately 60 d are needed to accurately determine RFI in cattle. Data from future Ram Tests are needed to further elucidate the time period needed to accurately determine RFI measurement; however, current Ram Test periods are likely more than sufficient.

Implications

The GrowSafe System used for the University of Wyoming Ram Test demonstrates that data generated can be used for RFI research. Future studies will incorporate the use of the GrowSafe System to collect individual animal intake and behavior data. This information will be used in conjunction with reproductive and genetic research to elucidate the effects of selecting for RFI in sheep.

These preliminary results indicate that carcass characteristics, growth traits, and fleece weight are not adversely affected by selecting for RFI in sheep. However, more research in sheep is needed to confirm these findings. Future RFI analyses will include other traits of interest routinely measured as part of the Ram Test, including performance index scores and fleece characteristics (staple length, fleece, clean wool, belly wool, and fiber diameter).

The economic benefits of selecting for RFI in beef cattle have been estimated to improve profitability by 9 – 33% (Archer et al., 2004). Though the economic benefits of selecting for RFI in sheep have yet to be determined, it is hypothesized that they would be similar in scale to those reported in beef cattle. Currently, the University of

Wyoming Ram Test requires producers to pay $0.15 per animal per day to use the GrowSafe System, and dual-purpose breeds utilize the full 140 d to generate intake data for feed efficiency estimations. This preliminary research suggests that the GrowSafe System will only need to be utilized for half that period to estimate performance and feed intake traits, saving producers (who on average submit 5 rams per test) approximately $50 per test.

Acknowledgements

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EFFECTS OF ADDED DIETARY FAT TO POST WEANED HOLSTEIN BULL CALVES ON GROWTH PERFORMANCE

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ABSTRACT: Objective was to determine if adding fat (0-6%) to rations of Holstein starter calves could improve average daily gains (ADG), feed efficiency, and total gains from weaning to 125 kg body weight. The added fat portion of the diets were taken out of the percentage of steam-flaked corn in the diet to evaluate adding fat to existing growing rations. Higher fat diets had greater caloric density. Sixty post-weaned Holstein bull calves with an average weight of 76.99 kg (n = 60, 76.99 ± 8.72 kg initial BW, P = 0.99) were sorted by weight into 5 blocks from heavy to light. Calves were randomly assigned to one of three diets of 0% (**CON**), 3% (**3AF**), or 6% (**6AF**) added dietary fat within their block for a total of 15 groups with 4 calves in each group and 5 pens per treatment. Pen was the experimental unit. The calves were hand fed using the slick bunk method 2 times per day. Calves were fed until the first group reached the average weight of 125 kg. No differences were detected in average daily gains between treatments (P = 0.27), but the contrast between CON and 3AF was nearing a trend (P = 0.12). Feed efficiency was measured using gain to feed ratio. Analysis of overall gain to feed identified a difference between treatments (P = 0.04) with the 3AF having the greatest impact on gain to feed (CON vs. 3AF; P = 0.02, CON vs. 6AF; P = 0.91, and 3AF vs. 6AF; P = 0.03). No differences were detected in the total weight gained (**TW**) per calf between treatments with CON = 99.64 kg, 3AF = 103.99 kg, and 6AF = 101.60 kg (P = 0.85). It was concluded that adding 3% dietary fat to the starter diet of Holstein bull calves significantly improved gain to feed ratios but had no effect on average daily gains or end weight.

KEYWORDS: added fat, Holstein calves, performance

Introduction

Raising Holstein calves presents many issues unique to dairy cattle. Growing programs include heifer calves to replenish milking stock, bull calves for breeding and steers for beef production. This portion of growth is costly to the producer and subject to health and performance factors (Graham et al., 2010). After parturition calves are typically given milk or milk replacer from a bucket or bottle, but not permitted to nurse from the dam. The period after weaning, to the production point is the growing phase (DeNise et al., 1989) of Holstein and other dairy breeds of cattle.

Growing methods represent a vital period in the life of Holstein cattle. Malnourishment or morbidity may alter production in fertility, milk production, feed efficiency, and time on feed in steers (Van Amburgh, 2003).

Adding dietary fat to the diets of neonatal Holstein claves before weaning has been studied in divers aspects. The studies covered a wide spectrum including effect of fat on cold temperatures (Jaster et al., 1992), composition of milk replacer on growth and nutrient retention (Hill et al., 2008), fatty acid requirements for neonatal calves (Hill et al., 2009), the relationship between cold temperatures and different levels of added dietary fat (Scibilia et al., 1987), and rate of passage in with different sourced protein and fat (Gaudreau and Brisson, 1980). A two part study from Cornell looked at the amount and later the energy density (using different sources of fat) of feed on plasma leptin in relation to puberty (Block et al, 2003).

Several advantages to adding fat to the growing diet of dairy calves include increasing caloric density while diminishing the amount of corn fed to the animal. However, little work has been published regarding the effect of adding fat to the diets of post-weaned dairy calves. We hypothesize that adding fat to the diets of weaned Holstein bull calves will improve feed efficiency while maintaining or improving other growth parameters. Therefore, our objective was to determine the effect of different levels of added fat on growth performance of post weaned dairy steers.

Materials and Methods

Diets, facilities, and practices.

All procedures in this study were approved by the University of Arizona Institutional Animal Care and Use Committee. Sixty (60), post weaned Holstein bull calves from a single source dairy (average BW = 77.0 ± 8.72 kg) were used. Animals were housed at the University of Arizona West Campus Agricultural Center in 15 group pens with 4 calves per pen. Pens provided adequate room for animals to move freely, and calves were supplied with fresh, clean *ad libitum* water and fed twice daily (0600 and 1700) using a slick bunk method with 2 challenge days per week to ensure maximum consumption of feed. Pen groups were first blocked according to weight (heavy, medium, and light) and then randomly assigned 1 of 3 treatments within block: a diet containing 0, 3, or 6% added fat. Diets were steam-flaked corn based with Rumensin® to improve feed efficiency and prevent coccidiosis. Feed was not isocaloric, because the added fat portion replaced steam-flaked corn. The fat source

was yellow grease derived from restaurant waste oil with some rendered animal fat.

Feed call was done every morning with refusals weighed. Feed was then weighed by pen and divided into 2 portions for both morning and evening feedings. Adequate bunk space was allocated to minimize competition. Animals were monitored once daily for health and thriftiness.

Calves were weighed biweekly until the first group reached a mean BW of 125 kg, a weight commonly accepted for inclusion into commercial feedlot operations (Duff and McMurphy, 2007). Along with biweekly BW, feed efficiency and ADG were calculated.

Statistics. Results were analyzed as a completely random block design using the GLM procedure of SAS (Cary, NC) with pen considered the expermental unit. Means and partitioning were across diets were generated using the LSMEANS, PDIFF, STDERR, and CONTRAST options.

Results

Five calves (3 CON and 2 3AF) were removed from the study due to morbidity. There was no mortality during this trial.

Although no differences (P = 0.85; Table 2) were detected in total weight gained, Figure 1 shows the differences in the weight gained within each treatment throughout the trial. After the first 2 wks, the 3AF group had the numerically lowest gains of all of the groups at 2.45 kg. This is approximately 50% of the 6AF group that gained 4.76 kg, and lower than the CON group that gained 3.49 kg.

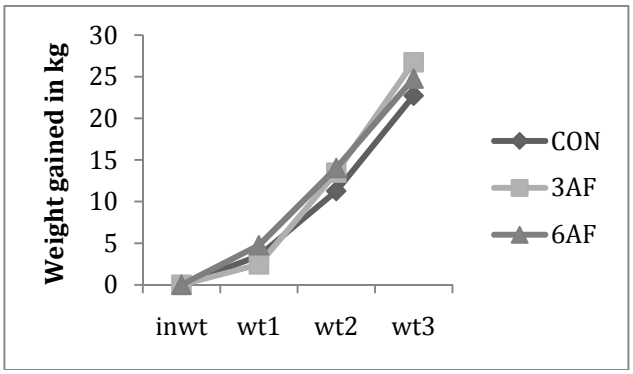

Figure 1. Differences between treatments over time in the amount of weight gained. Bottom scale measures 2 week increments. Diets labeled: CON = control, 3AF = 3% added fat, 6AF = 6% added fat.

Average daily gains were near a trend (P = 0.11) but not significant between CON and 3AF, and not significant (P = 0.27) over all treatments.

Gain to feed ratios yielded a difference (P = 0.04) between treatments with the 3AF having the greatest difference (CON vs. 3AF; P = 0.02, CON vs. 6AF; P = 0.91, and 3AF vs. 6AF; P = 0.03).

DMI did not differ (P = 0.73) between treatments shown in Table 2.

Discussion

Indicated in Figure 1, the first period of time did not show favorable gains, and the overall weight gain did differ significantly. This may be explained by difference in time between weaning and start of trial. The calves had been weaned for approximately 2 weeks and were fed the control diet prior to the trial start. Calves were not given an adaptation diet because the diet was planned to commence directly after weaning. It was hypothesized that following a 22/22 protein / fat milk replacer that calves would have the ability to metabolize higher levels of fat. Within the first week calves fed both added fat diets (3AF and 6AF) experienced diarrhea followed by loose stool the following week. This, however, does not explain the low gains for the CON group in the first period.

In addition, calves fed the 6AF had the best gains in every period except the last. More research should be conducted to determine if the effect of added fat is temporary, or quickly plateaus. Compensatory gains for the CON and 6AF may have followed. A step-up diet that allowed the calves to adjust to the higher levels fat could also slow retention time in the gastro intestinal tract during transition (Gaudreau and Brisson, 1980).

Average daily gains were better in the 3AF group compared to the CON and the 6AF. A larger sample over a longer period of time would help determine if the difference is significant. Average daily gains were nearing a trend (P = 0.11) for the difference between CON and 3AF.

Morbidity surfaced prior to the trial due to issues with *Mycoplasma bovis* during the preweaning phase of the calves. During the first 60 days after the birth of these calves *M. bovis* was diagnosed and the calves were treated and monitored daily. At the beginning of the study only healthy calves were used, but nearly a 9% relapse occurred during the trial and some calves were, treated, removed and segregated from the calves in the study. All incidences of morbidity were similar to *Mycoplasma* and all other calves were free of symptoms of morbidity.

Results indicated G:F had an overall difference (P = 0.04) across treatments. A difference (P = 0.02) was detected between CON and 3AF and also between 3AF and 6AF (P = 0.03). There was no difference (P = 0.91) between CON and 6AF. With 3AF having greater efficiency, more research could narrow the range of added fat in effective amount in diets.

Implications

The findings of this study indicate that the addition of dietary fat at 3% of the diet can improve feed efficiency in growing, post-weaned Holstein bull calves, but the addition of 6% dietary fat had no significant improvement over the control diet. Further research is needed to understand effect on heifer development and fertility in relation to added fat to growing diets.

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Table 1. Diet and nutrient composition of research diets

	Diet[1]		
Item	CON	3AF	6AF
Ingredient, %DM			
Steam flaked corn	59.5	56	52.5
Alfalfa	14	14	14
SBM, 48	12	12.5	13
Beet pulp	5	5	5
Mineral premix	2.5	2.5	2.5
Pre-mix[2]	1	1	1
Molasses	6	6	6
Fat	0	3	6
Nutrient			
DM	88.6	88.7	89.1
CP	15.8	15.2	15.5
NDF	10.4	11.7	13.6
ADF	6.1	6.1	7.9
Ash	6.3	6.7	6.8
Starch	28.3	28.5	28.2

[1]Diet labeled: CON = Control diet with no added fat; 3AF = 3% added fat; 6AF = 6% added fat.

[2]300 mg $\cdot$animal$^{-1}\cdot$d^{-1} of Rumensin 90 and 100 mg $\cdot$animal$^{-1}\cdot$d^{-1} of Tylan 40 (Elanco Animal Health, Greenfield, IN) in a ground corn carrier.

Table 2. Growth performance of Holstein bull calves fed diets differing in levels of added fat

	Diet[1]				
Item	CON	3AF	6AF	SEM	*P* value
Initial BW, kg[2]	77.1	77.4	77.0	3.97	0.99
Weight 2, kg	80.6	79.9	81.8	4.36	0.96
Weight 3, kg	88.4	90.9	91.0	5.11	0.92
Final BW, kg	99.8	104.2	101.8	5.38	0.85
Total gain, kg	22.8	26.8	24.8	1.68	0.28
ADG, kg	0.69	0.81	0.75	0.051	0.27
DMI 1, kg[3]	23.1	23.9	27.0	2.12	0.42
DMI 2, kg	19.0	21.1	23.7	1.82	0.23
DMI 3, kg	46.2	46.5	45.8	3.84	0.99
Total DMI, kg	88.3	91.5	96.5	7.23	0.73
G:F	0.26	0.29	0.26	0.010	0.04

[1]Diet labeled: CON = Control diet with no added fat; 3AF = 3% added fat; 6AF = 6% added fat.
[2]Average individual BW (n = 60). Weight 2 and weight 3 labeled according to biweekly weigh date.
[3]DMI labeled according to periods between weigh dates by pen (n = 15).

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CXCL12 AND CXCR4 EXPRESSION IN PERIPHERAL BLOOD FROM PREGNANT AND NON-PREGNANT SHEEP: IMPLICATIONS IN PREGNANCY DIAGNOSIS

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ABSTRACT: In ruminants, implantation of the blastocyst in the uterus and subsequent placentation is a prolonged process. This complex progression involves interplay between sex steroids and local signaling molecules, many of which have immune function. Chemokines and their receptors are pivotal factors in implantation and vascularization of the placenta. Chemokine receptor 4 (CXCR4) is up regulated in human endometrium during implantation and has only one recognized ligand, CXCL12. Activation of CXCR4 causes recruitment of leukocytes to the uterus of pregnant females and stimulates trophoblast proliferation and invasion. Based on known roles for CXCL12 and CXCR4 during early pregnancy, we hypothesized that expression of CXCL12 and CXCR4 would increase in peripheral blood during implantation and placentation in sheep. The objective of the study was to determine if mRNA for CXCL12 and CXCR4 is differentially expressed using real-time PCR (qPCR) in blood from pregnant and cyclic ewes. Jugular and uterine vein blood samples were collected from ewes on d 12 to 15 and d 35 and 50 of pregnancy. In jugular blood samples, the greatest expression of CXCL12 mRNA was on d 35 of gestation and was significantly ($P< 0.05$) elevated compared to all days tested. A similar expression pattern of CXCL12 was observed in uterine vein samples with the greatest expression on d 35 of pregnancy. Expression of CXCR4 was detected on all days but did not differ. Ruminant pregnancy is characterized by changes in immune cell populations in the periphery and these changes are likely important for conceptus protection. The increase in CXCL12 in peripheral blood is interesting as it correlates with placentation in sheep. The CXCL12/CXCR4 system may affect migration of immune cells into the uterus and aid in fetal-maternal tolerance. In summary, gene expression of CXCL12 is increased in peripheral blood cells from pregnant sheep during critical fetal-maternal communication and it is conceivable that detection of CXCL12 in blood could serve as a pregnancy diagnostic tool.

Keywords: Chemokine, Pregnancy, Sheep

Introduction

Implantation in ruminants is a sophisticated prolonged process, starting on d 15 to18, with completion by d 50 to 60 of pregnancy. To ensure successful pregnancy, a specific communication must exist between fetus-derived trophoblast cells and differentiated maternal cells in the endometrium. This communication relies on cellular interactions, such as chemokines functioning through their receptors, to maintain survival and health of the fetus during early pregnancy (Aplin and Kimber, 2004; Dimitriadis et al., 2005). Chemokines are small proteins that exert their function through G-protein coupled receptors (Mortier et al., 2008) and act as chemoattractants, triggering immune cell migration (Laing and Secombes, 2004). Chemokine receptor 4 (CXCR4) only has one defined ligand, CXCL12 and upon binding assists in leukocyte homeostasis and trafficking. During early pregnancy, changes in immune function occur to prevent immunological destruction of the conceptus. In ruminants, an increase in leukocytes such as macrophages, γδT cells, T_{reg} cells (CD4+, CD25+), and natural killer cells occurs within the uterus and this migration of cells may be due to CXCL12/CXCR4 interactions (Tekin and Hansen, 2004; Oliveira and Hansen, 2008). In addition to its immunological role, CXCL12 also suppresses apoptosis in trophoblast cells and promotes trophoblast cell survival, further highlighting the importance of CXCL12/CXCR4 signaling during early pregnancy (Jaleel et al., 2004). Due to the many functional roles of CXCL12/CXCR4 during early pregnancy in other species, we hypothesized that expression of CXCL12 and CXCR4 would increase in peripheral blood during implantation and placentation in sheep. The objective of this study was to determine if mRNA for CXCL12 and CXCR4 is differentially expressed using real-time PCR (qPCR) in blood from pregnant and cyclic ewes.

Materials and Methods

Blood Collection. All procedures involving animals within this study were approved by the Colorado State University Animal Care and Use Committee. Blood samples were collected by jugular and uterine venipuncture from pregnant and non-pregnant ewes (n = 6 ewes/day) on days 12 to 15, and days 35 and 50 of pregnancy. Samples were collected in anticoagulant tubes, and centrifuged at 2500 x *g* for 30 min at 4°C to obtain the white blood cell layer (buffy coat).

RNA Extraction. Total RNA was extracted from buffy coats using Tri Reagent BD (Molecular Research Center Inc., Cincinnati, OH) according to manufacturer's

directions. RNA was eluted in RNAse-free water and treated with TURBO DNA-free kit (Ambion, Foster City, CA) to eliminate genomic DNA. The quantity and purity of RNA were determined using a NanoDrop ND-1000 Spectrophotometer and subsequently stored at -80°C.

Real-Time Polymerase Chain Reaction (qPCR). Synthesis of cDNA was accomplished by using the iScript cDNA Synthesis Kit employing the reverse transcriptase (RT) RNAse H+ (BioRad, Hercules, CA) per manufacturer's instructions. The RT products were diluted to a final volume of 100 µL. Real-time PCR (qPCR) was performed using a LightCycler 480 PCR Detection System (Roche Applied Science, Indianapolis, IN) and components of iQ SYBR Green supermix (BioRad, Hercules, CA), 0.525 µM forward and reverse specific primers and 2 µL of cDNA. The specific primers employed included: GAPDH forward primer, 5'-TGACCCCTTCATTGACCTTC-3', GAPDH reverse primer, 5'-CGTTCTCTGCCTTGAC TGTG-3', CXCL12 forward primer, 5'–CCTTGCCGA TTCTTTGAGAG-3', CXCL12 reverse primer, 5'-GGT CAATGCACACTTGCCTA-3', CXCR4 forward primer, 5'-AAGGCTATCAGAAGCGCAAG-3', CXCR4 reverse primer, 5'-GAGTCGATGCTGATCCCAAT-3'. The qPCR conditions were 95ºC for 3 min. followed by 40 cycles of 95ºC (30 sec), 55ºC (30 sec), 72ºC (15 sec) and then a melt curve was performed per manufacture's conditions. The GAPDH amplicon did not change across days or pregnancy status and was used to normalize each target mRNA by using the ΔCt (target Ct - GAPDH Ct) values (Schmittgen and Livak, 2008). Data are represented by graphing $2^{-\Delta Ct}$ values calculated for each gene of interest. For each mRNA target, the amplicon was sequenced to ensure that each gene of interest was correctly amplified. Amplification efficiencies were determined using a 10-fold dilution series of cDNA for each primer set and each amplified at 95 to 110% efficiency.

Statistical Analysis. Significant changes for the qPCR data were determined at $P < 0.05$ using an unpaired, two-tailed Student's t-test on normalized Ct values using Prism (Version 5 from GraphPad Software, Inc.).

Results

The expression of mRNA for CXCL12 and CXCR4 were evaluated using qPCR. Expression of CXCL12 mRNA from jugular vein blood samples was detected in non-pregnant and pregnant ewes on d 12, 13, 14, and 15 but did not differ. However, on d 35 of pregnancy, expression of CXCL12 was greater ($P < 0.05$) compared to d 13, 14 and 15 (Figure 1). In uterine vein blood samples, expression of CXCL12 displayed a similar pattern with up regulation ($P < 0.05$) of CXCL12 on d 35 of gestation compared to d 15 and 50 of gestation (Figure 2). In blood samples from both jugular and uterine vein, the expression of CXCL12 mRNA had decreased by d 50 of pregnancy similar to expression observed on d 12 to 15 (Figure 3).

Expression of CXCR4 mRNA in jugular vein blood samples was detected on all days evaluated, but did not differ ($P > 0.05$) in pregnant or non-pregnant ewes. Likewise, in uterine vein blood samples, CXCR4 mRNA expression was similar to jugular vein blood samples with no differences ($P > 0.05$) noted between pregnant and non-pregnant ewes on the days tested (Figure 4).

Discussion

The implantation process in ruminants consists of three stages; 1) a pre-attachment period in which the conceptus elongates, 2) an apposition stage, and 3) an adhesion stage, during which development of the placenta occurs (Igwebuike, 2009). These stages are critical, and without proper cellular communication between fetal and maternal tissues, pregnancy tribulations could arise. Increased expression of CXCL12 from d 15 to 35 of pregnancy in sheep suggests CXCL12 may play an important role during implantation and placentation. The chemokine, CXCL12 may affect immune cell migration into the endometrium, thereby aiding in fetal survival. Despite no significant changes in blood expression of CXCR4, we are more interested in the role of CXCR4 within placental and endometrial tissue. Peripheral blood studies of CXCR4 are lacking, but research conducted by Martin et al. (2003) demonstrated a low expression of CXCR4 in human blood neutrophils and murine blood and bone marrow neutrophils, similar to CXCR4 expression observed in our study. Tissue analysis studies of CXCR4 expression have provided intriguing results however, with up regulation of CXCR4 in early human placental tissue, as well as expression within embryonic tissues (McGrath et al., 1999; Kumar et al., 2004), further cementing CXCR4's role during early pregnancy.

At approximately d 35 of pregnancy in ruminants, placentation occurs, which is characterized by extensive angiogenesis. As disruption of CXCL12 or CXCR4 in mice causes lethality during late gestation, with defects including vasculature deformities (Nagasawa et al., 1996;Tachibana et al., 1998), it is quite probable that the CXCL12/CXCR4 pathway is important for placental vascularization. Also, an increased expression of CXCL12 in platelets has been reported during vascular injury studies, signifying that CXCL12 is a crucial chemokine for vasculature repair and recruitment (Massberg et al., 2006; Schober, 2008).

Changes in immune cell populations in the periphery occur during pregnancy for protection of the conceptus. Migration of immune cells into the uterus may involve the CXCL12 and CXCR4 system to aid in fetal-maternal tolerance. Little is known about CXCL12 and CXCR4 expression within peripheral blood cells or their functions in ruminants. Identifying specific immune cells expressing CXCL12 could shed light on cellular functions occurring during implantation and early pregnancy. With these results, further knowledge can be applied to understanding mechanisms during implantation and placentation in ruminants and potentially reduce early pregnancy loss within the livestock industry.

Implications

Ruminant pregnancy is characterized by changes in immune cell populations in the periphery and these changes are likely to be important for conceptus protection. The

CXCL12 and CXCR4 system may affect migration of immune cells into the uterus and aid in fetal-maternal tolerance. Because the CXCL12/CXCR4 signaling system is important during early pregnancy in other species, the increase in CXCL12 gene expression in peripheral blood cells may symbolize similar functions in pregnant sheep. As expression of CXCL12 was observed early in gestation, it is plausible that detection of CXCL12 in the blood could serve as a pregnancy diagnostic tool.

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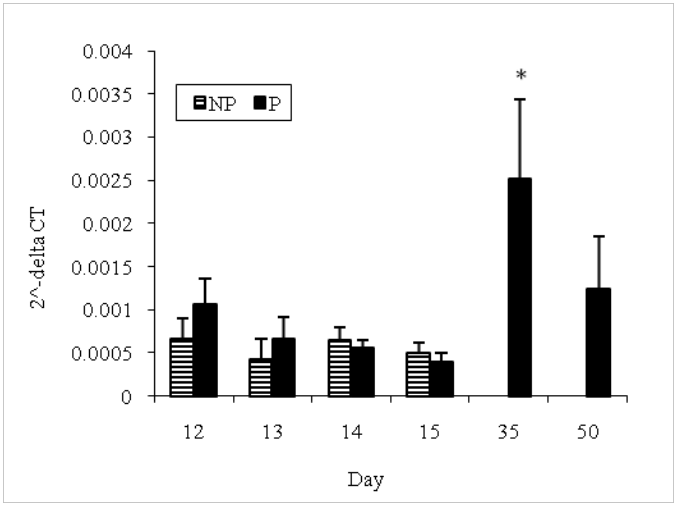

Figure 1. CXCL12 mRNA is up regulated in jugular vein blood samples from pregnant (P) compared to non-pregnant (NP) ewes. Expression of mRNA for CXCL12 was significantly (*P < 0.05) elevated on d 35 compared to d 12, 13, 14, or 15.

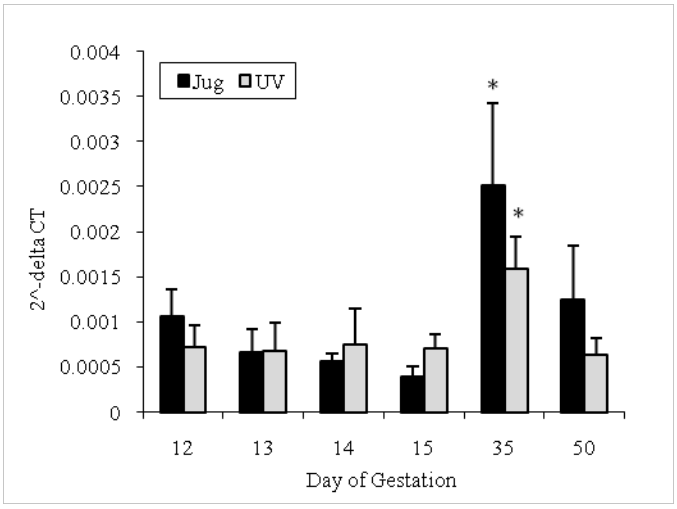

Figure 3. CXCL12 mRNA is up regulated in jugular (Jug) and uterine vein (UV) blood samples from pregnant ewes. Expression of mRNA for CXCL12 was significantly (*P < 0.05) elevated on d 35 compared to d 12,13,14 or 15.

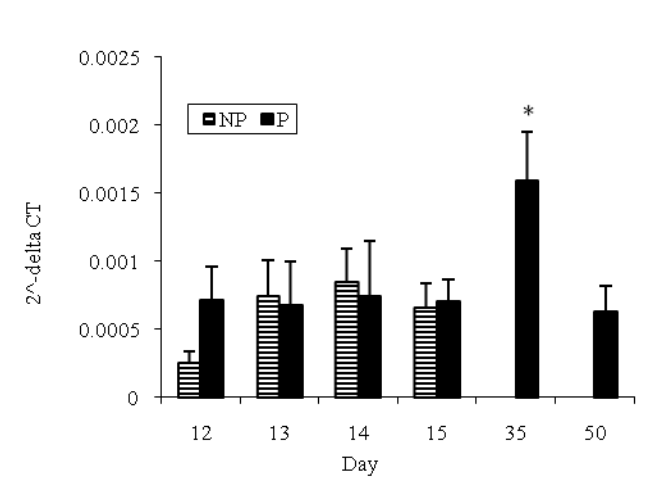

Figure 2. CXCL12 mRNA is up regulated in uterine vein blood samples from pregnant (P) compared to non-pregnant (NP) ewes. Expression of mRNA for CXCL12 was significantly (*P < 0.05) elevated on d 35 compared to d 15 and 50.

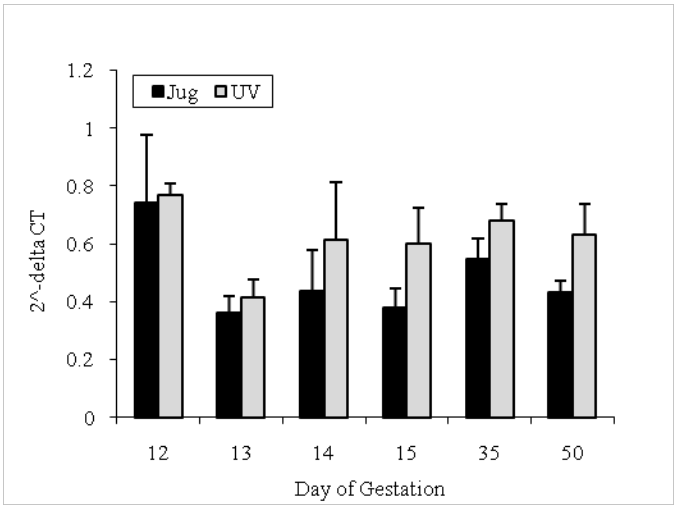

Figure 4. Expression of CXCR4 mRNA in jugular (Jug) and uterine vein (UV) samples from pregnant ewes remained constant across days tested.

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RELATIONSHIP BETWEEN BEHAVIORAL TRAITS AND FEEDLOT PERFORMANCE IN FINISHING STEERS

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ABSTRACT: Our objectives were to evaluate the relationship between subjective behavioral scores with objective exit velocity, and the relationship between behavioral traits and feedlot performance. Crossbred steers (n = 186, 394.7 ± 40.95 kg) received a high energy ration for 70 d prior to slaughter. Steers were weighed every 2 wk at which time 5-point subjective chute score (1 = very calm, 5 = very aggressive), 4-point subjective gait score (1 = walk, 2 = trot, 3 = run, 4 = fall), objective exit velocity, time in chute and vocalization incidence data were collected. Animal handling prior to entering the squeeze chute was not directly measured in this study, nor was information on the handler(s) collected or accounted for in the analysis. However, consistent low-stress animal handling techniques were employed when animals were processed. There was a positive correlation (r = 0.32, $P < 0.01$) between chute score and gait score. Both chute score and gait score were positively correlated (r = 0.31, $P < 0.01$ and r = 0.63, $P < 0.01$, respectively) with exit velocity. Exit velocity was negatively correlated (r = -0.07, $P < 0.03$) with ADG. There was no correlation ($P > 0.10$), between chute score and ADG, nor was there a correlation between gait score and time in chute ($P > 0.10$). These data suggest an effect of behavior as measured by subjective gait score on ADG. Steers categorized in the slowest gait scores (1 and 2) exhibited higher ($P < 0.05$) ADG than steers categorized in fastest categories (3 and 4). Average chute chore was different ($P < 0.05$) across all 4 gait score categories. Steers with a gait score of 1 exhibited a chute score 0.35 points higher ($P = 0.05$) than gait score 4. In conclusion, subjective gait score can represent objective exit velocity as a method of characterizing behavior. Additionally, steers with faster exit velocity had lower ADG.

Key Words: ADG, Chute score, Exit velocity, Feedlot steers, Gait score

Introduction

Human-animal interactions in production settings can have an effect on the performance of the animal. Cattle that observe to be "flightier" have subpar performance in both gain (Voisinet et al., 1997) and carcass quality (Gruber et al., 2010). Measuring the degree of flightiness is an abstract idea, but objective exit velocity has been proven as a possible means of objectively measuring stress response to handling (Curley et al., 2006). It has also been found that subjective chute score can be a measure of predisposition to flighty behaviors, as animal excitement is amplified by restraint (Grandin, 1994). The objectives of this study were to determine the relationships between subjective measures of behavior and objective exit velocity, as well as between measures of behavior and performance in finishing steers.

Materials and Methods

Bos indicus and *Bos taurus* crossbred feedlot steers (n = 186) were housed at the Colorado State University Agricultural Research Development and Education Center Feed Intake Unit (Fort Collins, CO). All steers weighed 394.7 ± 40.95 kg when first processed. Steers were weighed approximately every 14 d for 70 d. Due equipment failure, behavioral data were not collected on d 28.

Steers were fed an ad libitum total finishing diet of corn silage, cracked corn and alfalfa hay. Feed intake was measured using an RFID-linked feed intake behavior system (Grow Safe Systems Ltd., Airdrie, AB). Steers were housed in groups of approximately 30 steers.

Steers were restrained in a hydraulic chute to be weighed and/or assessed for subcutaneous fat deposition, longissimus dorsi area and intramuscular fat every 14 d. Upon restraint steers were designated a subjective chute score (**SCS**) of 0 to 5 on a line scale, (0 = calm, 5 = aggressive) as described by Gruber et al., 2009.

Time in the chute (**TIC**) was collected along with an objective chute exit velocity (**OEV,** m/s) beginning 1.892 m from the head catch and ending 1.892 m beyond that point using a infrared-sensor timing system (FarmTek Inc., North Wylie, TX) .

A subjective gait score (**SGS**) was also determined using a 4-point scale (1 = walk, 2 = trot, 3 = run/jump, and 4 = fall). Due to equipment failure behavioral data were not collected on d 28 of the feeding period.

Animal handling prior to entering the squeeze chute was not directly measured in this study, nor was information on the handler(s) collected or accounted for in the analysis. However, consistent low-stress animal handling techniques were employed when animals were processed.

Statistical Analyses. Data were analyzed using the mixed procedure of SAS (SAS Institute, Cary, NC) with OEV as the dependent variable. Person's correlations for SCS, SGS, OEV, ADG and TIC were determined using the correlation procedure of SAS. And, SGS was analyzed using a model that included OEV, ADG and SCS

Results and Discussion

During the trial, mean ADG among steers was 1.59 ± 0.274 kg/d and mean dry matter intake was 10.10 ± 1.285 kg/d (DM basis).

As demonstrated in Table 1, there were correlations between subjective and objective behavioral measurements. There was a positive correlation (r = 0.32, $P < 0.01$) between SCS and SGS. Both SCS and SGS were positively correlated (r = 0.31, $P < 0.01$ and r = 0.63, $P < 0.01$, respectively) with OEV. And, subjective gait score, TIC and OEV were negatively correlated (r = -0.07, $P = 0.03$, r = -0.11, $P = 0.02$; r = -0.07, $P = 0.03$ respectively) with ADG.

Least square means of behavioral traits and performance by gait scores in Table 2, indicate differences in OEV among SGS 1, 2 and 3 ($P < 0.05$), while there was no difference ($P > 0.05$) between SGS 3 and 4. Greater ($P < 0.05$) ADG was observed among gait scores 1 and 2 versus gait scores 3 and 4. There was no difference in ADG within the groupings of slower SGS (1 and 2) and faster SGS (3 and 4). Chute scores among gait scores were greater with faster exit gaits. The greatest difference in SCS was seen between SGS 2 and 4 with SGS 4 steers being 0.35 points on the SCS scale greater than ($P < 0.05$) SGS 4.

Mean SGS and OEV across processing dates, as plotted in Figure 1, decreased from d 0 to d 70. Average OEV did not differ ($P > 0.05$) between d 0, d 14 and d 56, but d 56 and d 70 differed ($P < 0.05$) from all other dates. Average SGS did not differ ($P > 0.05$) between d 0, d 14, d 42 and d 56, while we observed the slowest average exit velocity on d 70, but it was not different ($P > 0.05$) from any other dates.

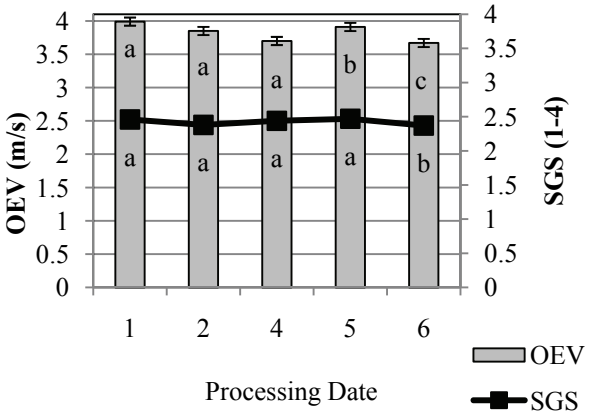

Figure 1. Mean subjective gait score (**SGS)** and objective exit velocity (**OEV)** plotted across d 0 (1), d 14 (2), d 42 (4), d 56 (5), d 70 (6)[1,2].

[1]Subjective gait score- 1 = walk, 2 = trot, 3 = run/jump and 4 = fall.

[2]Objective exit velocity- velocity from 1.892 m to 3.784 m past head gate of the squeeze chute.

[a,b,c] Within each speed measurement, means without common superscripts are different ($P < 0.05$)

In the current study, there was no correlation (r = 0.01, $P = 0.74$) between SCS and ADG unlike what was reported in a similar study (Voisinet et al., 1997). This discrepancy could be attributed the employment of different personnel before animals entered the chute, despite the consistent use of gentling handling practices. Unlike results of Voisinet et al. (1997), our study did not address genetic influence (*Bos indicus* vs. *Bos taurus*), but did account for behavioral parameters beyond SCS, Additionally, the current study included behavioral observations every 14 d, whereas Voisinet et al. (1997) collected temperament scores every 28 d.

Subjective chute score was positively correlated with OEV (r = 0.31, $P < 0.001$), which was similar to previous research (Curley et al., 2006). Data indicate that OEV is an accurate assessment of temperament as it correlates (r = -0.07, $P = 0.03$) to ADG. Ranking of SGS indicates that there are clear differences among SGS and OEV. These rankings also revealed a trend that cattle with greater SGS experience reduced ADG.

A similar study that examined behavioral traits and stress with performance and carcass parameters (Gruber et al., 2009) indicated that SCS was negatively correlated with ADG (r= -0.02, $P < 0.05$). In contrast the current study, Gruber et al. (2009) examined a wide array of physiological responses, but did not measure OEV or SGS.

Industry implications from this study are consistent with data from previous studies that poor temperament cattle have subpar performance to contemporaries with calm temperaments. Exit velocity could be used as a predictor of feedlot performance in addition to overall temperament (Curley et al., 2006). Given that poor temperament scores and stress response have a negative effect on carcass quality (Knowles, 1999; Gruber et al. 2009), there is potential for OEV to be used as a predictor of carcass quality.

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Table 1. Correlations of subjective chute score (SCS), subjective gait score (SGS), time in chute (TIC), objective exit velocity (OEV) and ADG in finishing steers

	SCS[1]	SEG[2]	TIC[3]	OEV[4]	ADG
SCS		0.31 ($P < 0.001$)	0.08 ($P = 0.01$)	0.31 ($P < 0.001$)	0.01 ($P = 0.74$)
SEG			-0.02 ($P = 0.50$)	0.63 ($P < 0.001$)	-0.07 ($P = 0.03$)
TIC				-0.01 ($P = 0.76$)	-0.11 ($P = 0.02$)
OEV					-0.07 ($P = 0.03$)
ADG					

[1]Subject chute score: (0 = calm, 5 = aggressive).
[2]Subjective exit gait: 1 = walk, 2 = trot, 3 = run/jump and 4 = fall.
[3]Time in chute : time from an animal entering the squeeze chute until passing 1.892 m in front of head gate of the squeeze chute.
[4]Objective exit velocity : velocity (m/s) from 1.892 m to 3.784 m past the head gate of squeeze chute.

Table 2. Least square means for objective exit velocity (OEV), ADG and subjective chute score (SCS) by subjective gait score in finishing steers evaluated for temperament and performance.

SGS	OEV (m/s)	SE	ADG (kg/d)	SE	SCS	SE
1	2.4[a]	±0.11	1.5[a]	±0.09	1.2[a]	±0.07
2	3.7[b]	±0.06	1.6[a]	±0.04	1.3[b]	±0.03
3	4.3[c]	±0.06	1.5[b]	±0.04	1.5[c]	±0.03
4	4.9[c]	±0.43	1.4[b]	±0.37	1.5[abc]	±0.25

[1]Subjective gait score – 1 = walk, 2 = trot, 3 = run/jump and 4 = fall
[2]Objective exit velocity - velocity from 1.892 m to 3.784 m past head gate of hydraulic chute.
[3]Subject chute score - 0 to 5 (0 = calm, 5 = aggressive)
a, b, c Within a column, means without common superscripts differ ($P < 0.05$)

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IMPROVING TIMED AI PREGNANCY RATES IN BEEF HEIFERS BY SYNCHRONIZING FOLLICULAR WAVES WITH GnRH ON D 9 OF A 14 D CIDR PLUS CO—SYNCH PROTOCOL

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ABSTRACT: Presynchronization of reproductive cycles with long term progestins increases estrus synchrony in heifers, but also causes persistent follicles. Follicular waves may be synchronized with GnRH to eliminate persistent follicles. The first objective of this study was to compare Timed AI TAI) pregnancy rates in beef heifers between a 14 d controlled internal drug release (CIDR) protocol with GnRH on d 9, to a 5 d CO-Synch+CIDR control protocol. The second objective was to compare pregnancy rates between a 50 mg $PGF_{2\alpha}$ injection and a 6 h interval between two, 25 mg $PGF_{2\alpha}$ injections at CIDR removal within the 14 d CIDR protocol. Angus and Angus cross heifers (n = 710) at 4 locations were assigned to 3 synchrony of estrus treatments. Heifers in the 14-d 50 PG treatment (n = 242) received 100 µg GnRH im and a CIDR (1.38 g progesterone) on d 0, followed by 100 µg GnRH im on d 9, 50 mg of $PGF_{2\alpha}$ on d 14 at CIDR removal, and 100 µg GnRH im at TAI 72 ± 3 h after CIDR removal. Heifers in the 14-d 6h PG treatment (n = 233) received the same protocol except instead of receiving 50 mg of $PGF_{2\alpha}$ they received two 25 mg injections of $PGF_{2\alpha}$ im, one at CIDR removal and a second 6 h later. The control heifers (5-d CO Synch+CIDR) (n = 235) received 100 µg GnRH im and a CIDR on d 9, and 25 mg of PGF2 at CIDR removal on d 14 with TAI and 100 µg GnRH GnRH 72 ±2 h later. The average and SD BCS and weight of all heifers was 4.8±2.42 and 310±35.0 kg, respectively. Average weight and body condition score did not differ (P > 0.10) between treatments. Conception rates were determined on d 46±6 d (SD) by ultrasonography. Statistical analysis was done using proc GLIMMIX in SAS. The 14-d 50 PG TAI rate of 54.5% (132/242) was not different from the 14-d 6h PG TAI rate of 53.6% (125/233) (P = 0.57) nor the control 5-d CO Synch+CIDR TAI rate of 46.4% (110/235) (P = 0.20). This protocol appears to produce encouraging TAI rates. However additional research is needed to determine whether the d 9 GnRH addresses persistent follicles.

Key Words: Artificial insemination, Beef heifers, Estrus synchronization

Introduction

Oral progestin exposure improves estrus synchrony and conception rates in beef cows (Patterson et al., 1995) and is effective at inducing cyclicity in prepubertal heifers (Patterson et al., 1990). Unfortunately heifers under the influence of oral progestins develop persistent follicles due to a continued period of follicular growth (Sirois and Fortune, 1990).The controlled internal drug release (CIDR) Select protocol utilizes progestin presynchronization and has improved ovulation due to GnRH and estrus synchrony (Leitman et al., 2008) while also increasing Timed AI (TAI) compared to the CO-Synch+CIDR protocol (Busch et al., 2007). One potential drawback of this protocol is it takes 33 d to complete.

Several studies have compared a single dose of $PGF_{2\alpha}$ to 2 doses at CIDR removal within the 5-d CO Synch+CIDR protocol (Rabaglino et al., 2010; Peterson et al., 2011) and found that the single dose did not produce different TAI rates than heifers receiving 2 doses.

This research led to the creation of a shorter 14 d CIDR protocol with d 9 GnRH and to the hypothesis that follicular waves could be synchronized by d 9 GnRH and cause ovulation of persistent follicles and initiate a new wave of more viable follicles. The first objective of this study was to compare TAI rates between this 14 d CIDR protocol and the 5-d CO Synch+CIDR protocol, and the second objective was to compare TAI rates between 14 d CIDR protocols that differed in the prostaglandin treatment they received on d 14.

Materials and Methods

Experimental procedures with animals were approved by the Colorado State University Animal Care and Use Committee prior to initiation of the experiment.

Angus and Angus cross heifers (n = 710) at 4 locations (location 1, n = 89 location 2, n = 440 location 3, n = 147 location 4, n = 34) were randomly assigned to one of 3 treatments. Heifers assigned to the 14-d 50 PG treatment (n = 242) were a CIDR (EAZI-BREED™ CIDR®, Pfizer Animal Health, New York, NY) and GnRH im (100 µg Factrel, Fort Dodge Animal Health, Fort Dodge, IA) on d 0. On d 9 they received another injection of GnRH administered im. When CIDRs were removed on d 14, heifers received 50 mg im of $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health, New York, NY) at one injection site. These heifers were then given 100 µg GnRH and artificially inseminated 72 ± 2 h after CIDR removal. Heifers in the 14-d 6 h PG treatment (n = 233) similarly received GnRH and a CIDR on d 0 and GnRH on d 9, 25 mg im of $PGF_{2\alpha}$ at CIDR removal on d 14, another 25 mg im of $PGF_{2\alpha}$ 6 h later, and 100 µg GnRH when artificially inseminated 72 ± 3 h after CIDR removal. The 5-d CO-Synch + CIDR® treatment (n = 235) served as the

control. Heifers in this treatment received CIDR's and GnRH on d 9, 25 mg im of $PGF_{2\alpha}$ at CIDR removal, and GnRH at TAI 72 ± 2 h after CIDR removal. A diagram of all treatments is shown in Fig. 1. All heifers were scored for body condition (1=thin, 9=obese, Richards et al., 1986) on d 0 and given Estrotect™ estrus detection patches (ESTROTECT, Spring Valley, WI) at CIDR removal on d 14. These patches were then scored on a 1-3 scoring system at breeding on d 17. A patch with a score of 1 was not rubbed off at all, score 2 was half rubbed off, and score 3 was completely rubbed off. However at location 1 patches were only scored as 1 or 3.

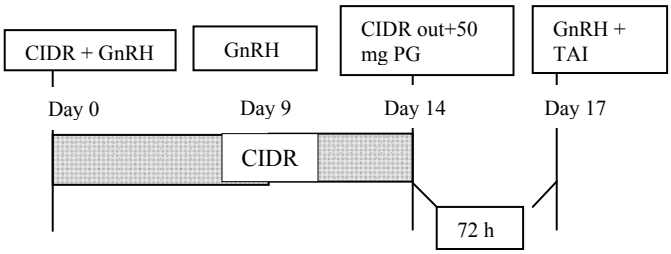
Pregnancy rates to TAI were determined through transrectal ultrasonography (5 MHz linear probe on an Aloka 500 console) 46 ± 6 d after TAI.

Statistical Analyses

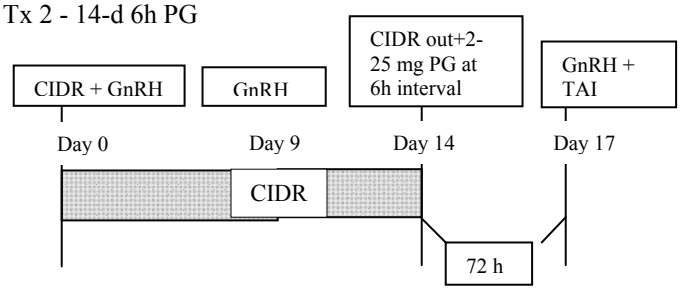
Differences in AI pregnancy rate were analyzed using PROC GLIMMIX of SAS.

Figure 1 – Treatments in the Study

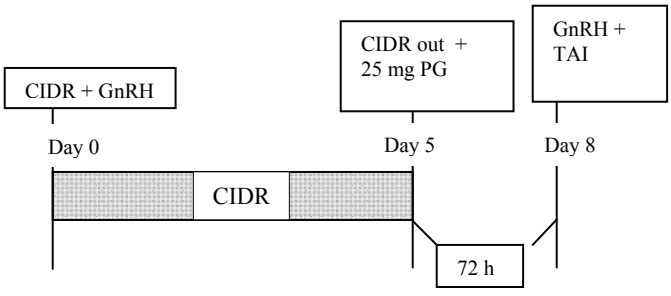
Tx 1 - 14-d 50 PG



Tx 2 - 14-d 6h PG



Tx 3 - 5-d CO-Synch + CIDR



Results

Information on heifer numbers, mean BW, and mean BCS are reported by location (Table 1) and treatment (Table 2). Estrous response by treatment is shown in Table 3. Both 14-d CIDR protocols had a lower percentage of unrubbed heat patches (P = 0.02) and a higher percentage of completely rubbed patches (P = 0.02) than the control. Timed AI pregnancy rates by patch score within treatment are shown in Table 4. There were no differences between TAI pregnancy rate within patch score across the 3 treatments.

Table 1: Number, BW, and BCS of heifers across locations (least squares means ± SE)

Location	n =	BW (kg)	BCS
1	89	311 ± 3.6	3.2 ^{w} ± 0.04
2	440	306 ± 1.7	4.9 ^{x} ± 0.02
3	147	323 ^{a} ± 2.8	5.1 ^{y} ± 0.03
4	34	-	5.8 ^{z} ± 0.07

^{a} Within a column, means without common superscripts differ ($P < 0.05$)

^{wxyz} means with different superscripts differ ($P < 0.01$)

Table 2: Number, BW, and BCS of heifers across treatments ((least squares means ± SE)

Treatment	n =	BW (kg)	BCS
14-d 50 PG	242	310 ± 2.3	4.7 ± 0.03
14-d 6 h PG	233	310 ± 2.4	4.8 ± 0.04
5-d CO Synch+CIDR	235	312 ± 2.4	4.8 ± 0.04

No differences ($P > 0.10$)

Table 3: Heat patch scores between treatments

	Heat Patch Score (as a percentage of total treatment)			
Treatment	1	2	3	Lost
14-d 50 PG	10^{x}	8 ^{x}	71 ^{x}	12 ^{x}
14-d 6 h PG	11^{x}	10	72 ^{x}	7
5-d CO Synch+CIDR	26^{y}	15^{y}	54 ^{y}	5 ^{y}

^{xy} means within a column with different superscripts differ ($P = 0.02$)

Table 4: TAI pregnancy rates across heat patch scores

	Heat Patch Score			
Treatment	1	2	3	Lost
14-d 50 PG	38%	52%	59%	39%
14-d 6 h PG	23%	48%	57%	65%
5-d CO Synch+CIDR	29%	46%	57%	27%

All significant variables ($P < 0.05$) were used in the statistical model to analyze pregnancy rate. Location ($P < 0.001$) had the largest effect.

Table 5: Pregnancy rates between treatments and locations

	Treatment					
	14-d 50 PG		14-d 6 h PG		5-d CO Synch +CIDR	
Location	Proportion	%	Proportion	%	Proportion	%
1	14/34	41	9/29	31	9/26	35
2	94/150	63[x]	90/144	63[x]	75/146	51[y]
3	16/48	37	18/49	37	17/150	34
4	8/10	80	8/11	73	8/13	62
Combined	132/242	55	125/233	54	110/235	46

[xy] means within a row with different superscripts differ ($P = 0.02$)

Timed AI pregnancy rates by treatment and location are shown in Table 5. There was no difference between the 14-d 50 PG treatment and 14-d 6 h PG treatment at Location 1 ($P = 0.86$), Location 2 ($P = 0.97$), Location 3 ($P = 0.72$), or Location 4 ($P = 0.69$). Because these 2 treatments were not different at any of the locations or overall ($P = 0.57$), we combined both treatments together and compared with the 5-d CO-Synch + CIDR® treatment.

The combined 14 d CIDR protocol was not different than the 5-d CO Synch+CIDR at location 1($P = 0.86$), location 3 ($P = 0.89$), or location 4 ($P = 0.36$). However at location 2 (the location with the largest number of heifers) the combined 14-d CIDR protocol had greater TAI rates than the 5 d control (63% vs. 51%; $P = 0.02$). Because treatment by location interaction was not significant ($P = 0.88$), TAI rates were combined across locations for an overall pregnancy rate for each treatment. The 14-d 50 PG treatment was not different than the 14-d 6 h PG treatment ($P = 0.57$), and the combined 14-d CIDR protocol was not different from the 5-d CO Synch+CIDR ($P = 0.20$).

Discussion

Timed AI results were mixed across the 4 locations in the study. However there were no treatment differences ($P > 0.10$) across all locations. The similar eficacy of the 50 mg dose of PGF to 2 injections 6 h apart has practical benefits for the beef producer facing time and labor constraints. This finding will prove important for the 14 d protocol.

Timed AI rates for all treatments at locations 1 and 3 were low. The 14 d CIDR treatment produced encouraging results at location 2 – the location with the largest sample size. The ability of these treatments to produce TAI rates above 60% in a protocol that takes 17 days to complete is encouraging given the fact producers cite time and labor as the primary reasons beef producers choose not to implement AI (NAHMS Survey, 2009).

The 14-d 50 PG TAI rate of 63% (94/150) compares well with other TAI protocol such as the CIDR Select TAI rate of 62% (67/108) (Leitman et al., 2008) and the 5-d CO-Synch + CIDR® TAI rate of 63.5% (47/74; (Bridges and Lake, 2011), especially considering the latter was the control in the present experiment. Results from the 14-d protocols at location 2 in this study are encouraging enough to call for future studies to further examine the robustness of the 14 d protocol with d 9 GnRH. Unfortunately this study only measured estrus response and pregnancy rate and therefore ultrasound data are not available to describe how the protocol affected ovarian dynamics. However, we can hypothesize why the protocol was successful.

One potential explanation for the success of the 14 d protocols relative to the 5 Day CO Synch+CIDR protocol is presynchronization. Administration of progestins induce peri-pubertal heifers into cycling status by increasing LH production (Anderson et al., 1996). The higher pregnancy rate in the 14 d protocols relative to the 5 day protocol could be due to the ability of the 14 d protocol to more effectively cause shallow anestrus heifers to cycle. However cycling status was not determined prior to protocol initiation, so this possibility remains speculative.

Another potential reason for success of the 14 d CIDR protocols could have been its ability to accurately synchronize follicular waves. Follicles grow in waves in cattle, and beef heifers typically have 2 or 3 waves of follicular growth (Sirois and Fortune, 1988) in each estrous cycle. However, progestin exposure has the potential to impede follicular waves through development of persistent follicles. Progestins are effective in producing negative feedback and preventing ovulation, but they can lead to persistent follicle formation (Kinder et al., 1996). It is likely that 14-d protocols eliminated persistent follicles due to d 9 GnRH. This could have produced a new follicle that ovulated a fertile oocyte.

The day of cycle upon protocol initiation also can affect responsiveness to GnRH (Geary et al., 2000) with only 14% of cattle on d 15 to 17 of their cycle responding to GnRH as opposed to a 100% response rate for cows receiving GnRH on d 0 to 5 of their estrous cycle. The 14-d 50 PG and 14-d 6 h PG treatments address this potential problem through the GnRH given on d 9. Heifers that begin 14 d protocols on d 15 of their estrous cycle would still be able to ovulate a fertile follicle at breeding because of the d 9 GnRH. Even if they do not respond to the initial d 0 GnRH given on d 15 of their estrous cycle, the d 9 GnRH would be given on d 3 of their estrous cycle, which should start a new follicular wave.

Further research that incorporates ultrasonography is needed to evaluate the ability of follicular waves to be synchronized by GnRH in the middle of a 14-d CIDR protocol. Such studies will help to clarify mechanisms that result in ovulating a fertile oocyte.

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COMPARISON OF PROTEIN AND COPPER SOURCES ON BIOAVAILABILITY IN RAINBOW TROUT

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ABSTRACT: Diets that provide adequate amounts of Cu for rainbow trout are necessary to maintain fish productivity. Few studies have compared inorganic and organic forms of supplemental Cu and source of supplemental protein on rate and efficiency of gain in rainbow trout diets. The objective of this study was to compare the effects of Cu source ($CuSO_4$ vs. Cu-Lys), dietary Cu level (0, 5, 10, 15 and 20 ppm) and diets with either fishmeal or plant-based protein fed for 14-wk. Diets were formulated to contain 40% CP and 20% crude lipid. Prior to feeding experimental diets, 576 juvenile rainbow trout (average 28g) were randomly allotted to 32, 168-L tanks and fed either the plant or fishmeal-based diets without supplemental Cu for a 2 wk depuration period. Following depuration, fish were offered the experimental diets for 12 wk. At 6 and 12 wk, 3-5 fish were removed from each tank and sacrificed to determine liver Cu concentrations. Fish fed plant-based diets had higher *($P < 0.05$)* ADG, and better *($P < 0.05$)* feed conversion ratio (FCR) than fish offered fishmeal-based diets. At both 3 and 6 wk, trout fed plant-based diets without Cu (0 ppm) had slower *($P < 0.05$)* growth rates and higher *($P < 0.05$)* feed intakes compared to fish fed Cu-supplemented plant-based diets. No growth differences were observed for trout fed any of the fishmeal-based diets. Liver Cu concentrations at 12 wk were higher *($P < 0.05$)* for fish fed plant-based protein compared to fishmeal-based protein. A quadratic response was measured *($P < 0.05$)* for increasing level of Cu supplementation at 6 wk. There was no effect *($P = 0.17$)* of Cu source on liver Cu concentrations. Supplemental Cu improved growth rate when trout were fed plant-based diets. No differences in liver Cu concentrations were detected due to Cu source at 12 wk.

Keywords: Copper, bioavailability, rainbow trout

Introduction

In order to meet the increased demand for aquaculture food products, sustainable, alternative protein-based diets are needed to support continued industry growth (Gatlin et al., 2007). However, replacement protein sources also must be examined for their potential effects on the uptake and utilization of other nutrients, including micronutrients such as vitamins and minerals (Barrows et al., 2008). To date, no research has examined the effects of plant-based diets on copper (Cu) uptake and utilization in rainbow trout.

Copper is a trace mineral involved in formation of hemoglobin and enzymes, bone growth and in immune responses (McDowell et al., 1992). Common indices of Cu status for rainbow trout include liver and whole-body composition, (Clearwater et al., 2002) plasma cerulosplasmin (Ammerman et al., 1995) and absence of growth suppression (Kamunde et al., 2002). The dietary Cu requirement for rainbow trout has been reported at 3 µg Cu/ g DM (Ogino & Yang et al., 1980).

Copper absorption in rainbow trout, is known to be affected by Cu source. Studies in rats (Guo et al., 2001) and heifers, (Rabiansky et al., 1999) found increased tissue Cu accumulation in Cu-Lysine fed animals when fed equal amounts of Cu-Lysine or $CuSO_4$. In contrast, other studies like those of Ward et al. (1993) with steers and a loading and depuration study by Kjoss et al. (2006) with rainbow trout, found that $CuSO_4$ and Cu-Lysine were equivalent in Cu bioavailability. However, in the Kjoss et al. (2006) study, the levels utilized were approximately 100 times the dietary requirement. Therefore, the objective of this study was to evaluate the effect of protein source (fishmeal or plant-based), Cu source (Cu-Lys or $CuSO_4$), and physiologically relevant levels of Cu on growth, feed efficiency and Cu uptake in rainbow trout during a 14-week growth trial.

Materials and Methods

Animals. The study was conducted over a 14- wk period from October 2010 to January 2011 at the Bozeman Fish Technology Center in Bozeman, Montana. A commercially available strain of juvenile rainbow trout (Trout Lodge, Seattle WA, USA) was used in the study. All procedures for fish care and handling were conducted in accordance with the USFWS, Bozeman Fish Technology Center Animal Care and Use Committee Guidelines.

Experimental Design: A factorial treatment design was employed to test the effects of dietary protein source (fishmeal or plant), Cu source ($CuSO_4$ or CuLys) and Cu level (0-20 Cu mg/kg) on Cu bioavailability in rainbow trout. A total of 32 -168 L tanks were stocked with 18 fish (average initial weight 28 g, (+/- 1.2 g per tank). Tank was the experimental unit for all response variables. Duplicate tanks were randomly assigned for each treatment. All tanks received 9.5 L of water per min and were connected via a recirculating water system with particulate and biological filtration to maintain optimum water quality. Water temperature was held constant at

15°C. Photoperiod was held constant at a 13:11 diurnal cycle.

Feeds and Feeding: Two basal diets (fishmeal vs plant-based) were formulated to contain 40% CP and 20% crude lipid. The protein sources for the plant-based diets included soy protein concentrate, corn protein concentrate, and soybean meal. The protein sources for the fishmeal-based diets were blood meal, menhaden meal, and soybean meal. Diets were formulated to contain either copper sulfate ($CuSO_4$) to provide 0, 5, 10, 20 ppm Cu or Cu-lysine to provide 0, 5, 10, 15 and 20 ppm Cu. Each diet was manufactured as a 3 mm sized pellet. Diets were processed using a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18 s exposure to 127°C in the extruder barrel. The die plate was water cooled to an average temperature of 60°C. Pressure at the die head varied from 200 to 400 psi. Pellets were dried in a pulse bed drier (Buhler AG, Uzwil, Switzerland) for 25 min at 102°C with a 10 min cooling period to a final moisture level of less than 10%. A topcoat of fish oil was added to the cooled feed using a vacuum-assisted top-coater (A.J. Mixing, Ontario, Canada). Diets were stored in plastic lined paper bags at room temperature until fed. Fish were fed twice daily to visual satiation, 6 d per wk. Weight gain and feed consumption was determined every three wk.

Tissue Sampling: Before initiation of experimental diet feeding, fish in each tank were randomly fed one of the two basal diets without Cu supplementation to "deplete" body stores. After depletion, fish within each basal diet group were randomly allocated to Cu source and level treatments and fed their respective diet for an additional 12 wks. At 6 wks and 12 wks, 3-5 fish, from each tank, were sampled to determine liver Cu levels. Copper levels were determined by inductively coupled plasma–optical emission spectroscopy following nitric acid digestion.

Statistical Analysis: Factorial analysis of variance (ANOVA) was used to identify the main effects of plant-based vs. fishmeal diets, Cu source ($CuSO_4$ vs. Cu-Lys), interactions of protein source by copper source, copper source by copper level, and regression analysis was used to examine the effects of increasing Cu levels (0, 5, 10, 15, 20 ppm). Differences were considered significant at $P < 0.05$.

Results and Discussion

Significant effects due to protein source and copper level were observed for ADG and feed conversion ratio (FCR). Fish fed plant-based diets had higher ($P < 0.05$) ADG (Figure 1), and better ($P < 0.05$) FCR (Figure 2) over the 12 wk growth period than fish fed the fishmeal-based diets. Protein source effects were significant ($P < 0.05$) by 3 wks. Trout fed plant-based diets, without Cu supplementation had lower ($P < 0.05$) growth rates and higher ($P < 0.05$) feed intakes compared to fish fed Cu-supplemented plant-based diets. It was inferred that plant-based diets with proper Cu supplementation can support growth equal or superior to traditional fishmeal-based diets (Gaylord et al., 2006). Results suggest that Cu supplementation did increase growth performance of rainbow trout fed plant-based diets, but no growth suppression was observed for trout fed the fishmeal-based diets without Cu supplementation. Barrows et al. (2008) found similar results with rainbow trout, where different vitamin premixes were necessary to optimize growth and FCR in plant-based diets compared to traditional fishmeal-based diets (Barrows et al., 2009).

An interaction between protein source and Cu source was observed for FCR. Fish fed fishmeal-based diets supplemented with $CuSO_4$, had higher growth rates ($P<0.05$) and lower FCRs, compared to fish fed fishmeal-based diets supplemented with Cu-Lysine at 6 wks; however, by 12 wks no significant ($P > 0.05$) effects due to Cu source were measured. Similar interactions between protein source and various nutrient supplementation have been demonstrated when rainbow trout have been offered plant-based vs fishmeal-based diets (Gaylord et al., 2006).

Dietary protein source and Cu level, but not Cu source, increased ($P < 0.05$) liver Cu stores in the present study. Fish fed Cu-supplemented plant-based diets had higher ($P < 0.01$) liver Cu concentrations than fish fed Cu-supplemented fishmeal-based diets at 6 wks. A quadratic response of increasing levels of Cu was measured at 6 wk ($P < 0.05$) irrespective of protein source or Cu source. Similar results were measured at 12 wks, with Cu- supplemented plant-based fed trout having higher ($P < 0.05$) liver Cu concentration levels (89.4 ppm) than trout fed Cu-supplemented fishmeal-based diets (65.1 ppm, Figure 3). Increased levels of dietary Cu resulted in increased liver Cu levels ($P < 0.05$), with a break point at approximately 10 ppm. No differences were measured in liver Cu concentrations due to Cu source ($P > 0.05$).

Implications

Trout fed plant-based diets over the 12 wk study had better rate and efficiency of growth than trout fed fishmeal-based diets. Liver Cu levels were higher in trout fed plant-based diets compared to trout fed fishmeal-based diets. No differences in growth rates or liver Cu concentration levels were observed for $CuSO_4$ or Cu-Lysine supplementation. Increasing levels of Cu supplementation resulted in higher liver Cu concentrations. In order to increase growth in trout fed plant-based diets, Cu supplementation between 5 and 10 ppm is recommended.

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Table 1. Ingredient Composition and Calculated Analysis of plant-based & fishmeal-based diets (g/100g).

Ingredients (%DM)	Plant-based	Fishmeal-based
Soy Protein Concentration	24.64	-
Fish Oil, Menhaden	15.59	16.75
Corn Protein Concentration	17.54	5.5
Wheat Flour	16.27	22.72
Soybean Meal, Solvent Extracted	13.3	12
Menhaden Meal, Mechanically	-	33.7
Blood Meal, Spray Dehydrated	-	7.43
Mono-Dical Phosphate	2.65	-
L-Lysine	1.99	-
Vitamin Premix ARS 702	1	1
Choline-CL	0.6	0.6
Potassium Chloride	0.56	-
Taurine	0.5	-
Dl-Methionine	0.5	-
Sodium Chloride	0.28	-
Threonine	0.23	-
Stay-C	0.2	0.2
Trace Mineral Premix[a]	0.1	0.1
Analyzed composition of basal diets		
Copper (mg/kg)	4.16	1.21
Formulated Composition		
Crude Protein (%)	40.1	40.1
Crude Fat (%)	20	20
Crude Fiber (%)	0.93	1.08

[a] Trace mineral premix; contributed in mg/kg of diet: zinc- 40; manganese – 17; and iodine – 6.

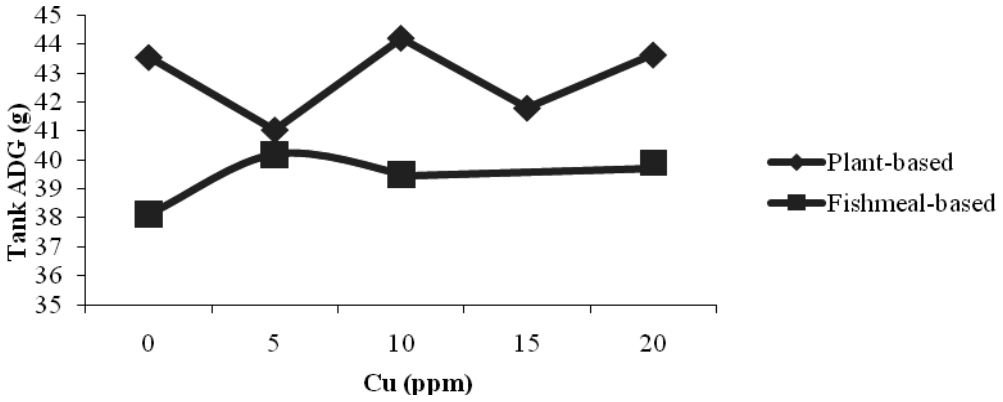

Figure 1. The effects of protein source (plant-based vs fishmeal-based) and level of dietary copper on daily gain by rainbow trout after 12 wk. Significant *($P < 0.05$)* effect due to protein source.

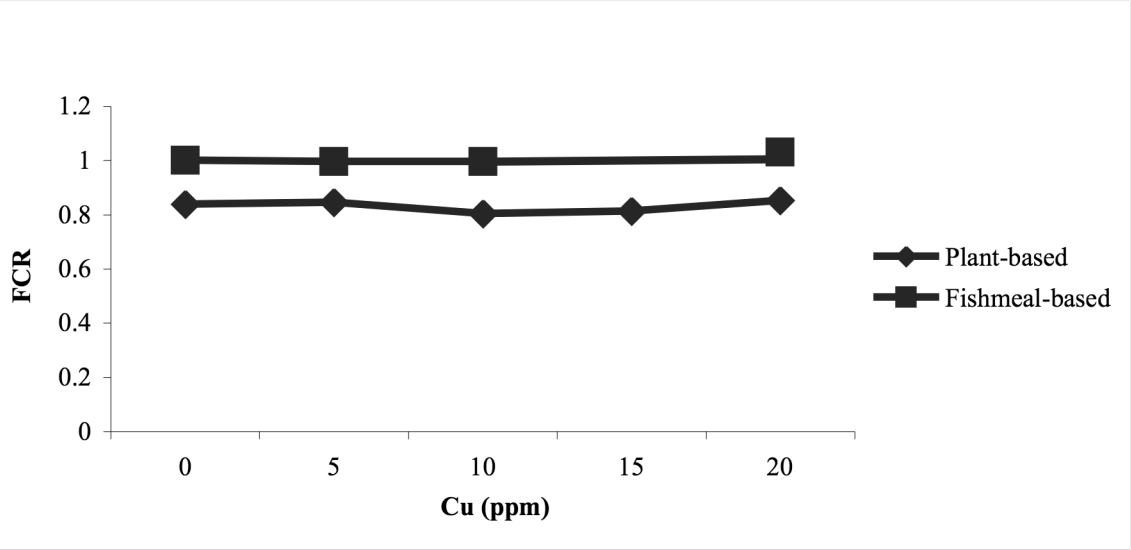

Figure 2. The effects of protein source (plant-based vs fishmeal-based) and level of dietary copper on feed conversion (FCR) by rainbow trout after 12 wk. Significant *(P < 0.05*) effect due to protein source.

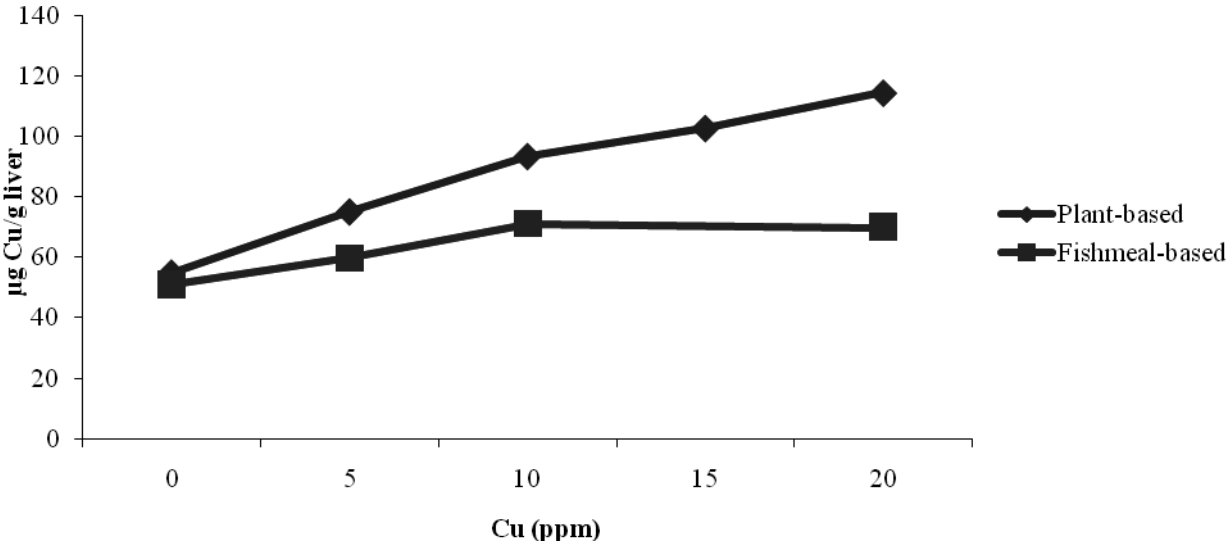

Figure 3. The effects of protein source (plant-based vs fishmeal-based) and level of dietary Cu on liver Cu concentrations in rainbow trout after 12 wk. Significant effect *(P < 0.05*) due to protein source and level of dietary copper.

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Effect of Selenium Source and Supplementation Rate in Ewes on Selenium Transfer from Ewe to Lamb and on Lamb Growth

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ABSRACT: Selenium (Se) is an essential micronutrient of sheep. Supplementation is important in young lambs to prevent Se-deficiency. The FDA regulates Se supplementation to ruminant diets at 0.3 mg/kg Se (as fed), however bioavailability differences between organic and inorganic Se sources exist, which may modify Se transfer to the newborn lamb, and subsequently affect lamb growth. To evaluate the effect of Se source and supplementation rate in ewes on Se status and growth of their offspring, 240 ewes (Suffolk, Polypay, and crossbred) were divided into 8 treatment groups and drenched weekly (at an amount equal to their summed daily intake) for one year, including during gestation and early lactation, with no Se (deficient); at recommended levels (0.3 mg/kg) with inorganic Na-selenite, Na-selenate, or organic Se-yeast (SeY); or at supranutritional levels (0.9 and 1.5 mg/kg) with Na-selenite or SeY. Selenium administered by weekly drenching of ewes during gestation and early lactation was effective at increasing Se concentrations in ewe colostrum and milk at 30 days in milk and in improving the Se status of lambs (whole-blood and serum-Se at birth, and skeletal-muscle Se at 14 days of age) ($P < 0.001$). Selenium concentrations in ewe milk and in lambs increased linearly with higher dosages of SeY ($P < 0.001$), whereas Se concentrations did not differ in ewes receiving 0.9 or 1.5 mg/kg of Na-selenite ($P > 0.05$). Lambs from ewes (in particular Suffolk ewes) receiving 1.5 mg/kg SeY did have greater 120-day body weights and growth rates than lambs from ewes receiving SeY at 0.3 mg/kg ($P = 0.10$; $P = 0.06$; respectively). We conclude that weekly oral drenching of ewes with SeY during gestation and early lactation is an effective method for improving Se status of lambs. Furthermore supranutritional SeY supplementation to ewes during pregnancy may improve growth performance of lambs

Key Words: Selenium, Sheep, Lambs, Se-Yeast, Na-Selenite.

Introduction

Selenium (Se) is an essential micronutrient in sheep and adequate Se transfer from ewes to lambs is important to prevent Se-responsive diseases such as nutritional myodegeneration and Se-responsive unthriftiness. Current FDA regulations limit the amount of dietary Se supplementation to 0.3 mg Se/kg diet (as fed), which is equivalent to 0.7 mg per head per day (FDA, 2009). Higher Se dosages are considered supranutritional. Current recommendations do not account for the chemical form of Se [e.g., Na-selenite (Na_2SeO_3), Na-selenate (Na_2SeO_4), or Se-yeast] and its effect on Se bioavailability, which may change with supplementation rate. Selenium sources can be classified into two categories: inorganic and organic. The most common inorganic Se sources are Na-selenite and Na-selenate, which are usually provided to sheep in mineral premixes or are injected. Organic Se sources are seleno-amino acids [e.g., selenomethionine (SeMet) and selenocysteine (SeCys)], which are found in Se-yeast or in feeds grown on Se-rich soils.

Provision of Se to the dam during gestation and early lactation is thought to be an effective method to meet Se requirements in newborn lambs. Selenium efficiently crosses the placental barrier into fetal tissues and enters mammary secretions, i.e., colostrum or milk (Abd El-Ghany et al., 2007; Rock et al., 2001), with a greater transfer efficiency for dietary organic Se versus inorganic Na-selenite (Rock et al., 2001; Taylor et al., 2009). The objective of this study was to further evaluate the effect of Se source and supplementation rate in ewes on the transfer of Se from ewes to their progeny and the influence on lamb growth.

Materials and Methods

Animals and Study Design. Procedures were approved by the Institutional Animal Care and Use Committees of Oregon State University. This was a prospective, placebo-controlled clinical trial of 12-months duration involving 240 mature ewes from three genotypes (Polypay, Suffolk, and crossbred). Ewes ranged in age and BW from 2 to 6 yr, and 51 to 93 kg, respectively. The experiments were conducted at the Oregon State University Sheep Center, Corvallis, Oregon. Ewes were randomly assigned to 8 treatment groups (n = 30 each) based on Se

supplementation rate (0, 0.7, 2.1, and 3.5 mg/d) and source [Na_2SeO_3, Na_2SeO_4 (0.7 mg/d only), and Se-yeast]. Treatment groups were blocked for footrot (FR) incidence and severity, breed, and age of ewe. The three Se dosages corresponded to 0.7 mg/d or 1x the FDA allowed supplementation rate (0.3 mg/kg as fed); 2.1 mg/d or 3x the FDA allowed supplementation rate (0.9 mg/kg as fed); and 3.5 mg/d or 5x the FDA allowed rate (1.5 mg/kg as fed).

Selenium Sources. Two inorganic Se sources were used: Na-selenite and Na-selenate, both from the same source Selenium supplements were solubilized in water and administered orally once weekly (at an amount equal to the summed daily intake). Thus, multiplying the daily rates of 0.7, 2.1 and 3.5 mg/d by 7 days, the amount of Se administered per week was calculated to be 4.9, 14.7, and 24.5 mg Se per ewe, respectively. A Se analysis of the individual doses of Na-selenite prepared was performed at the Center for Nutrition, Diagnostic Center for Population and Animal Health, Michigan State University (East Lansing, MI) and doses were found to be within expected analytical variance of their target concentrations (4.85, 14.85, and 24.6 mg Se, respectively). The weekly dose of inorganic Na-selenate was 82.5% higher (8.95 mg per dose or 1.27 mg/d) than the targeted concentration of 4.9 mg per dose.

Selenium Administration. Selenium treatments were administered individually by oral drench once weekly. Control ewes received water. The Se dose was suspended in a reasonable volume of water (5 mL for inorganic Se; more water was needed for the organic solutions, i.e., 11, 30 and 48 mL for 0.7, 2.1, and 3.5 mg/kg solutions, respectively), made up fresh each week, and administered with a dose syringe as sheep moved through a cutting chute. Color coding of Se sources to match ewe eartags was utilized to maintain dosing accuracy. Sheep were treated once weekly for a total of 52 weeks. Lambs did not receive any additional Se supplementation after birth.

Sample Collection. Jugular venous blood was collected from all ewes every 3 months, and from a subset of ewes (16 out of 30 ewes) in the control and all 0.7 mg/d dose groups, respectively. Immediately after parturition and before lambs had nursed, jugular venous blood was collected from lambs. Colostrum samples were collected immediately following parturition. Milk samples were collected again 30 d after parturition. Skeletal-muscle samples were collected from lambs at 14 days of age coinciding with tail docking. Tails were docked using a hot-docking method and skeletal muscle samples were collected from the coccygeal vertebrae.

Performance Measures. The 90 and 120-day weaning weights, ADG's, were calculated using the American Sheep Industry Association (ASI, 2002) formulas.

Selenium Analysis. Selenium concentrations in WB, colostrum, milk, and muscle samples were determined by a commercial laboratory (Center for Nutrition, Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI) using an ICP-MS.

Statistics. Statistical analyses were performed using SAS, version 9.1 (SAS, Inc., Cary, NC, USA) software. Ewes that did not give birth or rear a lamb were excluded from the statistical analysis. Values for multiple lambs from the same ewe were averaged because ewe was the experimental unit. The effect of source and amount of Se supplement on Se concentrations of WB, serum, colostrum, and 30-day milk in ewes; and on WB and serum in lambs at birth, and muscle at 14-days of age were analyzed using PROC GLM, 120 day weights were analyzed using Proc Mixed. Covariates in the model were FR-status (yes, no), breed (Polypay, Suffolk or crossbred), number of lambs born (1, >1). To evaluate whether FR-status, breed, number of lambs born, and number of lambs reared (1, >1) modified the effect of Se source and amount on WB- and serum-Se concentrations, data were additionally stratified by FR-status, breed, number of lambs born, and number of lambs reared, respectively. The effect of Se depletion on WB- and serum-Se concentrations was evaluated by comparing the estimated values of the no-Se control group with the estimated values of all Se groups receiving 0.7 mg Se/d (NRC-recommended Se dosage). The effect of Se source was evaluated by comparing the estimated values of groups receiving different Se sources at the same dosage. The effect of Se amount was evaluated by comparing the estimated values of groups receiving different Se dosages within the same Se source. Data are reported as least square means ± SEM. Statistical significance was declared at $P \leq 0.05$except for 120 day weights being declared significant at $P \leq 0.10$.

Results

Effect of Dietary Se Depletion on Se Transfer Efficiency. Ewes receiving no Se supplementation had lower WB-Se concentrations compared to ewes receiving 0.7 mg Se/d (64% lower compared to Na-selenite; 69% lower compared to Na-selenate; 73% lower compared to Se-yeast; Figure 1A) and lower serum-Se concentrations (55% lower compared to Na-selenite; 61% lower compared to Na-selenate; 63% lower compared to Se-yeast; Figure 1B) in blood samples analyzed within 30 days of lambing (all $P < 0.0001$). The Se decrease was greater in WB than in serum . Ewes receiving no Se supplementation also had lower colostral-Se concentrations compared to ewes receiving 0.7 mg Se/d (81% lower compared to Na-selenite; 86% lower compared to Na-selenate; 90% lower compared to Se-yeast; all $P < 0.0001$; Figure 1C) and similar, albeit smaller changes in milk-Se concentrations at 30 days in milk (35% lower compared to Na-selenite, $P = 0.17$; 47% lower compared to Na-selenate, $P = 0.02$; 61% lower compared to Se-yeast, $P < 0.0001$; Figure 1D). Lambs from ewes receiving no Se supplementation had lower WB-Se concentrations at birth (55% lower compared to Na-selenite; 62% lower compared to Na-selenate; 76% lower compared to Se-yeast) (all $P < 0.0001$; Figure 1E). Furthermore, lamb serum-Se concentrations were lower

(40% lower compared to Na-selenite; 46% lower compared to Na-selenate; 64% lower compared to Se-yeast (all $P < 0.0001$; Figure 1F), although not to the same degree as lamb WB-Se concentrations. Control ewes had similar WB-Se concentrations as their offspring (Figure 2C), although their serum-Se concentrations were higher than in their offspring, indicating that lambs of non Se supplemented ewes had higher Se concentrations in the non-serum WB fraction (Figure 2D).

Lambs from ewes receiving no Se supplementation had lower skeletal-muscle Se concentrations (-73%) than lambs from ewes receiving 0.7 mg/d Se-yeast ($P < 0.0001$), yet only numerically lower Se concentrations compared to lambs from ewes receiving Na-selenite (-37%; $P = 0.23$) or Na-selenate (-48%; $P = 0.05$; Figure 1G).

Effect of Dietary Se Source, Orally Drenched at 0.7 mg/d, on Se Transfer. At the FDA-recommended supplementation rate of 0.7 mg Se/d, organic Se-yeast resulted in higher WB- and serum-Se concentrations in ewes than inorganic Na-selenite (31% greater for WB; 22% greater for serum; both $P < 0.0001$) and Na-selenate (16% greater for WB, $P = 0.0005$; 7% numerically greater for serum, $P = 0.06$) (Figures 1A, 1B). Colostral Se concentrations in Se-yeast supplemented ewes were 83% higher compared to Na-selenite ($P < 0.0001$) and 33% higher compared to Na-selenate ($P = 0.02$; Figure 1C); and milk Se concentrations at 30 days in milk were 67% higher compared to Na-selenite ($P = 0.01$); and 37% numerically higher compared to Na-selenate ($P = 0.08$; Figure 1D).

When the two inorganic Se sources were compared, ewes receiving inorganic Se in the form of Na-selenate had significantly higher Se concentrations in WB and serum than ewes receiving inorganic Na-selenite ($P = 0.009$) $P = 0.001$) respectively (Figures 1A, 1B) and lacteal secretions (38% higher for colostrum, $P = 0.05$; 22% numerically higher for milk, $P = 0.40$) (Figures 1C, 1D). However, the Se dosage administered was 82% higher for Na-selenate than for Na-selenite.

Similar changes in WB- and serum-Se concentrations were observed in lambs as in their mothers (Figures 1E, 1F). Lambs of Se-yeast supplemented ewes had greater WB-Se concentrations than their mothers (7%), whereas lambs receiving inorganic Na-selenate (-20%) or Na-selenite (-24%) had lower WB-Se concentrations than their mothers (Figure 2C). Lambs from Se-yeast supplemented ewes had higher skeletal-muscle Se concentrations compared to lambs from ewes receiving inorganic Se (133% higher compared to Na-selenite, $P < 0.0001$; 92% higher compared to Na-selenate, $P = 0.0002$) (Figure 1G).

Effect of Supranutritional Dietary Se Supplementation on Se Transfer. Ewes receiving Na-selenite at supranutritional supplementation rates (combined 2.1 and 3.5 mg/d ewe treatment groups) had increased WB-Se concentrations (30% higher than in ewes receiving Na-selenite at 0.7 mg/d; Figure 3A) and increased serum-Se concentrations (34% higher than in ewes receiving Na-selenite at 0.7 mg/d; Figure 3B) (both $P < 0.0001$). Ewes receiving Se-yeast at supranutritional supplementation rates also had increased WB-Se (62% higher than in ewes receiving Se-yeast at 0.7 mg/d; Figure 3A) and increased serum-Se concentrations (38% higher than in ewes receiving Se-yeast at 0.7 mg/d; Figure 3B) (both $P < 0.0001$). When Na-selenite supplementation rate was increased from 2.1 to 3.5 mg/d, increases in WB-Se (5%; $P = 0.18$) and serum-Se concentrations (11%; $P = 0.0009$) were smaller, and for WB not significant. This was different when the Na-selenite supplementation rate was increased from 0.7 to 2.1 mg/d; both WB- and serum-Se concentrations increased 27% (both $P < 0.0001$; Figures 3A, 3B). In contrast, when Se-yeast supplementation rate was increased from 2.1 to 3.5 mg/d, similar increases in WB-Se (35%; $P < 0.0001$) and serum-Se concentrations (19%; $P < 0.0001$) were noted compared to when the Se-yeast supplementation rate was increased from 0.7 to 2.1 mg/d (38% increase in WB-Se and 26% increase in serum-Se concentrations; both $P < 0.0001$; Figures 3A, 3B). Selenium supplementation at 0.7 mg/d with Se-yeast resulted in similar WB-Se concentrations in ewes as did 3 or 5 times this rate of Se supplementation with Na-selenite (Figure 3A).

An increased supplementation rate from 0.7 to 3.5 mg/d with Se-yeast increased colostral-Se concentration 163% ($P < 0.0001$) and milk-Se concentration 144% ($P < 0.0001$) versus Na-selenite at the same supplementation rates increased colostral-Se concentration 110% ($P = 0.002$) and milk-Se concentration 53% ($P = 0.03$) (Figures 3C, 3D). The dosage range over which supplementation of ewes with Na-selenite was able to increase Se concentrations in lamb WB and serum was limited (Figures 3E, 3F). Whole-blood and serum-Se concentrations did not differ between lambs from ewes receiving 2.1 and 3.5 mg/d of Na-selenite (Figures 3E, 3F). In contrast, the dosage range over which supplementation of ewes with Se-yeast was able to increase Se concentrations in lamb WB and serum extended to at least 3.5 mg/d, as lamb WB-Se (25%) and lamb serum-Se (45%) concentrations continued to increase as supplementation rates in ewes increased from 2.1 to 3.5 mg/d (both $P < 0.0001$; Figures 3E, 3F). As a result, WB- and serum-Se concentrations in lambs from ewes receiving 0.7 mg/d of Se-yeast were 22% higher for WB ($P = 0.0003$) and 16% higher for serum ($P = 0.02$) than Se concentrations in lambs from ewes receiving five times as much (3.5 mg/d) Na-selenite (Figures 3E, 3F).

The lamb muscle-Se response to Na-selenite supplementation started to plateau in lambs from ewes receiving 2.1 mg/d of Na-selenite (442 ng/g at 2.1 mg/d versus 482 ng/g at 3.5 mg/d; $P = 0.66$; Figure 3G). In contrast, the lamb muscle-Se response to Se-yeast supplementation accelerated when ewe dosages were increased from 2.1 to 3.5 mg/d (71% increase, $P < 0.0001$), compared to the increase from 0.7 to 2.1 mg/d for Se-yeast (40% increase, $P = 0.003$) (Figure 3G). As a result, muscle-Se concentrations in lambs from ewes receiving 0.7 mg/d of Se-yeast were 47% higher ($P = 0.01$) than in lambs from ewes receiving five times as much (3.5 mg/d) Na-selenite (Figure 3G).

90 &120-day Body Weights and Average Daily Gains. Lambs born from ewes receiving Se as SeY or sodium selenite at 0.3mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not have higher averages for 90- and 120-day body weights than lambs from ewes not receiving Se (P >0.05). Lambs born from ewes receiving supranutritional Se as SeY at 1.5 mg/kg had greater 120-day body weights than lambs from ewes receiving SeY at 0.3 mg/kg (P =0.10). See Figure 1**.** This trend was also reflected in lamb average daily gain (ADG) to 120 days, (P =0.06). See Figure 4. When comparing 120-day body weights and ADG of lambs from Suffolk ewes receiving SeY at 1.5 mg/kg to the lambs from Suffolk ewes receiving SeY at 0.3 mg/kg, the 1.5 mg/kg group had greater 120-day body weights and ADG (P =0.07 and P =0.09, respectively). See Figure 4. In contrast to the SeY groups, lambs born from ewes receiving supranutritional Se as sodium selenite at 0.9 mg/kg and 1.5 mg/kg did not have higher averages for 90- and 120-day body weights and 90- and 120-day ADG than lambs from ewes receiving sodium selenite at 0.3 mg/kg. Finally, lambs born from ewes receiving inorganic selenite or selenate at 0.3 mg/kg had similar 90- and 120-day body weights and 90- and 120-day ADG.

Discussion

Weekly oral Se-yeast drenches administered at the FDA-recommended concentration of 0.7 mg/d was as effective at improving Se status in ewes as supranutritional supplementation with 3.5 mg/d Na-selenite, and even more effective at improving Se status of lambs, highlighting the superior bioavailability of Se-yeast. Thus, organic Se is transferred over a wide range of supplementation rates more efficiently from ewe to lamb than inorganic Se.

Others have shown that Se status of newborn lambs is closely correlated to Se status of their mothers (Abd El-Ghany et al., 2007). Thus, transplacental transfer of Se is the primary source of Se in newborn lambs prior to ingestion of colostrum. In our study, transplacental transfer of Se from ewe to lamb was affected by both the source of Se (chemical form) as well as the dose of Se administered. This was best illustrated in WB- and serum-Se measurements of lambs born to ewes receiving supranutritional doses of Se-yeast. Lamb WB- and serum-Se concentrations in our study are also consistent with findings of Davis et al. (2006b) and Juniper et al. (2008), although our study is unique in that different sources and rates of Se supplementation were studied to assess their effects on Se status of newborn lambs. Additionally, our study compared supranutritional Se dosages to ewes that were closer to the current 0.7 mg/d NRC recommendations (i.e., 2.1 and 3.5 mg/d) compared to other studies that were aimed at determining maximum tolerable concentrations (Davis et al., 2006a; Tiwary et al., 2006). Findings from our study suggest that Se concentrations of milk are low at 30 days of lactation, and contribute less to Se status of the lamb than colostral Se (981.8 ng/mL).

This study has two major implications for the sheep industry. First, drenching of ewes with Na-selenite, although relatively cheap, is an ineffective method to improve Se status of newborn lambs. This is likely because of its low bioavailability and limited half-life (Whanger, 2002). Second, Se-yeast drenching of pregnant ewes, although potentially more expensive, provides an effective method to improve Se status of newborn lambs. The benefits for lambs born with adequate levels of Se are enhanced absorption of maternal antibodies in colostrum (Rock et al., 2001) and modulation of immune function, including better responses to vaccination (reviewed in Rooke et al., 2004). In addition, organic Se stored as SeMet in skeletal muscle provides a reserve pool such that Se-yeast drenching prior to the lambing period may eliminate the need for costly injections or mineral Se pre-mixes of lambs marketed within 210 days of birth (Juniper et al., 2008).

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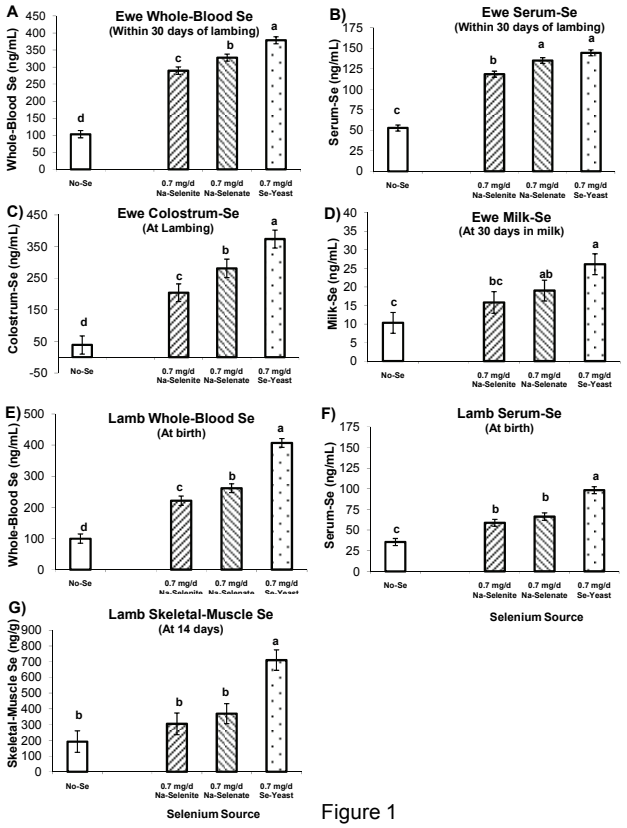

Figure 1

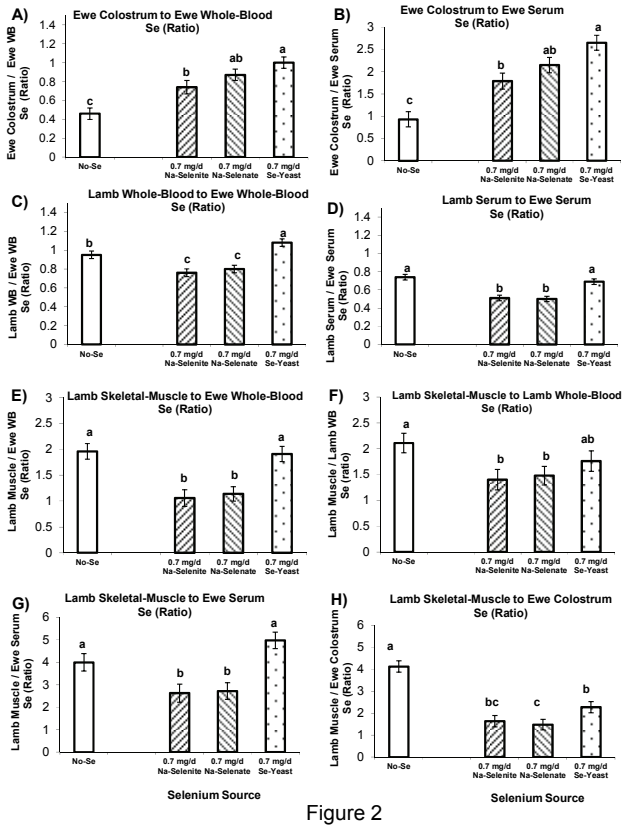

Figure 2

Figure 1. Effect of dietary Se depletion (no Se supplementation) and Se supplementation at the FDA-recommended dietary Se concentration (0.7 mg/d) with either inorganic Na-selenate, inorganic Na-selenite, or organic Se-yeast on Se concentrations (least-squares mean ± SEM) of **A**) ewe whole-blood (within 30 days of lambing), **B**) ewe serum (within 30 days of lambing), **C**) ewe colostrum, and **D**) ewe milk (at 30 days in milk); and **E**) lamb whole-blood (at birth), **F**) lamb serum (at birth), and **G**) lamb skeletal-muscle (at 14 days). [a,b,c,d] Bars without a common superscript differ ($P < 0.05$).

Figure 2. Effect of dietary Se depletion (no Se supplementation) and Se supplementation at the FDA-recommended dietary Se concentration (0.7 mg/d) with either inorganic Na-selenate, inorganic Na-selenite, or organic Se-yeast on Se-transfer efficiency ratios (least-squares mean ± SEM) of **A**) ewe colostrum to ewe whole-blood, **B**) ewe colostrum to ewe serum, **C**) lamb whole-blood to ewe whole-blood, **D**) lamb serum to ewe serum, **E**) lamb skeletal-muscle to ewe whole-blood, **F**) lamb skeletal-muscle to lamb whole-blood, **G**) lamb skeletal-muscle to ewe serum, and **H**) lamb skeletal-muscle to ewe colostrum. [a,b,c] Bars without a common superscript differ ($P < 0.05$)

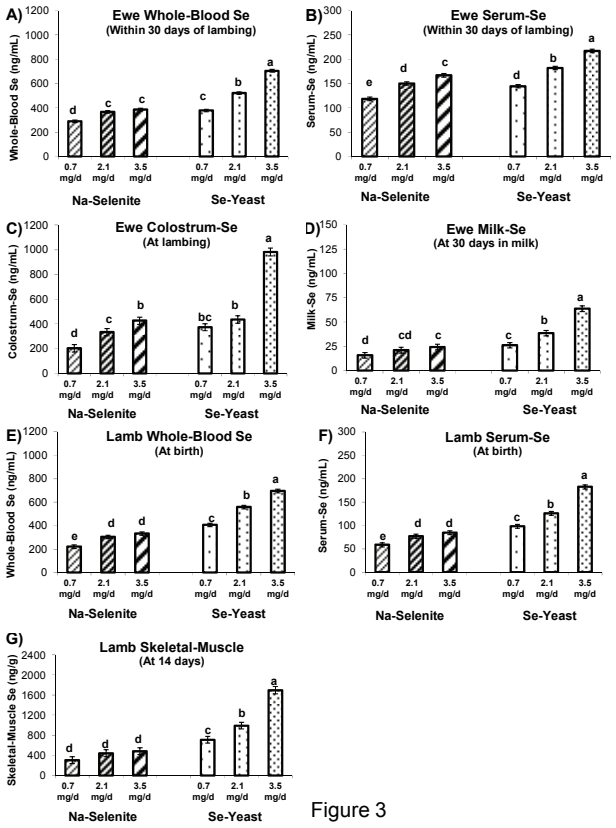

Figure 3

Figure 3. Effect of the FDA-recommended dietary Se concentration (0.7 mg/d) and supranutritional dietary Se supplementation (2.1 and 3.5 mg/d) with either Na-selenite or Se-yeast on Se concentrations (least-squares mean ± SEM) in **A**) ewe whole-blood (within 30 days of lambing), **B**) ewe serum (within 30 days of lambing), **C**) ewe colostrum, and **D**) ewe milk (at 30 days in milk); and **E**) lamb whole-blood (at birth), **F**) lamb serum (at birth), and **G**) lamb skeletal-muscle (at 14 days). [a,b,c,d,e] Bars without a common superscript differ ($P < 0.05$).

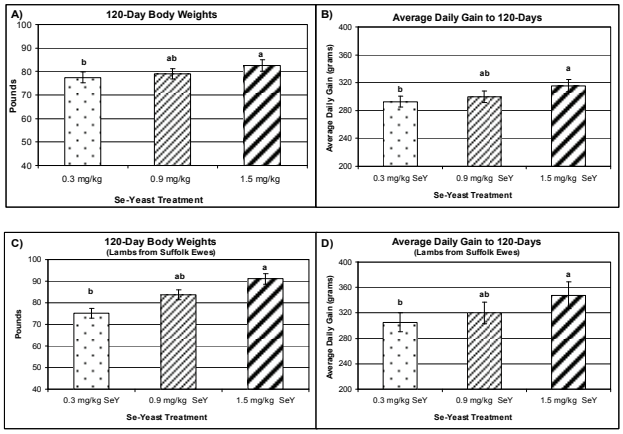

Figure 4. A) Effect of the FDA-recommended dietary Se concentration (0.7 mg/d) and supranutritional dietary Se supplementation (2.1 and 3.5 mg/d) with Se-yeast (least-squares mean ± SEM) on 120-day body weights, **B)** 120-day ADG, **C)** 120-day body weights of lambs from Suffolk ewes, **D)** 120-ADG of lambs from Suffolk ewes. [a,b,] Bars without a common superscript differ ($P \leq 0.10$).

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THE EFFECT OF FLUOXETINE ON EARLY LACTATION AND LAMB GROWTH IN SHEEP[†]

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ABSTRACT: Fluoxetine (a selective serotonin reuptake inhibitor; **FLX**) has been shown to delay the onset of lactogenesis stage II when taken during pregnancy and lactation in women. A study was conducted to evaluate if ewes would be an appropriate model to determine the effects of FLX on milk production. Eighteen ewes (BW = 91 ± 12 kg; BCS = 2.0 ± 0.5) in late gestation carrying twins were chosen and allotted to treatments by breeding date. Ewes were orally dosed daily at 0700 h by top dressing 454 g of ground corn with 0 or 80 mg FLX. Dosing began approximately on d 126 of gestation and continued until 3 wk post-lambing. Following parturition, ewes were allowed time to bond with their lambs before being moved to an indoor facility. Light hours mimicked the natural daylight hours, with lights on at 0700 h and off at 1900 h. Ewes were housed indoors for 3 wk, and were fed ground alfalfa hay twice daily at 0700 h and 1800 h. Forage intake was measured for 3 wk post-lambing (corn remained constant at 454 g/d). Milk yield was estimated on d 1, 2, 3, and 5 post-lambing at 1200 h and 1800 h, depending on the time of lambing. Milk yields were measured over a 3 h period during which lamb(s) were removed. Milk samples were collected at each milking, however only d 2 was analyzed for fat, protein, lactose, and somatic cell count. We observed no treatment differences (P = 0.59) or day effects on milk yield (P = 0.98). Lambs were weighed at birth (d 0), d 7 and d 14. We observed no differences (P > 0.05) between treatments in either birth weight or lamb gain. Milk composition did not differ (P > 0.05) among control and FLX ewes. Forage intake was similar amid control and FLX ewes (P = 0.32), but intake increased (P < 0.001) as days post-lambing increased. Overall, FLX had no effect on milk yield or composition, lamb birth weight or gain, or forage intake in ewes. Therefore, the ewe does not appear to be a suitable model to evaluate the FLX response in humans.

Key Words: fluoxetine, lactation, sheep

Introduction

Fluoxetine (FLX; Prozac, Eli Lilly & Co., Indianapolis, IN) and other selective serotonin reuptake inhibitors (SSRI) have become popular for the treatment of depression during pregnancy because of their safety, effectiveness, and lower occurrence of maternal side effects (Nonacs and Cohen, 2002; Simon et al., 2002). In 1987, FLX became the first SSRI introduced in North America (Catterson and Preskhorn, 1996; Hiemke and Härtter, 2000). Selective serotonin reuptake inhibitors act to increase extracellular serotonin (5-HT) levels sharply over a short period of time, while acting on serotonergic neurotransmissions on a continual basis. These events could lead to the reported adverse effects on pregnancy outcome and postnatal development in humans (Morrison et al., 2002; Laine et al., 2003). Serotonin's role in the mammary system and lactation has only recently been observed (Matsuda et al., 2004; Hernandez et al., 2008). It is likely that 5-HT is part of the autocrine-paracrine homeostatic feedback mechanism (feedback inhibitor of lactation), which resists endocrine stimulation of mammary development and milk secretion (Wilde et al., 1995; Matsuda et al., 2004). Thus, SSRI act to inhibit lactation by preventing reuptake of 5-HT, and its subsequent degradation into its metabolite 5-hydroxy indole acetic acid. Additionally, FLX has been shown to decrease food intake and body weight in humans (McGuirk and Silverstone, 1990; Foltin et al., 1996) and rats (Rowland et al., 1982; Wong and Fuller, 1987). Harding and Bocking (2001) noted that the fetal lamb and human are alike in physiologic functions. As a result, pregnant sheep are often utilized to evaluate maternal-fetal drug disposition and effects (Rurak et al., 1991). Our objective was to evaluate if ewes would be an appropriate model for studying the effects of FLX on lactation. We hypothesized that FLX would depress lactation and forage intake in ewes during the first 5 d of lactation, possibly decreasing lamb growth.

Materials and Methods

Animals, Facilities, and Diet. Procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Eighteen ewes (BW = 91 ± 12 kg; BCS = 2.0 ± 0.5) in late gestation were used in this experiment. Ewes were fed once daily 2.7 kg chopped alfalfa hay and 454 g ground corn during the last trimester of pregnancy and continued until parturition. Ewes were penned in dry lot pens with fence-line bunks where they received alfalfa hay. At 0700 h each day ewes were individually penned in 1.5 x 1.5 m pen and fed corn to facilitate the treatment administration until parturition. Following parturition, ewes were allowed time to bond with their lambs in a 1.5 x 1.5 m pen, before being moved to an indoor facility where they were individually penned in 3 x 2 m pens for 3 wk. Light hours mimicked the natural daylight hours, with lights on at 0700 h and off at 1900 h. While housed indoors, forage intake was measured. Ewes were fed ground alfalfa hay twice daily at 0700 h and 1800 h, while corn was fed once at 0700 h. Initially, ewes were fed 2727 g daily with half of the amount fed at each feeding and then were gradually increased by 113.6 g based on feed refusals

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from the previous day (< 50 g). Water was provided *ad libitum* and routinely cleaned multiple times daily. At birth, lambs were individually identified by unique premise ear tag and scrapie tag; their navels dipped with iodine and birth weights recorded. Lamb weights were measured on d 7 and 14 post-lambing, and average daily gain was calculated accordingly.

Design and Treatments. The experiment was a completely randomized design with ewe as the experimental unit. Ewes carrying twins (determined by external flank ultrasound (3.5 MHz probe; SSD-500V, Aloka Co., Tokyo, Japan) at approximately d 70 of gestation) were selected and stratified by breeding date to treatments. Ewes were separated into 2 pens, with 5 ewes from each treatment randomly assigned to each pen on approximately d 126 of gestation. Dosing also began on this day and continued until 3 wk post-lambing. Treatments consisted of no FLX (control), or 80 mg FLX. Ewes were orally dosed daily at 0700 h by top dressing 454 g of ground corn with 0 or 80 mg FLX. Fluoxetine capsules, 40 mg each, were opened and contents were dispensed on moistened corn (5 mL water) and were rinsed out with 5 mL water. Control corn was also top dressed with 10 mL water total.

Collections. Milk yield measurements and samples were taken on d 1, 2, 3, and 5 postpartum at either 0900 h and 1200 h or 1500 h and 1800 h, depending on the time of lambing. Milking times were selected by choosing the time closest to 24 h post-lambing. Milk collections followed procedures reported by Reynolds and Brown (1991), with the use of an oxytocic compound intravenously. Milk yields were measured over a 3 h period after lamb(s) were removed. At each milking, milk samples were collected and placed in a container that included a preservative pearl and were refrigerated until shipping, normally 3-5 d. Only the d 2 sample was analyzed for fat, protein, lactose, and somatic cell count at a commercial laboratory (Arizona DHIA, Tempe, AZ). Throughout the study, behavior changes were noted by visual assessment and recorded.

Statistical Analysis. The MIXED model of SAS (SAS Inst. Inc., Cary, NC) was used to analyze milk yield, milk composition, lamb birth weight, lamb ADG, and forage intake. Individual ewe was the experimental unit for all variables except lamb ADG in which lamb was the experimental unit. Milk production was analyzed as a split plot design with treatment in the whole plot and day, kg of lamb, and appropriate interactions in the sub plot. Forage intake was analyzed as a repeated measure over the 21 d time period.

Results

We observed no behavioral responses during the dosing period, parturition or lactation. No interactions were observed in milk yield and composition or forage intake, so only main effects are presented (Table 1). Daily milk yields were similar ($P = 0.59$) between control and FLX treatments. Surprisingly, milk yield was similar ($P = 0.98$) among the first 5 d of lactation. The daily milk yield values for d 1, 2, 3, and 5 were 351.3, 348.1, 365.4, and 347.8 g, respectively. Milk composition did not differ ($P > 0.25$) among control and FLX ewes. A treatment by day interaction ($P = 0.97$) was not evident for daily forage intake. Forage intake was similar between control and FLX ewes ($P = 0.32$), but intake increased ($P < 0.001$) as days post-lambing increased (Figure 1).

Lamb birth weight and ADG are presented in Table 2. Lambs were weighed at birth (d 0), d 7 and d 14. We observed no differences ($P > 0.65$) between treatments in either lamb birth weight or ADG.

Discussion

Serotonin has been previously reported to depress lactation in mouse, bovine, and human models (Matusda et al., 2004; Hernandez et al., 2008; Marshall et al., 2010). Matsuda et al. (2004) reported that 5-HT plays a role in mouse mammary gland development and homeostasis. When mouse mammary epithelial cells were treated with different levels of 5-HT, PRL-induced β-casein gene expression was repressed in a level-dependent manner. Also, upon inhibition of 5-HT synthesis through blockade of tryptophan hydroxylase I, milk protein gene expression was increased (Matsuda et al., 2004). Hernandez et al. (2008) reported similar findings through an in vitro experiment using cultures with lactogenic medium. They observed that 5-HT restricted milk protein mRNA expression in dairy cattle and suggested that 5-HT acts as a negative regulator of lactation.

Selective 5-HT reuptake inhibitors act to increase the bioavailability of 5-HT by preventing its reuptake into the cell, and subsequent degradation into its metabolite. In lactating mice, a local treatment of the lactating mammary gland with FLX resulted in involution of the mammary gland (Marshall et al., 2010). Additionally, a delay was noted in the onset of lactogenesis stage II in humans who had taken SSRI during pregnancy and lactation. However, in a previous study conducted by our laboratory, (Black et al., 2010) a 40 mg oral dose during the last 3 wk prior to parturition did not depress lactation. We believe that the dosage level of 40 mg per day was too low to elicit a measurable decrease in lactation. In an earlier study conducted by our laboratory, (Yates et al., 2010), we observed that passage through the rumen reduced the level of FLX absorbed into the circulatory system by more than half (15 mg) of the 40 mg daily dose of FLX. Serotonin has been shown to elicit a biphasic effect on tight junctions, the junctional complexes that close at lactation and open at involution, with lower concentrations resulting in a decrease in tight junction permeability, and higher concentrations increasing tight junction permeability (Pai and Horseman, 2008). Thus, these previous studies led us to an increased dosage level of 80 mg and over a longer time period of 3 wk post-parturition. However, in the current study we found that our 80 mg dose still did not depress lactation. But in general terms, we did observe a numeric decrease (9.5%) in milk yield values of FLX-treated ewes.

Milk composition may be affected by the treatment of FLX. Our laboratory group conducted a previous experiment evaluating milk composition of ewes that were treated with a 40 mg dose of FLX orally for 3 wk prior to parturition. An FLX effect was observed on fat

content as treated ewes had significantly higher fat content compared to the control ewes; however protein, lactose, and somatic cell count were unaffected (P. Black, unpublished data). In the current experiment, we analyzed the d 2 milk sample and observed no treatment differences in composition. The previous data was the result of milk samples that were collected on d 1, 3, and 5 (post-lambing) that were frozen after collection for several months until analysis, whereas in the current study, milk samples were placed in a container with a preservative and refrigerated until shipping and analysis, usually 3-5 d. The difference in how the samples were treated may have caused the difference in the results.

While the direct effects of SSRI treatments are to increase the amount of extracellular serotonin, further side effects are possible. Weight loss is a common side effect when adults take FLX; researchers suggested that FLX may directly decrease weight gain in infants who receive FLX through breast milk (Chambers et al., 1999). Chambers et al. (1996) suggested that weight loss during pregnancy of FLX treated women could be linked directly to decreased birth weights due to lower maternal weight gain which would limit fetal growth. In humans, reduced birth weights and postnatal weight gain were observed when women were exposed to FLX during their third trimester (Cohen et al., 2000; Nordeng et al., 2001). A study conducted with rats, showed that pregnant rats receiving FLX had lower weight gain and delivered smaller pups (Vorhees et al., 1994). No differences were observed potentially due to differences in fetal numbers, as several treated and control ewes in the current study had a single or triplets, potentially making it difficult to actually quantify differences in birth weight. Additionally, prior to lambing, we experienced an abnormally cold weather pattern, possibly altering birth weights of all lambs due to shorten gestation periods. Similar lamb ADG may be due to the lack of differences in birth weight and milk production.

In humans, FLX has been shown to alter food intake. Foltin et al., (1996) observed that individuals receiving 40 mg daily of FLX had lower food intake due to a decreased number of eating occasions. McGuirk and Silverstone (1990) also found a similar result as study participants' intake decreased on d 1 and 8, following a 2 wk daily treatment of a 60 mg dose of FLX. They however found no depression of appetite on d 15 post-treatment. In rats, Uphouse et al., (2006) observed that treatment of 10 mg daily caused a depression of food intake. Researchers similarly observed that daily injection of FLX decreased food intake in rats (Rowland et al., 1982; Wong and Fuller, 1987). Potentially due to differences in the digestive physiology of monogastrics vs. ruminants, we did not find a difference in forage intake between control and treated ewes.

Implications

Fluoxetine when dosed orally to pregnant and lactating ewes did not alter milk yield or composition, or resulting lamb weight and gains. Furthermore, forage intake was not decreased in ewes receiving fluoxetine compared to controls. Thus, based on current and previous research we propose that sheep are not an appropriate model to assess the fluoxetine response in humans when administered orally.

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Table 1. Average daily milk yields during the first 5 d of lactation in Suffolk-cross ewes treated with 0 mg fluoxetine (Control) and with 80 mg fluoxetine (FLX) beginning on d 126 of gestation and continuing 3 wk post-lambing.

Item	Control	FLX	SEM[5]	*P*-value
Milk yield, g[1,2]	370.6	335.7	45.1	0.59
Milk composition[3]				
Fat, %	8.8	9.5	0.41	0.25
Protein, %	6.2	6.2	0.15	0.84
Lactose, %	4.4	4.3	0.10	0.42
Somatic cell count , cfu/mL	969.9	717.7	288.6	0.55
Forage intake, g[4]	3352.5	3050.8	157.1	0.32

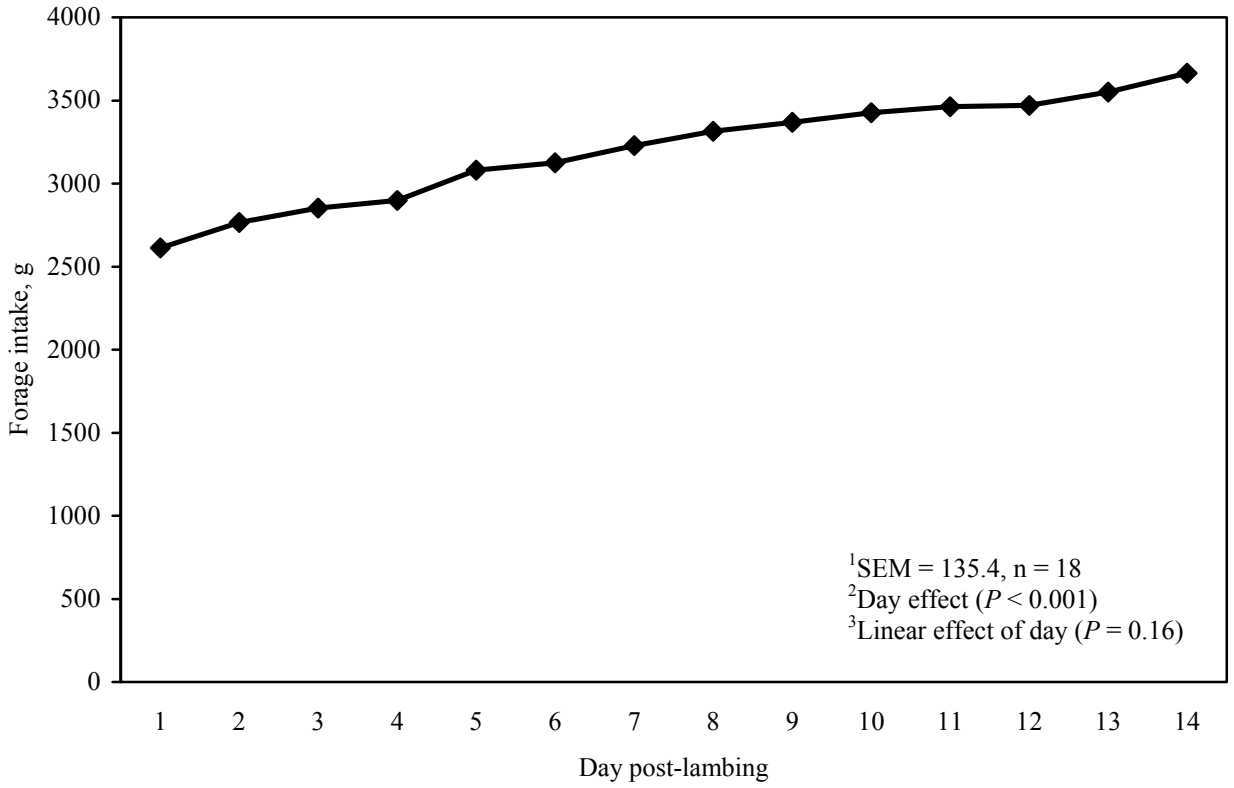
[1]Milk yields were measured over a 3 h period after lamb removal.
[2]A treatment by day interaction was not observed *(P* = 0.99); therefore, means are presented for treatments across days.
[3]Sample analyzed was d 2.
[4]Intake was average over a 14 d period post-lambing.
[5]Standard error of the mean, n = 18.

Table 2. Lamb birth weight and gain over a 14 d post-lambing period in Suffolk-cross ewes treated with 0 mg fluoxetine (Control) and with 80 mg fluoxetine (FLX) beginning on d 126 of gestation and continuing 3 wk post-lambing.

Item	Control	FLX	SEM[3]	*P*-value
Birth weight, kg	4.7	4.3	0.2	0.35
ADG d 1-7, kg[1]	0.32	0.29	0.02	0.65
ADG d 1-14, kg[2]	0.33	0.34	0.03	0.76

[1]Gain was calculated by taking d 7 body weight minus birth weight and dividing it by 7
[2]Gain was calculated by taking d 14 body weight minus birth weight and dividing it by 14
[3]Standard error of the mean, n = 30.

Figure 1. Daily forage intake over a 14 d post-lambing period in Suffolk-cross ewes.[1,2,3]



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EFFECTS OF FLAXSEED LEVEL AND PROCESSING ON SITE AND EXTENT OF DIGESTION IN BEEF COWS FED NATIVE HAY

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ABSTRACT[1]: The objective of this study was to evaluate the effects of flaxseed level (0.17 and 0.42% of BW) and processing (whole, rolled or ground) on site and extent of digestion in beef cows consuming chopped native grass hay (10% CP, 75% NDF). Six Angus cows (BW = 590 ± 26 kg) fitted with ruminal and duodenal cannulae were used in a 6 × 6 Latin square with a 2 × 3 factorial arrangement of treatments. Experimental periods were 21 d in length with each period consisting of 17 d for diet adaptation and 4 d of intensive sampling. There was a level × processing interaction ($P \leq 0.013$) for forage and total OM intake. However, no interactions ($P > 0.23$) were observed for duodenal and fecal OM flow. True ruminal OM digestibility (% of intake) was not different ($P = 0.49$) across treatments. Nevertheless, a tendency for a level × processing interaction was observed ($P = 0.08$) for total tract OM digestibility. There was a level × processing interaction ($P = 0.03$) for total N intake. As expected, total N flow to the duodenum was greater ($P = 0.009$) when flaxseed was fed at 0.42% of BW compared to 0.17%. Providing flaxseed at 0.42% of BW increased ($P = 0.013$) duodenal non-microbial non-NH_3 flow. True ruminal N and NDF digestibility did not differ ($P \geq 0.29$) due to flaxseed level or processing. Total tract NDF digestibility tended ($P = 0.07$) to be lower with processing, however, level had no affect ($P = 0.74$). Results from this study indicate that it is unnecessary to process flaxseed, irrespective of level, when supplemented to cows consuming forage-based diets.

Key Words: Digestion, Flaxseed, Processing

Introduction

Flaxseed is extensively used as a feed supplement in beef and dairy cattle in the northern plains of the United States and Canada, due to its high protein and oil content (Petit, 2010). Within flax, 40% of the total seed weight comes in the form of oil (Mustafa et al., 2002) with 50% of that oil being α-linolenic acid (18:3n-3; Petit 2002, 2003). Recently, researchers have looked at using flaxseed as a potential supplement to increase omega-3 fatty acids in milk (Soita et al, 2003) and fresh meat (Maddock et al, 2006). However, concerns about decreased digestibility and total OM intake exist due to the high lipid content of the flaxseed. Research has shown that flaxseed can be included in dairy cows diets up to 15% of total dietary DMI (Petit, 2010) and up to 8% of diet DM in feedlots with no effects on DMI (Maddock et al., 2006) without negatively impacting animal performance. Processing of oilseeds increases performance characteristics of cattle in feedlots. Specifically, Maddock et al. (2006) showed an increase ($P \leq 0.08$) in ADG and G:F in feedlot heifers when feeding rolled or ground flaxseed compared to the whole seed.

In forage-based diets, processing of oilseeds may not be as necessary due to the greater extent of mastication of the diet. Keele et al. (1989) suggested that the seed coat of whole cottonseeds provide protection from ruminal biohydrogenation due in part to extensive mastication of the seeds. Therefore, livestock producers wishing to provide flaxseed to cattle consuming forage-based diets could reduce feed costs by reducing processing costs. We hypothesized that the processing of flaxseed would not alter the digestion of flaxseed in beef cattle consuming a forage diet. Therefore, the objective of this study was to evaluate the effects of flaxseed level and processing on site and extent of digestion in beef cows consuming chopped native grass hay.

Materials and Methods

Animals and diets

All experimental procedures were reviewed and approved by the Northern Great Plains Research Laboratory, Animal Care and Use Committee. Six Angus cows (BW = 590 ± 26 kg) fitted with ruminal and duodenal cannulae were used in a 6 × 6 Latin square with a 2 × 3 factorial arrangement of treatments. Chopped native grass hay (10% CP, 75% NDF, DM basis) was offered at 5% above the previous day's intake. Flaxseed was fed at two levels (0.17% or 0.42% of BW, DM basis) with three methods of processing (whole, rolled or ground). Processing of flaxseed was conducted using a roller mill (Peerless F6S9, Peerless International Inc., Joplin, MO) with roller gap set at 1 cm, which resulted in approximately 50% of all seeds being crushed. A hammer mill (Bearcat 1830C, Western Land Roller, Hastings, NE) with a 3 cm screen was used to grind the flaxseed. Animals were randomly assigned to treatment. Experimental periods were 21 d in length with 17 d for diet adaptation and 4 d of intensive sampling. Animals were housed in a temperature controlled barn within a 3.3 m × 2.7 m pen equipped with water cups. Cows were fed twice daily at 0700 and 1900. Hay and flax were fed in separate feeders and orts were recorded each day. Flax seed was

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always completely consumed before each feeding. Gelatin capsules (Torpac Inc, Fairfield, NJ) containing 5 g of TiO_2 were dosed intraruminally at each feeding as a marker for digesta flow. Animals were given free choice access to trace mineralized salt (American Stockman Trace Mineralized Salt, North American Salt Co., Overland Park, KS; NaCl >95.5%, Zn >3,500 mg/kg, Fe >2,000 mg/kg, Mn >1,800 mg/kg, Cu >280 mg/kg, I >100 mg/kg, Co >60 mg/kg). Chemical composition of hay and flaxseed is presented in Table 1.

Table 1. Chemical composition of hay, whole flaxseed, rolled flaxseed and ground flaxseed fed to beef cows[1]

Item	Hay	Whole flaxseed	Rolled flaxseed	Ground flaxseed
DM	95.0	95.3	95.4	95.3
OM, % of DM	91.1	94.0	92.7	92.8
CP, % of OM	10.0	22.8	22.4	22.5
NDF, % of OM	75.0	25.6	28.1	24.6

[1]Samples for nutrient analysis were taken on d 17 through d 21 of each period.

Sampling and laboratory analysis

On d-18, starting at 0400 of each sampling period, duodenal (200 mL) and fecal (50 mL) samples were taken every 4 h. On d-19, collection times were advanced 2 h to allow for samples to represent every 2 h in a 24 h period. Fecal samples were dried in a 55°C forced-air oven, ground (Wiley mill, 1-mm screen) and composited within cow for each period. Equal amounts of duodenal digesta samples were composited within cow for each period and immediately frozen. Duodenal samples were then lyophilized (Freezemobile 25SL Freeze Dryer, The VirTis Co., Gardiner, NY) and ground (1-mm screen).

On d 20, prior to the 0700 feeding, 200 mL of Co-EDTA (5 g of Co; Uden et al., 1980) was dosed intraruminally and whole rumen contents were collected (0 h) and again at 3, 6, 9, 12, 15, 18, 21 and 24 h post dosing. Ruminal pH was immediately collected using a combination electrode (Orion Research Inc., Boston, MA) and samples were processed as described by Scholljegerdes and Kronberg (2008).

All feed, microbe, duodenal digesta, and fecal samples were analyzed for DM and ash (AOAC, 1990). Nitrogen content of feed, microbes, duodenal digesta, and feces were determined using a Carlo Erba Model NA 1500 Series 2 N/C/S analyzer (CE Elantech, Lakewood, NJ). Neutral detergent fiber of feed, duodenal digesta, and feces were determined using an ANKOM 200 fiber analyzer (ANKOM Technology, Fairport, NY). Duodenal and fecal samples were analyzed for TiO_2 according to the procedures of Myers et al. (2004).

Duodenal and isolated bacteria samples were analyzed for purine concentration as described by Zinn and Owens (1986) using a plate reader (Synergy HT, Bio-Tek Instruments, Inc., Winooskie, VT)

Ruminal fluid samples were centrifuged at 10,000 × *g* for 20 min at 4°C, and a 2.5 mL aliquot was added to 0.5 mL of 25% metaphosphoric acid containing 2 g/L of 2-ethyl-butyric acid (Goetsch and Galyean, 1983). These samples were analyzed for concentrations of VFA using a Varian 3800 GC equipped with a 15 m × 0.533 mm (i.d.) column (Nukol, Supelco, Bellefonte, PA). Approximately 100 mg of duodenal digesta were reconstituted to 3% DM using 0.1 *N* HCl for subsequent analysis of NH_3 concentration (Hannah et al., 1991). Reconstituted duodenal digesta and rumen fluid NH_3 concentration was determined by the phenol-hypochlorite procedure (Broderick and Kang, 1980) using a spectrophotometer (DU-640, Beckman Instruments Inc., Fullerton, CA). Ruminal fluid Co concentrations were determined using an air-plus-acetylene flame by atomic absorption spectroscopy (model 3110, Perkin Elmer Inc., Norwalk, CT).

Calculations and statistical analysis

Digesta flow was calculated by dividing the amount of TiO_2 administered by the concentration of TiO_2 in the duodenal or fecal sample. Duodenal flow of N and NDF equaled the nutrient concentration in duodenal OM multiplied by duodenal OM flow. The microbial purine:N ratio was calculated by dividing microbial purine content by the amount N in bacteria. Duodenal non-microbial non-NH_3 N was calculated by subtracting duodenal microbial N and ammonia N flow from total duodenal N flow. Ruminal fluid passage rate was determined by regressing the natural logarithm of Co concentration against sampling time (Uden et al., 1980).

All data were analyzed using the MIXED model of SAS (SAS Inst. Inc., Cary, NC) as a 6 × 6 Latin square experiment with a 2 x 3 factorial arrangement of treatments. The model included period, level, processing method, and level × processing method. Animal was used in the random statement. All time course data included the effects of period, level, processing method, time and all possible interactions. Autoregressive order one was determined to be the most desirable covariance structure according to the Akaike's information criterion. Treatment differences were considered significant at an alpha of $P < 0.05$.

Results and Discussion

OM intake and digestibility

There was a level × processing interaction ($P \leq 0.013$) for forage and total OM intake (Table 2). Forage and total OM intake was greatest for cows consuming whole flaxseed at 0.17% of BW and least for cows consuming rolled flaxseed. This did not hold true at the 0.42% of BW level as the intake was highest for rolled flaxseed and lowest for cows fed whole flaxseed. Increasing whole flaxseed in forage-based diets decreased forage intake (Scholljegerdes and Kronberg, 2008). The differences observed between the current experiment and that of Scholljegerdes and Kronberg (2008) are likely due to the fact that whole flaxseed was fed at increasing levels and was compared to an unsupplemented control. In the present experiment, there was a numerical decline in forage intake for cows fed whole flaxseed at 0.17 to 0.42% of BW. However, it is not

completely clear as to why intake did not respond similarly when flaxseed was processed. It is possible, that by rolling the flaxseed, plant proteins were more available to ruminal bacteria, with some of the fatty acids being partially protected. Whereas, the lack of difference observed between increasing levels of ground oilseeds has been reported previously (Murphy et al., 1987).

No interactions ($P > 0.23$) were observed for duodenal and fecal OM flow. True ruminal OM digestibility (% of intake) was not different ($P = 0.49$) across treatments. This response is similar to that reported previously by our laboratory (Scholljegerdes and Kronberg, 2008) where flaxseed was fed at similar levels. Dietary total fatty acid content was 3.2 and 5.8% for 0.17 and 0.42% of BW, respectively. A tendency for a level × processing interaction was observed ($P = 0.08$) for total tract OM digestibility.

N intake and digestibility

A level × processing interaction ($P = 0.03$) was noted for total N intake, which was due to differences observed for OM intake (Table 3). There was also an increase ($P < 0.001$) of N intake at higher levels of supplementation. As expected, total N flow to the duodenum was greater ($P = 0.009$) when flaxseed was fed at 0.42% of BW compared to 0.17%. Providing flaxseed at 0.42% as compared to 0.17% of BW increased ($P = 0.013$) duodenal non-microbial non-NH_3 flow. This agrees with previous work performed by Scholljegerdes and Kronberg (2008) whom observed an increase in duodenal non-microbial non-NH_3 flow when beef heifers were supplementing 0.91 and 1.82 kg/d whole flaxseed and consumed native grass hay. Despite a numerical decline in non-microbial non-NH_3, no differences were detected ($P = 0.361$) across processing methods. True ruminal N digestibility did not differ ($P = 0.323$). However, there was a level × processing interaction for total tract N digestibility ($P = 0.05$). Total tract N digestibility declined with processing at the 0.17% of BW level, whereas, N digestibility increased with processing and a higher flax inclusion level.

NDF intake and digestibility

A level × processing interaction was observed for NDF intake ($P = 0.01$), with a tendency for an interaction ($P = 0.06$) being noted for total tract NDF digestibility (Table 4). Cows consuming whole flaxseed at 0.17% of BW had greater total tract NDF digestibility then cows consuming rolled flaxseed. Whereas cows fed rolled flaxseed at 0.42% of BW had greater total tract NDF digestibility then cows consuming whole flaxseed. This increase in NDF digestibility is likely responsible for the increase observed in OM intake. da Silva et al. (2007) confirmed that the processing of flaxseed increased OM digestibility. True ruminal NDF digestibility did not differ across treatments ($P = 0.29$). Total tract NDF digestibility tended ($P = 0.07$) to be lower with processing, however, level had no affect ($P = 0.74$). This was most likely caused by the increase in release of oil from the seed during processing. Scott et al. (1991) reported decreased fiber digestibility as a result of grinding soybeans.

Ruminal fluid passage rate, pH, and VFA

Level × processing interactions were detected for ruminal pH ($P = 0.037$) and NH_3 ($P = 0.015$). Despite differences being observed, all ruminal pH values are within the optimal range needed for fiber digestion with a range of 6.33 to 6.61 (Hoover, 1986). Ruminal NH_3 levels increased in magnitude as the extent of processing increased. Ruminal NH_3 concentrations were lower ($P < 0.001$) at the 0.17% level than at the 0.42% level. Ruminal NH_3 concentration were greater ($P < 0.001$) when flaxseed was processed. Gilbery et al. (2008) showed a decrease in ruminal NH_3 concentrations (4.60 vs. 6.40 m*M*) in Holstein steers when whole flaxseed replaced linseed meal and corn at 8% of diet DM.

There was no level × processing effect on fluid passage rate ($P = 0.945$). Neither level nor processing affected fluid passage rate ($P \geq 0.295$) in beef cows fed flaxseed and consuming native grass hay. Total ruminal VFA concentrations did not differ ($P \geq 0.804$) with level or processing, which resulted in no ($P = 0.245$) level × processing interaction. Price et al. (2008) saw similar results when supplementing lipids from oilseeds to add an additional 3% fat in the diet of sheep.

Providing greater quantities of flaxseed in the diet decreased ruminal molar proportions of acetate ($P < 0.001$) while increasing propionate ($P = 0.001$). As a result, acetate:propionate was lower for cattle fed flaxseed at 0.42% of BW. The observation that increasing levels of fat increase the ruminal molar proportion of propionate is well documented (Krysl et al., 1991; Whitney et al., 2000; Scholljegerdes et al., 2004).

Implication

Processing of whole flaxseed will not alter intake or digestion and thus the cost of processing can be considered an unnecessary expense for ranchers whom desire to include flaxseed in forage-based diets.

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Table 2. Influence of supplemental flaxseed level and processing on OM intake, flow, and digestibility in beef cows consuming native grass hay[1]

	0.17%			0.42%				Contrasts[3]		
Item	Whole	Rolled	Ground	Whole	Rolled	Ground	SEM[2]	Level	Proc[3]	L×P[3]
OM Intake										
Forage	11427	10001	10460	10139	10940	10432	536	0.69	0.54	0.011
Total	12317	10904	11328	12305	13089	12597	549	0.002	0.53	0.013
OM flow, g/d										
Duodenal	3429	3287	3845	3857	3841	3526	263	0.31	0.89	0.23
Microbial	413	548	595	449	431	379	98.4	0.225	0.808	0.457
Fecal	2171	2340	2356	2493	2563	2578	104	0.005	0.29	0.82
OM digestibility										
True ruminal, % of intake[4]	75.0	73.6	70.5	72.1	73.4	73.9	2.69	0.97	0.82	0.49
Lower tract, % of duodenal flow										
	34.2	28.5	37.9	33.5	34.2	26.2	3.98	0.50	0.82	0.11
Total tract, % of intake	81.8	77.5	78.7	79.6	80.2	78.0	1.25	0.76	0.146	0.082

[1]Treatments: Level = 0.17% and 0.42% of BW (DM basis) and processing = whole, rolled or ground flaxseed.
[2]n = 6.
[3]Proc = Processing, L × P = Level × Processing interaction.
[4]Corrected for microbial OM.

Table 3. Influence of supplemental flaxseed level and processing on N intake, flow, and digestibility (OM basis) in beef heifers consuming native grass hay[1]

	0.17%			0.42%				Contrasts[3]		
Item	Whole	Rolled	Ground	Whole	Rolled	Ground	SEM[2]	Level	Proc[3]	L×P[3]
N intake, g/d	223	203	208	252	259	256	9.67	<0.001	0.39	0.03
Duodenal N flow, g/d										
Total	114	116	120	136	138	127	7.17	0.009	0.89	0.50
Microbial	37.8	48.6	51.5	38.9	35.8	31.5	8.47	0.137	0.893	0.46
NH_3	23.8	25.6	29.4	26.1	35.6	37.4	2.10	<0.001	0.002	0.19
Non-microbial non-NH_3[4]	50.7	43.2	39.6	72.5	66.6	56.5	9.39	0.013	0.361	0.94
Fecal N flow, g/d	45.0	45.3	47.1	60.0	57.3	51.5	2.17	<0.001	0.20	0.04
N digestibility										
True ruminal, % of intake[5]										
	77.5	77.6	79.3	71.4	74.0	77.2	4.74	0.323	0.709	0.91
Lower tract, % of duodenal flow										
	60.5	60.2	60.5	55.3	58.8	58.4	2.45	0.157	0.756	0.71
Total tract, % of intake	79.2	76.3	77.0	76.1	77.8	79.3	1.25	0.82	0.62	0.05

[1]Treatments: Level = 0.17% and 0.42% of BW (DM basis) and processing = whole, rolled or ground flaxseed.
[2]n = 6.
[3]Proc = Processing, L × P = Level × Processing interaction.
[4]Nonammonia, nonmicrobial N.
[5]Corrected for microbial N and NH_3.

Table 4. Influence of supplemental flaxseed level and processing on NDF intake, flow, and digestibility (OM basis) in beef heifers consuming native grass hay[1]

	0.17%			0.42%				Contrasts		
Item	Whole	Rolled	Ground	Whole	Rolled	Ground	SEM[2]	Level	Proc[3]	L×P[3]
NDF intake	8593	7497	7863	7623	8233	7864	410	0.76	0.57	0.01
Duodenal NDF flow, g/d	598	336	718	543	340	79.5	190	0.15	0.47	0.20
Fecal NDF flow, g/d	1725	1876	1924	1817	1866	1982.0	82	0.48	0.06	0.77
NDF digestibility										
Ruminal, % of intake	70.9	69.1	67.0	67.9	72.9	71.7	2.97	0.44	0.79	0.29
Lower tract, % of duodenal flow										
	23.3	13.3	23.3	20.3	13.4	3.2	6.39	0.15	0.34	0.25
Total tract, % of intake	78.9	73.5	74.5	75.5	76.7	73.5	1.55	0.74	0.07	0.06

[1]Treatments: Level = 0.17% and 0.42% of BW (DM basis) and processing = whole, rolled or ground flaxseed.
[2]n = 6.
[3]Proc = Processing, L × P = Level × Processing interaction.

Table 5. Influence of supplemental flaxseed level and processing on ruminal pH, NH_3, fluid passage rate, and VFA in beef heifers consuming native grass hay

	0.17%			0.42%				Contrasts[3]		
Item	Whole	Rolled	Ground	Whole	Rolled	Ground	SEM[2]	Level	Proc[3]	L×P[3]
Ruminal pH	6.59	6.61	6.52	6.50	6.33	6.36	0.05	<0.001	0.020	0.037
Ruminal NH_3, m*M*	6.65	7.39	7.47	8.14	10.69	10.75	0.42	<0.001	<0.001	0.015
Fluid passage rate, %/h	4.51	4.36	4.89	4.97	5.20	5.38	0.69	0.295	0.779	0.945
Ruminal total VFA, m*M*	52.59	49.11	50.14	47.94	52.28	50.09	2.50	0.804	0.967	0.245
Ruminal VFA, mol/100 mol										
Acetate	71.76	71.17	71.51	70.80	69.81	69.96	0.27	<0.001	0.018	0.513
Propionate	15.94	16.38	16.22	17.28	17.15	17.11	0.27	0.001	0.804	0.435
Butyrate	8.75	8.34	8.60	8.98	9.19	9.24	0.20	0.001	0.653	0.172
Isobutyrate	1.31	1.40	1.37	1.41	1.40	1.40	0.04	0.147	0.525	0.374
Isovalerate	1.49	1.64	1.59	1.64	1.70	1.68	0.06	0.037	0.122	0.639
Valerate	0.781	0.777	0.779	0.844	0.854	0.810	0.02	<0.001	0.278	0.360
Acetate:propionate	4.51	4.35	4.42	4.11	4.08	4.09	0.07	<0.001	0.323	0.528

[1]Treatments: Level = 0.17% and 0.42% of BW (DM basis) and processing = whole, rolled or ground flaxseed.
[2]n = 6.
[3]Proc = Processing, L × P = Level × Processing interaction.

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EFFECT OF SPAYING AND TYPE OF IMPLANT DURING GRAZING ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF HEIFERS

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ABSTRACT: Fall-weaned crossbred heifer calves (n = 580; 197 ± 24 kg) were utilized in a split plot design to determine the effect of spaying and type of grazing implant on feedlot performance and carcass characteristics. At the beginning of winter grazing, half of the heifers were vaginally spayed using the K-R instrument and placed on two native range pastures with equal numbers of intact and spayed heifers per pasture. At the end of winter grazing (114 d), half of the heifers within each spay treatment were implanted with Revalor-G (**REV-G**) or Component E-H with Tylan (**COMP**) for a 106-d (April 7 – July 22) summer grazing period. Following summer grazing, heifers within each spay x implant treatment group were split into light and heavy weight blocks. The light weight block was allotted to one of 24 feedlot pens (6 pens/treatment) at the research feedlot and fed for 165 d. The heavy weight block was fed at a commercial feedlot (1 pen/treatment) for 130 d. All heifers were implanted with Revalor-IH on d 0 of finishing and re-implanted ~76 d prior to slaughter with Revalor-200. Melengestrol acetate was not fed at either feedlot location. Winter ADG of intact and spayed heifers was 0.11 and 0.10 kg/d. For ADG during summer grazing, there was a trend ($P = 0.09$) for a spay x implant interaction because of greater ADG for COMP compared with REV-G in spayed heifers, but not in the intact heifers. There was a trend ($P = 0.09$) for a spay x implant interaction for feedlot ADG and DMI, but only spaying influenced ($P < 0.05$) G:F (0.162 vs. 0.156). Spayed heifers had greater ADG compared to intact heifers when implanted with REV-G, but not with COMP. Implanting with REV-G tended ($P < 0.10$) to decrease DMI compared with COMP for intact heifers, but not spayed heifers. HCW tended ($P < 0.10$) to be decreased for REV-G compared with COMP in intact heifers but not spayed heifers, whereas, LM area was lower ($P < 0.05$) for REV-G compared with COMP in spayed heifers but not intact heifers. Overall carcass maturity tended ($P < 0.13$) to be decreased by spaying and use of REV-G grazing implant. In summary, spaying heifers in combination with different types of grazing implant affected grazing and feedlot performance; however, effects were inconsistent. Spaying improved feed efficiency and decreased maturity scores. Implanting spayed heifers with COMP may be more beneficial due to increased gain during grazing and increased LM area without affecting carcass quality.

Key Words: carcass characteristics, feedlot performance, grazing implants, heifers, spaying

Introduction

Spaying heifers has been a management tool producers have utilized for over a hundred years. Spaying is used to eliminate the chance of pregnancy and diminish the negative effects of estrous activity on feed efficiency in the feedlot (Hamernik et al., 1985). In a summary of a Feeder/Packer survey, Bennett (1985) showed approximately 17% of the heifers on feed are pregnant having a $30.32 lower value compared with open heifers. Field et al. (1996) reported that spaying heifers decreased overall maturity scores when age at slaughter was approximately 32 months, which falls within the USDA's age range for cattle to produce "B" maturity carcasses. In a recent Beef Quality Audit (Garcia et al., 2008), 3% of carcasses from 10,000 steers, heifers, cows, and bullocks graded either "B" or "C" maturity.

The use of an anabolic implant has been shown to improve performance of heifers during the stocker and feedlot phases of production. ZoBell et al. (1993) reported ADG was improved 9.4% in implanted heifers grazing brome grass/alfalfa pastures compared to non-implanted heifers. Rupp's (1987) summary of multiple studies showed that leaving heifers intact results in a 7.9% improvement in ADG compared to spayed heifers, but performance losses due to spaying were recovered when given an anabolic implant. Cameron et al. (1977) showed spayed heifers implanted with either Ralgro or Synovex-H had similar rates of gain during the grazing phase compared with intact, implanted heifers. Therefore, the hypothesis of this study is that the combination of spaying and use of anabolic implants can be used to improve feed efficiency and reduce maturity scores of yearling feedlot heifers while maintaining rate of gain. Therefore, the objective was to examine the effect of spaying and type of grazing implant on feedlot performance and final carcass characteristics in fall-weaned heifer calves grazing tallgrass native range.

Materials and Methods

Winter Grazing. Five-hundred eighty fall-weaned crossbred heifer calves (197 ± 24 kg) were utilized in this experiment. Heifers were sired by Simbrah, Brangus, or Braford bulls bred to 3/8 Brahman cows. Heifers were initially processed on one of two consecutive days (Dec. 14 and 15) where each heifer was weighed and tagged using an individual electronic identification and visual treatment tag. Every other heifer was vaginally spayed by an experienced veterinarian using the K-R instrument as described by Rupp and Kimberling (1982).

Heifers that were spayed also received an injection of 10-mL of Penicillin G Procaine (injectable suspension containing 300,000 units per ml; Bimeda Inc., Le Sueur, MN) to prevent any postsurgical infection. At the end of each processing day, the heifers were placed on one of two 388 ha tallgrass native range pastures with equal numbers of intact and spayed heifers and allowed to graze for 113 or 114 d. Pastures predominately consisted of big bluestem (*Andropogon gerardii* Vitman), little bluestem (*Schizachyrium scoparium* [Michx.] Nash), indiangrass (*Sorghastrum nutans* [L.] Nash), and switchgrass (*Panicum virgatum* L.). Heifers were supplemented with 1.36 kg/d of a 20% protein supplement on a prorated 5 d/wk feeding schedule. Protein source of the supplement consisted of either dried distillers grains with solubles, cottonseed meal, or soybean meal depending upon the cost and time of year.

Summer Grazing. At the end of winter grazing on April 7, heifers were ranked by Dec. BW within spay treatment and randomly allotted to one of two implant treatments. Implant treatments consisted of: Revalor®-G (**REV-G**; 40 mg TBA and 8 mg estradiol; Intervet Inc., Millsboro, DE) and Component® E-H with Tylan® (**COMP**; 200 mg testosterone propionate USP, 20 mg estradiol benzoate, and 29 mg tylosin tartrate; Ivy Animal Health Inc., Overland Park, KS). Additionally, heifers were individually weighed, vaccinated with Ultrabac® 7/Somubac® (Pfizer Animal Health, Exton, PA), and dewormed with 5-mL of Ivomec® Plus injection (Merial Limited, Duluth, GA). Heifers remained in the same pasture groups and were rotated to adjacent, fresh pastures (388 ha each) that had been burned for spring growth. Heifers grazed these two summer pastures for 106 d.

Feedlot Phase. At the end of summer grazing on July 22, heifers were split within each treatment group into light and heavy weight blocks. The heavy weight block was transported 502 km to a commercial feedlot in Garden City, Kansas. Heifers were sorted by treatment group and placed into one of four pens (1 pen/treatment) and were fed for 130 d. The light weight block was transported 84 km to the Willard Sparks Beef Research Center in Stillwater, Oklahoma. Heifers were sorted within treatment group and randomly allotted to one of 24 pens (6 pens/treatment) and were fed for 165 d. Pens were blocked to provide equal treatment groups across the feedlot to decrease any effect of feedlot pen location. Heifers were fed a standard receiving diet (38.75% cracked corn, 20% wet distillers grains with solubles, 35% roughage, and 6.25% of a supplement) and were transitioned to the finishing diet using a two-ration blending method for 24 d (Burken et al., 2010). The finishing diet (DM) included 53.75% cracked corn, 30% wet distillers grains with solubles, 10% hay, and 6.25% of a supplement to provide vitamins and minerals, 353 mg•hd^{-1}•d^{-1} of monensin, and 11 mg•hd^{-1}•d^{-1} of tylosin. The dry-matter composition of the finishing diet was 16.05% CP, 58.24 Mcal NEg/cwt, 18.75% NDF, and 5.81% fat. Pen weights were measured every 28 d during the finishing phase.

On d 0 of the finishing phase, the light and heavy weight blocks were implanted with Revalor®-IH (80 mg TBA and 8 mg estradiol; Intervet Inc., Millsboro, DE), vaccinated with Express™ 5 (Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO), Vision® 7 with SPUR® (Intervet Inc., Millsboro, DE), and dewormed with Ivomec® Plus injection. Heifers were re-implanted with Revalor®-200 (200 mg TBA and 20 mg estradiol; Intervet Inc., Millsboro, DE) and re-vaccinated with Titanium® 3 (Agri Laboratories Ltd., St. Joseph, MO) at 70 and 82 d prior to slaughter for heavy and light weight blocks, respectively. Melengestrol acetate was not included in the starter or finishing diets at either feedlot location. Heifers were finished to a common visual endpoint at each location and then transported to the same commercial abattoir, where trained personnel from Cattle Trail Inc. (Johnson, KS) collected standard carcass data (HCW, 12th rib fat, LM area, marbling score, USDA quality, and yield grades) as well as carcass maturity scores.

Statistical Analysis. Performance and final carcass characteristics were analyzed in a split-plot design using a generalized linear mixed model (PROC GLIMMIX, SAS Inst. Inc., Cary, NC). Individual animal was the experimental unit for grazing performance and carcass characteristics, whereas, feedlot performance data were analyzed using pen as the experimental unit. The model for winter grazing performance contained only spay treatment and used Dec. BW as a covariate in the analysis. The model for summer grazing, feedlot performance, and carcass characteristics contained spay, grazing implant, and the spay x grazing implant interaction. Additionally, April BW x grazing implant was used as a covariate for summer grazing performance. Pasture and pasture x spay treatment were used as random variables for summer grazing performance. Individual pen ADG during the feedlot phase was computed by linear regression (PROC GLM; SAS Inst. Inc.). Feedlot performance of the light weight block used initial feedlot BW as a covariate and included block (pen location) as a random variable. Carcass characteristics utilized HCW as a covariate and feedlot location as a random variable. All covariates were removed from the model when not significant at $P < 0.05$. Quality and yield grades and maturity scores were analyzed with PROC GLIMMIX using a binomial distribution. Mean separation for all data was accomplished using LSD, and data were considered significant at $P < 0.05$ for the main effects and the spay x grazing implant interaction.

Results and Discussion

Grazing Phase. There was no difference in overall gain or ADG between spayed and intact heifers during winter grazing (Table 1). During the summer grazing phase, there was a tendency ($P < 0.09$) for a spay x implant interaction because of the increase rate of gain for COMP compared with the REV-G in spayed heifers, but not in the intact heifers (Table 2). Cameron et al. (1977) showed that spaying and implanting heifers with either Ralgro or Synovex-H while grazing summer mountain grassland in Montana had similar rates of gain compared to intact, implanted heifers. Shoop et al. (1984) found that spayed heifers that were implanted once or twice with Zeranol during summer grazing of shortgrass pastures had similar rates of gain compared to intact, implanted heifers. ZoBell et al. (1993) found heifers that were spayed and

implanted with Synovex-S had a 17.6% greater rate of gain compared to spayed, non-implanted heifers. Average daily gain was similar between spayed, implanted heifers and intact, implanted heifers. These data show the benefit of implanting spayed heifers to improve rates of gain in grazing programs to offset any detrimental impacts that spaying will produce.

Feedlot Phase. Feedlot performance data was only collected for the light weight block. The spayed heifers from the light weight block tended ($P < 0.07$) to be lighter entering the finishing phase (Table 3). However, there tended ($P < 0.09$) to be a spay x implant interaction for ADG because of the increased rate of gain for spay compared with intact heifers implanted with REV-G, but not COMP. Dry matter intake had a spay x implant tendency ($P < 0.09$) for the intact, COMP heifers to have higher intake levels compared with the intact, REV-G heifers. There was an improved ($P < 0.03$) G:F ratio for the spayed heifers compared with the intact heifers (0.162 vs. 0.156). In contrast, Garber et al. (1990) reported that vaginally spaying and implanting heifers with Synovex-H or –S implants at feedlot entry improved feedlot ADG compared with intact, implanted heifers. In addition, spayed, non-implanted heifers had lower growth rates further emphasizing the need to implant spayed heifers. Adams et al. (1990) showed that using the K-R instrument to spay feedlot heifers had no detrimental effect on performance with the spayed, implanted (Synovex-H) heifers having similar rates of gain compared with intact, implanted heifers. Hamernik et al. (1985) showed that spayed heifers via the left paralumbar fossa, produced no detrimental effects on rate of gain even though the heifers were not implanted with an anabolic implant. However, there was a three week adaptation period following the ovariectomy prior to the beginning of the study.

Carcass Characteristics. Carcass characteristics were measured on the light and heavy weight blocks. Spaying heifers at the beginning of the grazing phase had no effect ($P > 0.46$) on 12th rib fat or marbling score (Table 4). Hot carcass weight tended ($P < 0.10$) to be decreased for REV-G compared with COMP in intact heifers, but not in spayed heifers. Longissimus muscle area was smaller ($P < 0.01$) for REV-G compared with COMP in spayed heifers, but not intact heifers. This suggests that the additional rate of gain provided by the COMP during the summer grazing phase may have been necessary to achieve adequate muscle growth of spayed heifers. Spayed heifers had a higher ($P < 0.01$) yield grade compared to the intact heifers. Spayed heifers tended ($P < 0.13$) to have lower skeletal and overall maturity scores compared with intact heifers. The COMP heifers had a higher ($P < 0.02$) lean maturity score and tended ($P < 0.07$) to have higher overall maturity scores compared with the REV-G heifers. Research has shown that implanted heifers will have greater HCW and LM area compared to non-implanted heifers (Garber et al., 1990). However, the researchers did not see any difference between type of implant used (Synovex-H vs. Synovex-S). Additionally, Garber et al. (1990) showed no effect of spaying x type of implant interaction on marbling score or quality grade distribution which is similar to the current study. Adams et al. (1990) also showed no effect on marbling score when heifers were spayed with the K-R instrument and implanted with Synovex-H. The researchers did see a decrease in LM area in the spayed, non-implanted heifers; however, there were no differences when an anabolic implant was used in the spayed heifers. Hamernik et al. (1985) showed no effects on carcass characteristics when heifers were spayed via the left paralumbar fossa.

The distribution for skeletal maturity was similar for "A" and "C" maturity carcasses (Table 5). There was a tendency ($P < 0.10$) for the intact, REV-G heifers to have a higher percentage of "B" maturity carcasses compared with the intact, COMP heifers; however, this difference was only numerically different ($P = 0.14$). There were no differences among treatments for overall skeletal maturity score or quality grade distribution. For lean maturity, all carcasses graded in the "A" category so there were no distributions to report. Klindt and Crouse (1990) showed that ovariectomized heifers had lower skeletal and overall maturity scores compared with intact heifers. Intact heifers had a greater ($P < 0.03$) percentage of yield grade 2 carcasses with a tendency ($P < 0.07$) to have a lower percentage of yield grade 3 carcasses compared with the spayed heifers. For yield grade 4, there was trend ($P < 0.06$) for a spay x grazing implant interaction because of the greater percentage of yield grade 4 carcasses for spayed heifers compared with intact heifers implanted with REV-G, but not with COMP.

Implications

Early research demonstrated that ovariectomy had negative effects on performance; however, most of those studies used flank spaying without an anabolic implant. There are multiple procedures to spay beef heifers which can have differing effects on performance and carcass quality depending upon the use of an anabolic implant. In the current experiment, using the K-R instrument to spay heifers produced no detrimental effects on performance or carcass quality. The spayed, COMP heifers actually had greater rates of gain during summer grazing while producing carcasses of similar quality and a lower overall maturity score compared to other treatment groups. Given the significant interactions between spaying and type of grazing implant, and the relatively little published data evaluating implant regimens in spayed heifers, more research is needed to determine the optimum implant program in spayed and intact heifers.

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Table 1. Effect of spaying on winter grazing performance of heifers grazing tallgrass native range

	Treatment			
Item	Intact	Spay	SEM	*P*-value
Heifers, No.	285	295	-	-
Dec. BW, kg	198	196	1.39	0.26
April BW[1], kg	209	208	0.81	0.52
Gain/heifer[1], kg	12.00	11.35	0.71	0.51
ADG[1], kg/d	0.11	0.10	0.01	0.51

[1] Dec. BW was a significant covariate ($P < 0.05$).

Table 2. Effects of spaying and grazing implant on summer grazing performance of heifers grazing tallgrass native range

	Treatment[1]							
	Intact		Spay			*P*-value		
Item	REV-G	COMP	REV-G	COMP	SEM	Spay	Implant	S*I[2]
Heifers, No.	142	143	145	150	-	-	-	-
April BW, kg	208	212	207	208	3.48	0.28	0.24	0.37
July BW[3], kg	331[a]	331[ab]	330[a]	335[b]	10.58	0.23	0.25	0.09
Gain/heifer[3], kg	122[a]	122[ab]	121[a]	126[b]	10.58	0.23	0.25	0.09
ADG[3], kg/d	1.15[a]	1.15[ab]	1.14[a]	1.19[b]	0.10	0.23	0.25	0.09

[1] REV-G = Revalor-G; COMP = Component E-H with Tylan.

[2] S*I = spay x grazing implant interaction.

[3] April BW x grazing implant was a significant covariate ($P < 0.05$).

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

Table 3. Effects of spaying and grazing implant on feedlot performance of heifers[1]

	Treatment[2]							
	Intact		Spay			*P*-value		
Item	REV-G	COMP	REV-G	COMP	SEM	Spay	Implant	S*I[3]
Heifers, No.	72	73	72	75	-	-	-	-
Pens/Treatment	6	6	6	6	-	-	-	-
Initial BW, kg	295[ab]	296[b]	285[a]	292[ab]	3.32	0.07	0.24	0.39
Final BW, kg	524	536	538	536	4.31	0.15	0.25	0.12
ADG, kg/d	1.45[a]	1.51[ab]	1.57[b]	1.53[ab]	0.03	0.03	0.71	0.09
DMI, kg	9.40[x]	9.71[y]	9.64[xy]	9.56[xy]	0.11	0.67	0.30	0.09
Gain:Feed	0.155[a]	0.156[ab]	0.163[b]	0.160[ab]	0.003	0.03	0.80	0.45

[1] Feedlot performance data only contains the light weight block that was fed in Stillwater, OK.
[2] REV-G = Revalor-G; COMP = Component E-H with Tylan.
[3] S*I = spay x grazing implant interaction.
[a,b] Within a row, means without a common superscript letter differ ($P < 0.05$).
[x,y] Within a row, means without a common superscript letter differ ($P < 0.10$).

Table 4. Effects of spaying and grazing implant on final carcass characteristics of heifers[1]

	Treatment[2]							
	Intact		Spay			*P*-value		
Item	REV-G	COMP	REV-G	COMP	SEM	Spay	Implant	S*I[3]
Heifers, No.	142	142	142	148	-	-	-	-
HCW, kg	341[a]	349[b]	347[b]	348[b]	9.24	0.23	0.06	0.10
12th rib fat[6], cm	0.39	0.39	0.40	0.40	0.03	0.46	0.94	0.78
LM area[6], cm^2	89.06[b]	88.21[b]	85.35[a]	87.36[b]	1.83	0.01	0.38	0.03
Yield grade[6]	2.88[a]	2.90[a]	3.06[b]	3.05[b]	0.16	0.01	0.89	0.76
Marbling score[4,6]	440	433	436	430	15.10	0.52	0.29	0.93
Maturity score[5,6]								
Skeletal	163[ab]	167[b]	159[a]	161[ab]	5.75	0.10	0.26	0.71
Lean	138[a]	141[b]	138[a]	139[ab]	1.05	0.17	0.02	0.21
Overall	151[a]	159[b]	150[a]	152[ab]	2.81	0.13	0.07	0.25

[1] Heifers from both the light and heavy weight blocks were used in the analysis.
[2] REV-G = Revalor-G; COMP = Component E-H with Tylan.
[3] S*I = spay x grazing implant interaction.
[4] Marbling grid: 400 = Small[00]; 500 = Modest[00].
[5] Skeletal, lean, and overall maturity grid: 100 = "A" maturity; 200 = "B" maturity.
[6] HCW was a significant covariate ($P < 0.05$).
[a,b] Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 5. Effects of spaying and grazing implant on the distribution of carcass maturity scores, and quality and yield grades of heifers[1]

	Treatment[2]							
	Intact		Spay			*P*-value		
Item	REV-G	COMP	REV-G	COMP	SEM	Spay	Implant	S*I[3]
Skeletal Maturity								
A	94.35	92.35	95.90	94.69	0.52	0.32	0.42	0.94
B	2.36	0.46	0.92	1.82	1.54	0.76	0.50	0.10
C	2.21	7.14	2.82	2.72	0.58	0.58	0.44	0.42
Overall Maturity								
A	94.28	92.98	96.55	95.68	0.53	0.16	0.63	0.94
B	3.51	1.73	2.86	2.23	0.98	0.96	0.32	0.64
C	1.47	5.00	0.00	1.36	0.71	0.97	0.97	0.97
Quality Grade								
Prime	0.00	0.70	0.70	0.00	1.00	1.00	1.00	0.97
Upper 2/3 Choice	20.19	18.79	19.53	12.71	0.33	0.25	0.17	0.34
Low Choice	49.29	47.88	47.18	54.06	0.17	0.62	0.51	0.32
Select	30.08	32.21	31.42	32.78	0.33	0.81	0.65	0.92
Standard	0.00	0.00	0.70	0.00	1.00	0.99	0.99	0.99
Yield Grade								
1	5.33	5.94	6.51	2.75	0.85	0.42	0.29	0.17
2	53.54[b]	44.38[ab]	38.72[a]	41.19[a]	0.19	0.03	0.43	0.16
3	36.46[a]	40.71[ab]	42.90[ab]	49.38[b]	0.26	0.07	0.20	0.81
4	3.49[a]	7.68[ab]	9.79[b]	6.05[ab]	0.49	0.24	0.66	0.06
5	0.00	0.00	0.70	0.00	1.00	0.99	0.99	0.99

[1] Heifers from both the light and heavy weight blocks were used in the analysis.
[2] REV-G = Revalor-G; COMP = Component E-H with Tylan.
[3] S*I = spay x grazing implant interaction.
[a,b] Within a row, means without a common superscript letter differ ($P < 0.05$).

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THE EFFECT OF FOLLICLE AGE ON PREGNANCY RATE IN BEEF COWS

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ABSTRACT: The objective of this study was to test the effect of age of the ovulatory follicle on fertility in beef cows. Ovulation was synchronized with the 5 d CO-Synch + CIDR program in multiparous (n = 171) and primiparous (n = 130) postpartum beef cows in two groups (G1 and G2) before application of treatments. Cows in G1 received estradiol benzoate (EB; 1mg/500kg BW, i.m.) 5.5 d (n = 162) and G2 received a similar dose of EB 6.5 d (n = 139) after the final GnRH of the synchronization program to create follicular turnover. Within group, PGF (25 mg, i.m.) was administered either 5.5 d ("young" follicle, YF; n = 155) or 9.5 d ("mature" follicle, MF; n = 146) after EB. In the MF treatment, estrous detection and AI were performed for 3 d after PGF, and timed-AI (TAI), coupled with GnRH administration, was performed at 72 h after PGF for cows not detected in estrus. In the YF treatment, estrous detection was performed for 4 d, with TAI at 96 h after PGF if estrus was not detected. Ovarian ultrasonography was performed in YF and MF at EB, PGF and AI, and 5.5 d after EB (MF only). Cows that failed to initiate a new follicular wave after EB (G1, n = 6; G2, n = 5) were excluded from further analyses. Also, cows in the MF treatment that initiated a second follicular wave after EB, but before PGF (G1, n = 25; G2, n = 22) were excluded from further analyses. Within the first 72 h after PGF, more MF cows (76.6%) than YF cows (48.3%; $P < 0.01$) exhibited estrus. Throughout the estrous detection period, proportion detected in estrus and interval from PGF to estrus were greater ($P < 0.01$) in the YF than MF treatment (88.6 vs. 76.6%, 79.0 ± 0.7 vs 56.7 ± 1.7 h, respectively). Diameter of the ovulatory follicle was greater ($P < 0.01$) with estrus-AI (13.3 ± 0.1mm) than TAI (12.6 ± 0.2mm) but did not differ between treatments (MF, 13.1 ± 0.2 mm; YF, 13.0 ± 0.1 mm). Pregnancy rate in the MF (72.3%) and YF (67.1%) treatments did not differ, however, pregnancy rates in estrus-AI (75.1%) was greater ($P < 0.01$) than in TAI (55.4%). In summary, age of the ovulatory follicle resulted in a longer interval to estrus and to AI in cows with young follicles but did not influence pregnancy rate.

Keywords: follicle age, beef cows, pregnancy rate

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Introduction

Cows generally have 2 or 3 waves of follicular growth during an estrous cycle and number of follicular waves during an estrous cycle would affect the interval from follicle emergence to estrus (age of the follicle). Bleach et al. (2004) reported that cows with 2 follicular waves resulted in ovulatory follicles approximately 3 d older compared to cows with 3 follicular waves, and was also associated with a lower pregnancy rate per AI (Townson et al., 2002). The decrease in fertility observed after a longer period of follicle dominance was associated with decreased oocyte quality (Revah and Butler, 1996) and embryo development (Ahmed et al., 1995), however those studies used a longer period of dominance than would occur spontaneously. Research using the Ovsynch protocol to synchronize the emergence of a new follicular wave, showed that cows that ovulated in response to the first GnRH injection had improved ovulation response following the second injection of GnRH (Vasconcelos et al., 1999; Rutigliano et al., 2008), and greater pregnancy per AI (Chebel et al., 2006; Rutigliano et al., 2008). Therefore, ovulation in response to the first GnRH injection of the Ovsynch protocol may increase fertility by inducing ovulation of a younger follicle after the second GnRH injection.

A recent study (Cerri et al., 2009) in which duration of dominance was controlled, reported that decreasing the follicular dominance by increasing the response to the first GnRH resulted in greater embryo development. Furthermore, Santos et al. (2010) demonstrated that decreasing the period of follicular dominance by decreasing the interval between first injection of GnRH and PGF_{2a} from 7 d to 5 d in lactating dairy cows produced greater pregnancy rate. This experiment was based upon a study with beef cattle (Bridges et al., 2008) in which the proestrus length was increased 12 h and a younger follicle was induced to ovulate (5-d CO-Synch + CIDR vs 7-d CO-Synch + CIDR), resulting in a greater estradiol concentrations prior to AI and pregnancy rate. Perry et al. (2005) reported a positive relationship between ovulatory follicle size and estradiol concentration at AI, however relationship between follicle size and age of the ovulatory follicle was not investigated. The objective of the present study was to evaluate the effect

of age of the ovulatory follicle on fertility in beef cows. Our hypothesis was that cows ovulating a younger follicle would require a longer proestrus period, but have increased fertility.

Material and Methods

Animals and treatments. All procedures involving animals used in this research were approved by the Fort Keogh Livestock and Range Research Laboratory Animal Care and Use Committee. Crossbred postpartum beef cows (multiparous, n = 171; primiparous, n = 130) at USDA-ARS, Fort Keogh Livestock and Range Research Laboratory in Miles City, MT, were assigned by calving date and parity into two experimental groups (G1, n = 162; 493 ± 5.2 kg; G2, n = 139; 528 ± 5.0 kg). Ovulation was pre-synchronized in all cows with the 5 d CO-Synch + CIDR program (CIDR + GnRH [100µg, i.m.] followed 5 d later with CIDR removal and 2 injections of PGF [25 mg each, i.m.] and 3 d after PGF with GnRH [100 µg, i.m.]) to improve manipulation of ovulatory follicle age during application of treatment (Figure 1). Cows were assigned to receive estradiol benzoate (EB; 1 mg/500kg BW, i.m.) 5.5 d (G1) or 6.5 d (G2) after the final GnRH injection of the pre-synchronization program to create follicular turnover and initiate a new follicular wave. Within group, PGF (25 mg, i.m.) was administered either 5.5 d ("young" follicle, YF; n = 155) or 9.5 d ("mature" follicle, MF; n = 146) after EB (Figure 1). After PGF, estrous detection was performed for 3 d (MF) or 4 d (YF) with AI approximately 12 h after estrus (estrus-AI). Cows not detected in estrus received timed-AI (TAI), coupled with GnRH, at 72 h (MF) or 96 h (YF). Approximately 10 d after AI, cows were exposed to bulls for the remainder of a 55 d (G1) or 43 d (G2) breeding season.

Ultrasonography Transrectal ultrasonography was performed using an Aloka 500 with a 7.5 MHz linear probe to characterize ovarian structures in all cows at time of EB, PGF and AI, and 5.5 d after EB (MF only). Follicle size was measured by averaging follicular diameter at the widest point and perpendicular to the first measurement. Follicles and CL were recorded on ovarian maps during each examination.

Pregnancy Determination Pregnancy diagnoses were determined using an Aloka 500 ultrasound with a 5.0 MHz transrectal linear probe approximately 34 d and 88 d (G1) or 74 d (G2) after TAI.

Statistical Analysis Cows that failed to initiate a new follicular wave after EB (G1, n = 6; G2, n = 5) were excluded from statistical analyses. Also, in the MF treatment, cows in which the new, EB-induced follicular wave became atretic, and a second wave emerged before PGF (G1, n = 25; G2, n = 22) were excluded from further analyses. This response created a unique (and unplanned) group of cows (MF2) that ovulated a very young follicle (approximately 4 d of age) in response to GnRH at 72 h. Diameter of the ovulatory follicle and pregnancy rate was determined for the MF2 group.

Estrous response within the first 72 h after PGF, overall estrous response and pregnancy rate were analyzed using a model that included group, treatment (MF, n = 94; YF, n = 149), age and their interaction, with the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC, USA, version 9.2). Interval from PGF to estrus within the first 72 h, overall interval from PGF to estrus and follicle size at AI were analyzed with the MIXED procedure of SAS.

Results and Discussion

A greater ($P < 0.01$) percentage of cows in the MF treatment (76.6%) exhibited estrus within the first 72 h after PGF than YF cows (48.3%). However, throughout the estrous detection period (Figure 2), proportion detected in estrus was greater ($P < 0.01$) in the YF (88.6%) than in the MF (76.6%) cows. Interval from PGF to estrus (proestrus) was greater ($P < 0.01$) in the YF (79.0 ± 0.7 h) than in the MF (56.7 ± 1.7 h) cows, which suggests that YF cows required a longer proestrus for ovulatory follicles to achieve maturity. However, the ovulatory follicles were still approximately 3 d younger in the YF than MF treatment at either spontaneous or induced ovulation. All of the MF2 cows (n = 47) received TAI, but 7 of these cows were in estrus at 72 h (TAI).

Diameter of the ovulatory follicle at AI (MF, 13.1 ± 0.2 mm; YF, 13.0 ± 0.1 mm) did not differ between treatments ($P > 0.10$). This finding indicates that in the YF treatment, the rate of follicular growth was increased in response to regression of CL earlier in their growth phase as follicles were approximately 3 days younger at estrus/TAI. We estimate that the MF2 cows received the PGF injection when follicles were approximately 1 d of age and follicular diameter at approximately 4 d of age when GnRH was administered was 10.2 ± 0.2 mm. Pregnancy rate (MF, 72.3%; YF, 67.1%) did not differ between treatments ($P > 0.10$). These results conflict with findings in dairy cows in which ovulation of older follicles resulted in a lower pregnancy rate (Santos et al., 2010) and lesser embryo quality (Cerri et al., 2009). Apparently, in beef cows, if afforded sufficient time for a majority of cows to exhibit estrus, age of the follicle is not a significant source of variation in fertility. Across treatments, follicle diameter and pregnancy rate were greater ($P < 0.01$) with estrus-AI (13.3 ± 0.1 mm; 75.1%) than TAI (12.6 ± 0.2 mm; 55.4%), however, diameter of the ovulatory follicle was not related ($P > 0.10$) to pregnancy rate within type of AI. It has been reported numerous times that follicle diameter at induced ovulation is related to fertility (Vasconcelos et al., 2001; Perry et al., 2005, Mussard et al. 2007; Atkins et al. 2009). Ovulatory follicle diameter was not a significant source of variation affecting pregnancy to estrus AI or TAI in the present study, but there were an insufficient number of cows that were TAI to adequately evaluate the effect of follicle diameter on fertility. Interestingly, in the MF2 treatment, pregnancy rate to TAI was 51.1% in cows with follicles that were approximately 10 mm in diameter and emerged 4 d previously when afforded 72 h of proestrus. Bridges et al. (2008) demonstrated that increasing length of proestrus from 1.25 d to 2.25 d in cows that were induced with GnRH to ovulate follicles of similar age and diameter resulted in a higher pregnancy rate. In the present study when duration of proestrus was not limited by induction of ovulation in most cows, changing length of the

preovulatory period by inducing luteal regression when follicles differed in age did not influence fertility.

Implications

Manipulation of age of the ovulatory follicle in postpartum beef cows resulted in a longer interval to estrus and to AI in cows with young follicles, but did not influence pregnancy rate nor follicle size at AI. These data suggest that age of the ovulatory follicle is not a major contributor to variation in fertility among beef cows.

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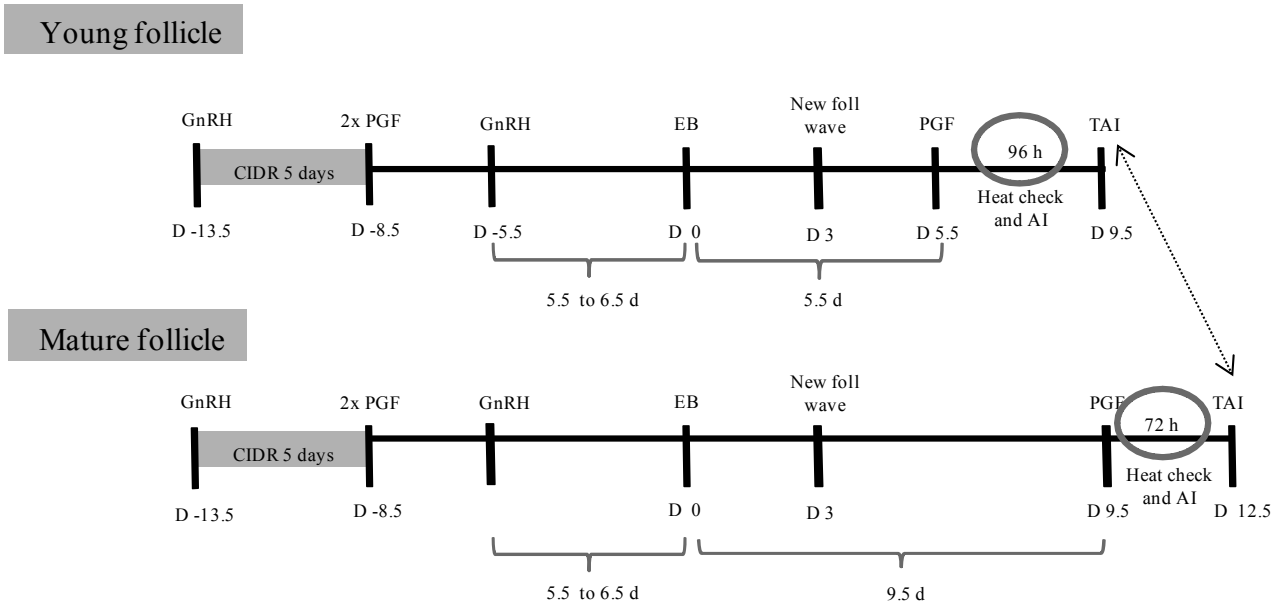

Figure 1. Diagram of activities of both treatments. In group 1, the interval from GnRH to EB injection was 5.5 d, and in group 2, this interval was 6.5 d. For clarity, the diagram is lined up on D 0 (EB), however, treatments were offset so that TAI was performed on the same date for cows in both treatments.

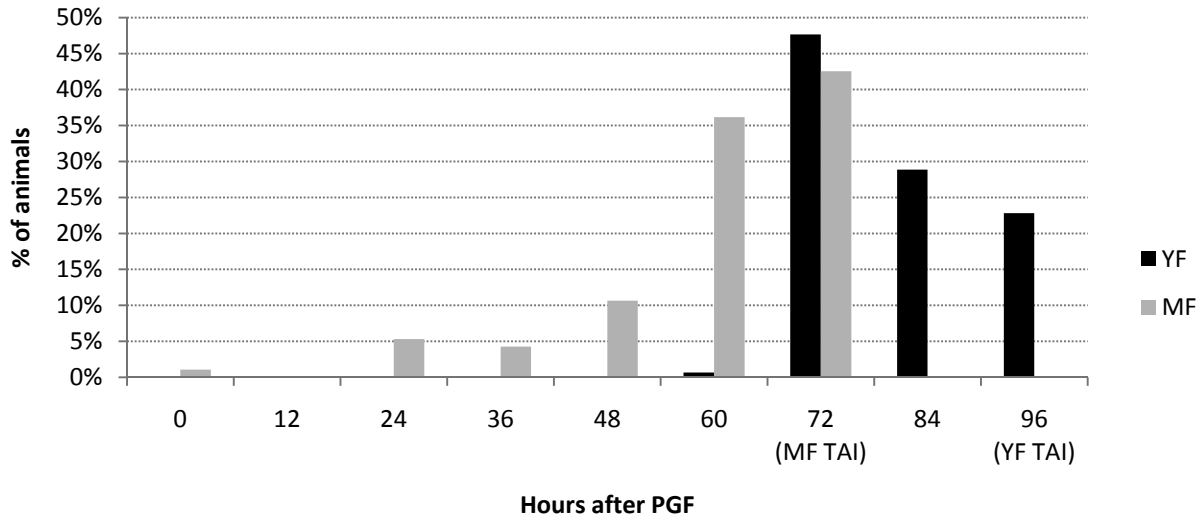

Figure 2. Distribution of estrus after PGF injection across treatments. TAI was performed at 72 h in MF and at 96 h in YF.

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THE EFFECT OF SUPPLEMENTAL MAGNESIUM ON MINERAL CONSUMPTION AND FEEDING BEHAVIOR BY PRIMIPAROUS BEEF HEIFERS

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ABSTRACT: Cattle rely on adequate intakes of Mg to meet metabolic requirements and to aid in the prevention of hypomagnesmia. Little critical evidence has been presented to show that cattle will consume mineral supplements based on nutritional wisdom. The objective of this study was to compare the preference for two free-choice mineral supplements with 0.0 or 10.0% Mg from MgO on consumption when animals either grazed pasture or were fed in drylot. In Dec., 23 Angus heifers were weighed (average BW = 513kg) and randomly assigned to one of two locations (11 heifers drylot, and 12 heifers pasture). The groups were rotated between locations after 15 d for 30 d of measurements. Individual mineral consumption (g/d), feeder attendance (trips/d), and feeding duration (s/d) were measured using a GrowSafe® individual feeding system. Heifers were offered barley hay (15.0% CP, 56.6% TDN, 0.39% Ca, 0.21% Mg, and 1.27% K) in both locations twice daily. Supplements were provided in feeders at each location, and were rotated among feeders every 5 d. Hay intakes were similar (P = 0.91). However, hay plus supplemental Mg intakes were 185% of NRC requirements for 513 kg gestating beef heifers. Heifers consumed 119% more (P < 0.01) 0.0% Mg supplement each day (121.6 g/d) than the 10.0% Mg (55.5 g/d). Heifers made almost twice as many (P < 0.01) trips to the feeder (3.1 vs. 1.8 trips/d), and spent an additional 91.3 s consuming the 0.0% Mg than the 10.0% Mg (186.4 vs. 95.1 s/d). Total mineral intakes were 87.3 % greater (P < 0.01) when supplemented on pasture (230.8 g/d) compared to drylot (123.4 g/d). During the first 15 d, total mineral intakes were higher (P < 0.01) than for the second 15 d (222 vs. 132.2 g/d). The amount of 0.0% Mg consumed was correlated (P < 0.01) with feeder attendance (r = 0.72) and duration (r = 0.60), but no correlation with 10.0% Mg was measured. The addition of MgO to the mineral decreased overall mineral consumption, feeding attendance, and feeding duration.

Key Words: Feed intake, magnesium, mineral preference

Introduction

Hypomagnesmic tetany is a metabolic disease that affects approximately 350,000 beef cows in the United States annually (Fontenot, 1979), with related losses predicted at $50-150 million (Mayland, 1993). Tetany is the result of either dietary Mg deficiency, or impairment in Mg utilization by the animal (Fontenot et al., 1973). Ruminants rely on daily intake of Mg to meet metabolic requirements and to aid in the prevention of hypomagnesia (Ritter et al., 1984). Free-choice mineral supplementation is the most widely used method of providing supplemental Mg and other minerals to grazing cattle, but this approach allows for more individual mineral consumption variation; (Greene, 2000; Tait and Fisher, 1996). Supplements must be formulated to supply minerals in adequate amounts and be provided in a palatable form to allow for sufficient intakes. The most common source of supplemental Mg is MgO. Magnesium oxide contains up to 60% Mg, and is the cheapest Mg supplement sold by the feed industry (Ammerman et al., 1972). However, Mg, P, and other mineral salts tend to reduce free-choice supplemental consumption due to decreased palatability (Greene, 2000). As a result, commercially available mineral supplements containing Mg may have inadequate amounts of Mg to protect against tetany because of palatability issues (McDowell, 1996). Little critical evidence has been presented to show that cattle will consume mineral supplements based upon metabolic requirements (Coppock et al., 1972). The objective of this study was to compare the preference for two free-choice mineral supplements containing 0.0 or 10.0% Mg from MgO on consumption when animals either grazed pasture or were fed in a drylot.

Materials and Methods

Animals and Treatments. Animal care procedures were approved by the Montana State University Institutional Animal Care and Use Committee (AA-07). In December 2010, twenty-three primiparious heifers were weighed (513 kg BW) and randomly assigned to one of two locations (11 heifers in the drylot, and 12 heifers on the pasture) to measure individual loose mineral consumption (g/d), feeder attendance (trips/d), and feeding duration (s/d) with a GrowSafe® individual feeding system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). The groups were rotated between locations after 15 d for 30 d of measurements. The drylot measured 22.5 x 11.0 m and contained 6 GrowSafe feeders, and two automatic waters located 10 m from the feeders. Heifers were fed barley hay (15.0% CP, 56.6% TDN, 0.39% Ca, 0.21% Mg, and 1.27% K) at a rate of 15.4 kg DM·hd·d^{-1} in the drylot. Barley hay was chopped to a length of 10 cm through a hammer mill, and fed twice daily at 0800 and 1500 using a Roto-Mix TMR Mixer/Feeder (Dodge City, KS). Heifers were allowed ad libitum access to hay, supplements, and water.

The pasture measured 3.0 ha and contained two GrowSafe feeders, and one automatic water located 30 m from the feeder. The pasture composition was primarily a mixture of timothy grass (*Phleum pretense)*, orchard grass (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.), legume subterranean clover (*Trifolium subterraneum* M. B.), and weeds. Forage availability was predicted at 2930

kg/ha at the initiation of the study. However, the pasture was covered with snow for the entire study, so heifers were fed barley hay (15.0% CP, 56.6% TDN, 0.39% Ca, 0.21% Mg, and 1.27% K) at a rate of 20.5 kg DM·hd·d^{-1}. All GrowSafe feeders were covered by an open sided barn to prevent scale disturbances from wind or precipitation.

Supplements (0.0% or 10.0% Mg; Table 1) were provided in two separate GrowSafe® feeders at each location, and were rotated among feeders every 5 d. Supplements were observed daily, and additional supplement was added as needed. Due to high supplement intakes on the first two days, 50% salt was added to both mineral mixtures on d-3 and for the remainder of the experiment. Hay intake was measured in the drylot with four GrowSafe feeders.

Measurements and Collections. Individual DM hay and preference for supplement (g/d), feeder attendance (trips/d), and feeding duration (s/d) were recorded daily using the GrowSafe® system. Body weight measures were taken on d 0, d 15, and d 30.

Statistical Analysis. Heifer was considered the experimental unit, because individual intakes and feeding behavior were measured. Mineral consumption preference, feeder attendance, and feeding duration were analyzed by location, period, and location x period interaction with ANOVA using the PROC MIXED model of SAS (SAS Inc. Cary, NC). Location and period were fit as fixed effects with mineral consumption, feeder attendance, and feeding duration considered as random effects. Least square means were separated using LSD procedures when $P < 0.05$.

Results and Discussion

There was no difference (P=0.33) in BW gain between animals or periods. Daily mineral consumption was variable between animals, supplements, and location. However, no interactions were detected between animals, supplements, and location. Mineral consumption in the drylot ranged from 14 to 384 g·hd·d^{-1} and 0 to 106 g·hd·d^{-1} for the 0.0% Mg and 10.0 % Mg supplements, respectively (Table 2). Pasture supplement intakes ranged from 0 to 1100 g·hd·d^{-1} for the 0.0% Mg supplement and from 0 to 274 g·hd·d^{-1} for the 10.0% Mg supplement. Total mineral intakes were 87.3% greater ($P < 0.01$) for heifers on the pasture (230.8 g/d) when compared to drylot heifers (123.4 g/d). The CV's for the drylot compared to the pasture were not different (P=0.59) for either supplement. Although the CV's were not different, the large range and variability of consumption of 0.0% Mg on the pasture may be the cause of the large numerical CV (Figure 1). Heifers consumed 119% more ($P < 0.01$) 0.0% Mg supplement each day (121.6 g/d) than the 10.0% Mg (55.5 g/d). Coppock et al. (1972) reported similar variation in daily mineral consumption (0 to 1000 g·hd·d^{-1}) by lactating dairy cattle offered free-choice dicalcium phosphate. Other investigators (Cunha, 1987; McDowell, 1996) have reported increased supplemental mineral intakes during the winter or dry season due to decreases in forage quality and mineral availability. However, in the present study there was not a decrease in mineral availability as hay plus supplemental Mg intakes accounted for 185% of the daily NRC requirements for 513 kg gestating beef heifers. Other research found no differences in total mineral consumption between cattle supplemented with four mineral blocks which contained differing Ca to P ratios during the grazing months when compared to the winter months (Chladek and Zapletal, 2007).

Total daily mineral intakes were higher ($P < 0.01$; Table 3) during the first 15 d when compared to the second 15 d (221.9 g/hd vs. 132.2 g/hd). Garossino et al. (2001) reported greater mineral supplement intake by calves during the second 14 d when compared to the first 14 d. In the present study heifers were not adapted to the hay or supplements prior to the initiation of the study, which may account for increased intakes in the first 15 d. The observed CV of supplement intakes for the first 15 d compared to the second 15 d were not different (P=0.59) for either supplement. However, the large numerical difference observed in the CV between the 0.0% Mg and 10.0% Mg supplement in both the first 15 d and the second 15 d is most likely was the result of high mineral intakes before the addition of 50% salt to both supplements on d 2. Similarly, Cockwill et al. (2000) reported that increasing the level of salt from 9.8% to 22.5% decreased daily mineral intakes from 241.6 g/d to 183.5 g/d.

Heifers made almost twice as many ($P < 0.01$; Figure 2) trips to the feeder (3.1 vs. 1.8 trips/d), and spent an additional 91.3 s ($P < 0.01$; Figure 3) consuming the 0.0% Mg than the 10.0% Mg (186.4 vs. 95.1 s/d). The amount of 0.0% Mg consumed was correlated ($P < 0.01$) with feeder attendance (r = 0.72) and duration (r = 0.60), but no correlation of 10.0% Mg with these variables was measured. Consumption differences and behavior between the two mineral supplements may be the result of decreased palatability with the addition of Mg to the mineral. Greene (2000) reported that addition of Mg, P, and other inorganic mineral salts to supplemental minerals tended to reduce free-choice mineral consumption due to decreased palatability. Decreased palatability and consumption of MgO mixed in a 1:1 ratio with trace mineralized salt was also reported by Frye et al. (1977) when compared to MgO combined with trace mineralized salt and dry molasses in a 1:1:1 ratio.

Implications

When given a choice of consumption, results indicate that the addition of MgO to the mineral supplement decreased overall mineral consumption, feeding attendance, and feeding duration. Heifers consumed more mineral on the pasture than in the drylot and during the first 15 d compared to the second 15 d. The relationship between salt and MgO concentration in mineral supplements warrants further investigation.

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Table 1. Composition of mineral supplements.

	Mineral Supplements	
Item	0.0% Mg	10.0% Mg
Ca	12.0	12.0
P	6.0	6.0
Mg		10.0
NaCl	50.0	50.0

Table 2. Average daily intakes, range, and coefficient of variation of mineral supplements containing 0.0% or 10.0% Mg by heifers offered in drylot or on pasture.

	Drylot[a]					Pasture				
Item	Avg.	Min.	Max	CV%	SE	Avg.	Min.	Max	CV%	SE
0.0% Mg[b]	87.8	14.0	384.0	79.2	76.6	155.2	0.0	1108.0	120.0	76.6
10.0% Mg	35.5	0.0	106.0	76.4	22.9	75.5	0.0	274.0	68.0	22.9

[a] Main effect due to location , $P < 0.05$.
[b] Main effect due to level of Mg, $P < 0.05$.

Table 3. Average daily intakes, range, and coefficient of variation of mineral supplements containing 0.0% or 10.0% Mg by heifers measured for the first 15 d or the second 15 d of a 30 d experiment.

	d0 – d15[a]					d16 – d30				
Item	Avg.	Min.	Max	CV%	SE	Avg.	Min.	Max	CV%	SE
0.0% Mg[b]	151.9	14.0	1108.0	123.7	76.6	91.2	0.0	384.0	75.0	76.6
10.0% Mg	70.0	2.0	274.0	74.0	22.9	41.0	0.0	154.0	80.1	22.9

[a] Main effect due to 15 d period, $P < 0.01$.
[b] Main effect due to level of Mg, $P < 0.01$.

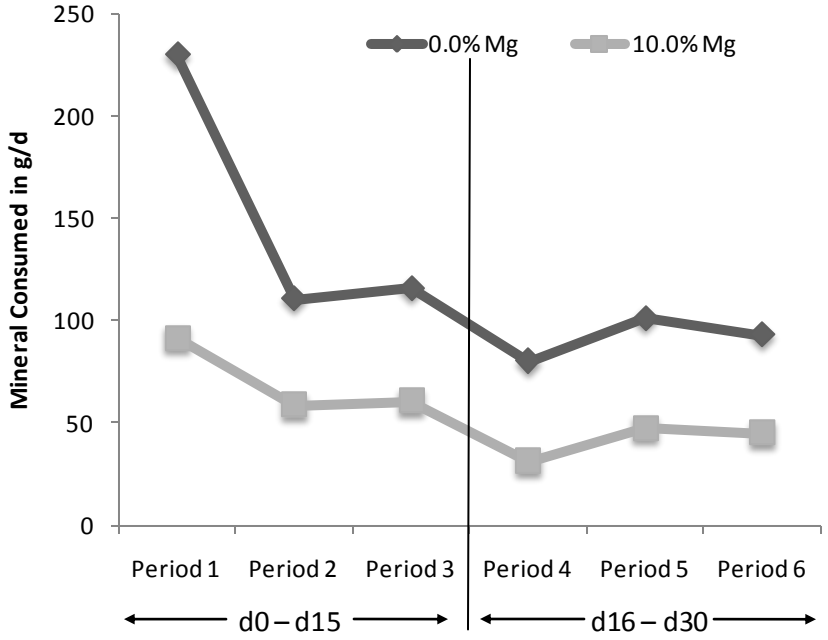

Figure 1. Comparison of preference for mineral supplements containing either 0.0% or 10.0% Mg by heifers. Main effects were measured due to period and level of Mg, ($P < 0.01$).

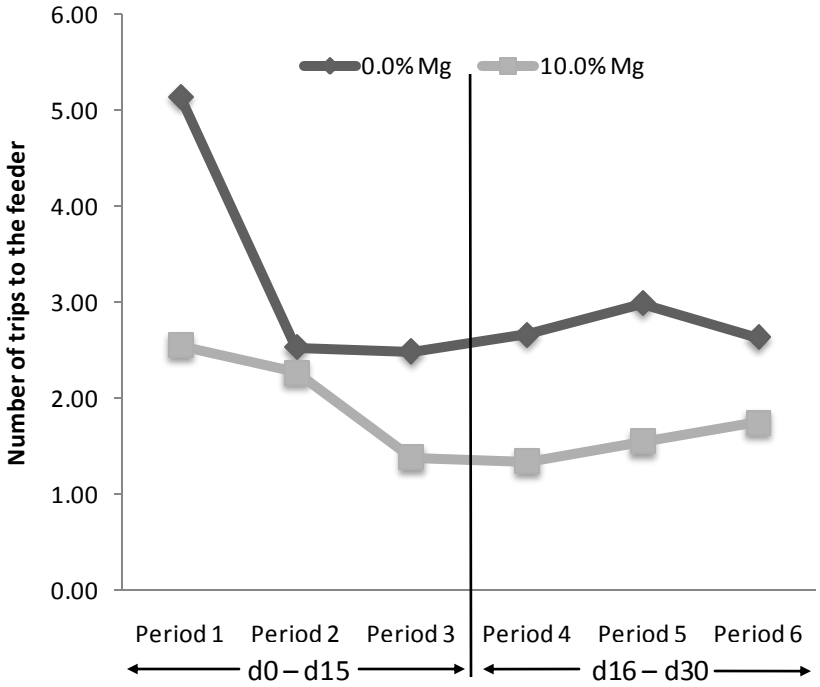

Figure 2. Comparison of the average number of trips by heifers to the mineral feeder when supplements contained either 0.0% or 10.0% Mg. Main effects measured were due to period and level of Mg, ($P < 0.01$).

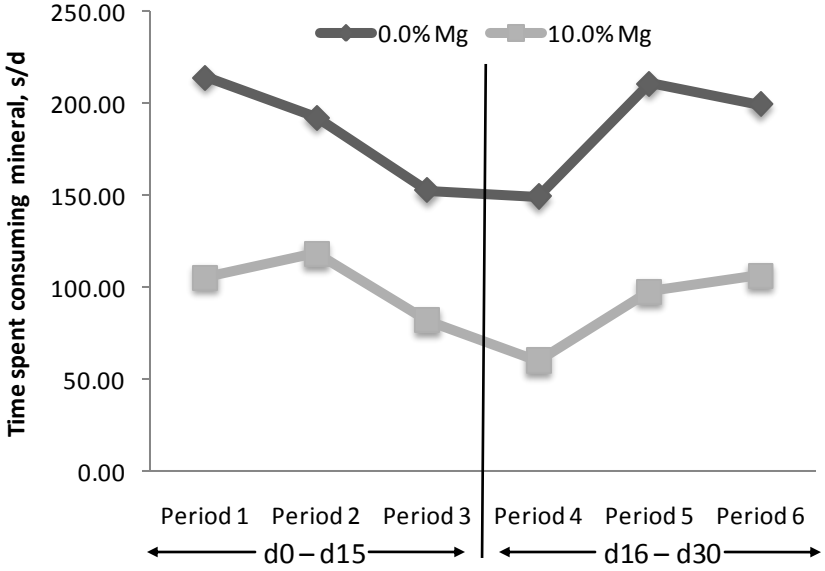

Figure 3. Comparison of the average number of seconds spent consuming mineral when supplements contained either 0.0% or 10.0% Mg by heifers. Main effects were due to period and level of Mg, ($P < 0.01$).

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EFFECT OF BIRD DEPREDATION ON NUTRIENT COMPOSITION OF CATTLE DIETS FED AT 2 SOUTHWESTERN RESEARCH FACILITIES

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ABSTRACT: Two trials were performed to determine the effect of bird depredation on nutrient composition of cattle diets at 2 Southwestern research feedlots. In trial 1 and over the course of 5 wks during the summer, samples from 9 different diets (concentrate level ranging from 52 to 77.5% as fed) were collected from feed troughs at 0, 6, and 24 h after feeding at a research beef feedlot and analyzed for nutrient composition. Dry matter, ash, and CP increased ($P < 0.01$) at 6 and 24 h after feeding. Starch decreased ($P < 0.01$) while fiber components did not differ ($P > 0.10$) over time. To determine the preference of the bird population on diet components, samples of ground corn, steam-flaked corn, whole corn, soybean meal, and a high-concentrate diet were sampled from a feed trough not accessible to cattle. All corn feedstuffs were preferred ($P < 0.01$) over soybean meal, and nearly 50% ($P < 0.01$) of the starch in the diet was consumed. In trial 2, samples of a calf pellet diet (59% corn, 25% commercial pellet, 10% beet pulp, 6% molasses) were collected from feed buckets inaccessible to adjacent individually-housed calves at 0, 8, and 24 h after feeding at a research dairy calf farm. Most components (DM, CP, fiber components, ash) increased ($P < 0.01$) while starch decreased ($P < 0.01$) between 0 and 8 after feeding. All components remained unchanged ($P > 0.10$) between 8 and 24 h after feeding). For trial 1, bird depredation was mainly from pigeon (*Columbia livia*) and mourning dove (*Zenaidura macroura*). Bird depredation on the starch component of the diet in trial 2 was observed from mourning dove, pigeon, and great-tailed grackle (*Quiscalus mexicanus*). The economical loss of bird depredation at a southwestern cattle operation can be attributed to both quantitative loss and loss of energy from starch.

Key Words: pigeon (*Columbia livia*), mourning dove (*Zenaidura macroura*), bird depredation

Introduction

Bird predation on grain crops has been researched for over 40 years (Besser et al., 1968). With the advent of confined animal feeding operations, feed predation has also spread to the livestock industry (Lee, 1987). Losses can occur directly through property damage and grain loss as well as indirectly through energy loss to the animals (Depenbusch et al., 2009).

Bird depredation studies have mainly occurred in the Midwestern United States, where bird predation in feedlots is caused mainly by European starlings (Besser et al., 1968; Depenbusch et al., 2009). Although present in the winter months elsewhere, starlings rarely summer in Southwestern United States. During this season, other species, including feral pigeons and doves, are more prevalent in Southwestern cattle operations.

A shift of research focus from agricultural impact to biosafety issues has also occurred. Feral birds are known to carry several pathogens and can spread disease-causing agents directly to livestock and humans through excrement contamination including *E. coli* and *S. enterica* (Pedersen and Clark, 2007).

Despite the specie population difference and shift in research focus, there has been no report on the effect of bird depredation on Southwestern cattle operations, nor has any reported the effect of time after feeding cattle diets on the severity of bird predation. We hypothesize that bird depredation can affect the nutrient composition of cattle diets fed in southern Arizona. Our objective, therefore, was to evaluate the effect of bird predation on cattle diets fed at 2 southwestern cattle research facilities.

Materials and Methods

Trial 1. Cattle (approximately 250 in 30 pens) in this study were used according to the University of Arizona Institutional Animal Care and Use Committee approval. Over the course of 5 weeks (May to June) at the University of Arizona West Campus Agriculture Center feedlot located in Tucson, AZ, 7 to 9 diets varying in nutrient concentration (Table 1) were fed to separate cattle groups, dependent on the cattle's primary research purpose. All cattle were fed once daily in the morning and managed by the slick bunk method. Diet samples were collected from designated feed bunks at 0, 6, and 24 hr after feeding once every 7 d (1 collection d per wk). One open bunk (no cattle) was also used to evaluate bird preference on various feedstuffs. These included whole, steam-flaked, and ground corn as well as soybean meal and the J5 diet (Table 1). Samples from the open bunk were collected 24 hr after feeding.

Trial 2. Pre-weaned calves (approximately 60 in individual hutches) were used according to the University of Arizona feedlot's Institutional Animal Care and Use Committee approval. Over a 4 wk period (October) at the University of Arizona Agricultural East Campus Center calf research barn, 2 calf pellet diets differing in amount of corn processing (Table 1) were fed to calves once daily. Diet samples were collected from feed buckets placed at an empty hutch inaccessible to adjacently-housed calves at 0,

8, or 24 hr after feeding. To keep the integrity of the amount of feed initially placed (1.0 kg/bucket), buckets were sampled either at 8 or 24 hr after feeding and not both.

Sample analysis. Diet samples from both trials were subjected to all of the following analyses: DM, ADF and NDF (Ankom 200, Macedon, NY), ash, and CP (TC400; Leco Corp.; St. Joseph, MI). Briefly, starch was analyzed by gelatinization, followed by amyloglucosidase digestion, and finished with measurement of glucose concentration (Zinn, 1990, with modifications per R.A. Zinn, personal communication, and J.D. Allen). Open feed bunk samples were dried and evaluated for weight loss on a DM basis.

Statistics. Results were evaluated as a completely random design using the PROC MIXED procedure of SAS (Cary, NC) with diet considered the experimental unit and collection hr considered the independent variable. Means and partitioning across time were generated using the LSMEANS and PDIFF options.

Results

Bird species. Although birds were not quantified and separated by specie, simple observation to identify specific bird species feeding from the feedbunks and buckets were noted. Overall, several thousand birds frequent the University feedlot during the summer. Primarily, pigeon and mourning dove were the most prevalent feeders in the bunks themselves. At the calf research barn, less than a thousand birds were present with an equal proportion of pigeon, mourning dove, and great-tailed grackle (*Quiscalus mexicanus*) feeding from the buckets.

Trial 1. Nutrient compositions of diets as a whole across time are given in Table 2. Because of the variety of feedlot diets, a diet interference was expected, so a diet × hr interaction was not calculated.

DM percentage increased ($P < 0.01$) for all diets as time of day increased. Overall, diets lost approximately 6 percent of moisture throughout the 24 hr period. Other than moisture content, starch was the only other nutrient to decrease ($P < 0.01$) during the 24 hr period with a third of the total starch content being lost to depredation. Fiber components (NDF and ADF) did not change ($P \geq 0.10$) with bird depredation. Increases ($P < 0.01$) in CP and ash were observed.

Feedstuffs from the open bunk are recorded in Table 3. Birds preferred ($P < 0.01$) corn to the protein-rich SBM. Type of corn processing did not differ ($P > 0.10$). Overall, the birds were able to consume over 60 percent of the corn available in a given 24 hr period.

All components of the J5 diet differed between hr 0 and 24, with DM increasing ($P < 0.01$; Table 3) approximately the same amount as diets in cattle-allowed bunks (Table 2). Ash also increased ($P < 0.01$) comparatively by 7 percent. In contrast to the cattle bunk data, fiber fractions increased ($P < 0.01$).

Trial 2. Nutrient compositions of the calf diets across time are given in Table 4. All components except for starch increased ($P < 0.05$) within the first 8 hr of feeding. Starch decreased ($P < 0.01$) by approximately one-third during the same time period. All components remained similar ($P > 0.10$) between hr 8 and 24.

Discussion

As shown in Table 1, diets crossed a wide array of cattle type and diet composition. Feedlots typically prefer to limit the variety of diets fed; however, the cattle industry in southern Arizona comprises a variety of cattle types, including dairy and beef cattle as well as dairy calves (Duff and Anderson, 2007).

Although most reports have centered on starling populations in the Midwest, bird depredation in any operation is dependent on the size of the operation, the number and type of birds present, and the diet composition (Palmer, 1976). This would suggest that a larger, commercial operation could experience a greater bird impact, as reported by Glahn and Otis (1986), than what was seen during the current study. In a dated Kansas survey where starlings are the major problem, not all animal operations reported having bird issues (Lee, 1987). In contrast, Depenbusch and others (2009) reported significant bird depredation in a commercial feedlot by starlings. This is not to conclude that all birds act and feed in the same manner. Clark (1976), for instance, compares and contrasts the idiosyncrasies of several bird species, including pigeon, found to predate on agricultural land in California. Since no report exists on mourning dove impact on a feeding operation, the current study with its southwestern location and variant bird composition proves novel to the literature.

As summer temperatures in southern Arizona can exceed 37^{o} C and can remain warm through mid-autumn (WRCC, 2010), moisture content was expected to diminish over a 24 hr period. An absence in fiber change could be representative of bird selection on other diet components, specifically starch. Corn was the only starch ingredient in all of the diets, which is the preferred grain by mourning doves (Armstrong and Noakes, 1981). An increase in fiber content of the open bunk J5 diet, however, suggests that cattle may have interfered with nutrient composition during or after bird predation by consuming predated portions that were already higher in fiber content. Regardless, from both the cattle-allowed and open bunk scenario, primary bird predation occurred mainly on the starch content.

A tri-fold increase in ash concentration is seen as a direct effect of starch loss. However, a one-third increase in CP could be explained by both starch loss or bird addition. During collections, bird waste matter was observed within the feedbunks. Nitrogen is a major component of bird waste (McNabb et al., 1971), so the addition of bird feces to the composition of the feed could likely increase CP.

Feedstuff results from the open bunk show a common trend across species to select grains over leguminous feeds (Besser et al., 1968). This study is comparative to other reports, with most bird species, including mourning dove and pigeon, preferring concentrates or starchy grains over fibrous forage and protein by-products (Clark, 1976; Armstrong and Noakes, 1981).

Results from the calf barn also show a preference of the resident birds for the corn portion of the diet.

However, bird predation appears to have occurred more rapidly as compared to the feedlot trial. This may be explained by the lack of calf interruption, the differing specie composition at either site, or a combination of both.

A loss of one-third of the corn in the cattle-allowed bunks should not be equated to total loss in diet fed. Cattle consume the diet throughout the day, diminishing the available feed in the trough. Therefore, a more accurate account of the potential of bird depredation can be seen in the analysis of the open bunk scenario (Table 3).

When cattle were removed from the bunk scenario, birds accounted for approximately 45 percent of the starch disappearance in the J5 diet. With corn comprising 77.5 percent of the diet, a maximum of nearly 350 g of corn per 1 kg of feed (as fed basis) could be predated by the bird population. At a corn price of $0.21/kg (price during trial) and a bunk containing 100 kg of the J5 diet, the economic loss could reach $7.35/bunk. If bird populations were not kept low, the loss over time could become exponential.

Implications

The results from the current study imply that bird populations consisting mainly of pigeons and mourning doves found in a southwestern cattle operation can have an effect on the overall nutrient composition of various diets within 6 and 24 hr after feeding to cattle. Furthermore, a significant economic loss over time can occur if bird depredation is not minimized. Although the current study shows significant starch loss, future research is needed to quantify bird population and composition to accurately estimate bird depredation on southwestern feedlots.

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Table 1. Dietary ingredients of research cattle diets (as fed basis)

	Diet[1]										
Item:	B1	B3	F0	F3	F6	J1	J5	H1	H2	C1	C2
Ingredient, %											
Corn	51	60	59.5	56	52.5	77.5	76.5	76.5	76.5	59	59
Alfalfa	39	30	14	14	14	9	9	9.5	9.5	-	-
Molasses	6.3	6.3	6	6	6	6.3	6.3	5.2	5.2	6	6
SBM, 48	-	-	12	12.5	13	4.2	4.2	-	-	-	-
Beet pulp	-	-	5	5	5	-	-	-	-	10	10
Mineral Mix[2]	2.2	2	2.5	2.5	2.5	2	2	2.2	2.2	-	-
Pre-mix[3]	1	1	1	1	1	-	1	1	1	-	-
Urea	0.5	0.7	-	-	-	1	1	1.2	1.2	-	-
Tallow	-	-	-	3	6	-	-	4.4	4.4	-	-
Calf Pellet	-	-	-	-	-	-	-	-	-	25	25

[1]Diets labeled: B1, B3 = Bull step rations; F0, F3, F6 = young dairy steer diet at 0% (F0), 3% (F3) or 6% (F6) added fat; J1, J5 = Finish beef diet with (J5) or without (J1) pre-mix; H1, H2 = Finish Holstein steer diet with heavy (512 g/L; H1) or light (448 g/L; H2) steam-flaked corn, C1, C2 = Calf diet with steam-flaked corn (C1) or whole corn (C2).

[2]Composition provided adequate amounts of Ca, NaCl, K, Mg, Zn, Cu, Fe, Se, I, Co, and Vitamins A and E.

[3]300 mg·animal^{-1}·d^{-1} of Rumensin 90 and 100 mg·animal^{-1}·d^{-1} of Tylan 40 (Elanco Animal Health, Greenfield, IN) in a ground corn carrier.

Table 2. Effect of bird depredation over a 24 hr period on the composition of diets fed at a southwestern feedlot[1]

	Hour after feeding				
Item, %:	0	6	24	SEM	*P* value
DM	88.5[a]	92.5[b]	94.2[c]	0.309	<0.01
CP	15.1[a]	19.6[b]	21.7[c]	0.466	<0.01
NDF	12.4	14.5	13.5	0.688	0.10
ADF	7.2	8.2	8.3	0.517	0.27
Ash	6.0[a]	10.7[b]	18.0[c]	0.403	<0.01
Starch	30.3[a]	24.5[b]	19.5[c]	0.811	<0.01

[a,b,c]Results in the same row without a common superscript differ ($P < 0.001$).
[1]Samples (n = 40 per hr; N = 120) were collected at 0, 6, and 24 hr after feeding. Dry matter basis.

Table 3. Effect of bird depredation on feedstuffs and nutrient composition over a 24 hr period in an open feedbunk at a southwestern feedlot[1]

	Feedstuff						
Item:	Diet J5	Whole corn	Steam-flaked corn	Ground corn	SBM	SEM	*P* value
Loss, %	69.6[a]	62.9[a]	63.1[a]	61.2[a]	28.2[b]	7.50	<0.01

	Diet J5[2]			
	Hr 0	Hr 24	SEM	*P*-value
DM	87.1	96.4	0.27	<0.01
NDF	10.7	15.3	0.92	<0.01
ADF	8.1	13.6	1.11	<0.01
Ash	5.1	12.7	0.81	<0.01
Starch	35.5	19.8	1.60	<0.01

[a,b] Feed losses in the same row without a common superscript differ ($P < 0.002$).
[1]Dry matter basis. N = 5 samples per feedstuff/diet. J5 diet = finishing beef diet.
[2]Diet was collected at hr 0 and 24 after feeding.

Table 4. Effect of bird depredation over a 24 hr period on the composition of calf pellet diets fed at a southwestern calf feedlot[1]

	Hour after feeding				
Item, %:	0	8	24	SEM	*P* value[2]
DM	89.6[a]	94.3[b]	93.8[b]	0.66	<0.01
CP	17.6[a]	25.7[b]	27.2[b]	1.98	<0.01
NDF	11.3[a]	14.0[b]	13.9[b]	0.87	0.03
ADF	4.1[a]	6.5[b]	6.8[b]	0.47	<0.01
Ash	5.4[a]	9.6[b]	9.7[b]	0.82	<0.01
Starch	29.0[a]	19.1[b]	15.3[b]	3.27	<0.01

[a,b]Results in the same row without a common superscript differ ($P < 0.01$).
[1]N = 3 to 11 per hr. Samples were collected at 0, 8, and 24 hr after feeding. DM basis.
[2]*P* value is indicative of a comparison within all 3 hourly collections.

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RANGE CATTLE WINTER WATER CONSUMPTION IN NORTHERN GREAT PLAINS

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ABSTRACT: Water consumption and DMI has been found to be positively correlated and may interact to alter range cow productivity. Environmental conditions can have a significant influence on water consumption during the winter. The objective of this study was to determine influences of water and air temperature on quantity and pattern of water intake. Six paddocks (320 ha) were grazed from December through February in 2009-2010 and 2010-2011 by 79 pregnant range cows at USDA-ARS Fort Keogh Livestock and Range Research Laboratory in Miles City, MT. Three paddocks provided cold (8.2 ±0.4°C) and three paddocks provided warm (31.1 ± 1.3°C) stock water. Warm water drinkers were heated by a Rheem outdoor tankless propane water heater. Water intake was measured daily for each paddock (0830) by an electronic water flow meter. Days were categorized by daily high temperature: warm (> -3°C), cool (-9.5°C to -3°C), and cold (< -9.5°C). In order to determine drinking patterns for each paddock a motion activated camera was set up at each water source to determine time of day water was consumed and the number of trips/d. Water temperature, daily high temperature, yr, and their interactions were evaluated and analyzed as a 2 × 3 × 2 factorial arrangement of treatments with paddock serving as the experimental unit. Cows in warm water paddocks consumed more water than cows provided cold water ($P < 0.01$; 27.7 and 19.5 ± 1.0 L/d for cows drinking warm and cold water, respectively). Year × water temperature × daily high temperature interactions ($P < 0.01$) were observed for number of trips to water and time at water per day. However, percent of cows drinking each day was not influenced by water temperature ($P = 0.56$; 65 and 68 ± 3% for cold and warm water, respectively). Results from this study shows that daily water intake is increased when heated water is provided to cows grazing winter range.

Key words: Cows, Water temperature, Water intake

Introduction

Water is the most important nutrient for range cattle. It is required for all life processes including; regulation of body temperature, reproduction and lactation, digestion, metabolism, elimination of waste materials, and maintenance of proper hydration and mineral balance (NRC, 2000). Multiple factors affect water intake such as physiological condition of the animal, DMI, water availability, quality of water, ambient temperature, and temperature of the water offered (NRC, 1981). In general, water intake decreases with decreasing ambient temperature. Studies have shown domestic animals to consume more water when offered warm water compared to cooler water. A two-trial study determined 14 ponies drank a mean of 40% more warm than ambient near-freezing water in January (Kristula and McDonnell, 1994). During winter in Missouri, non lactating dairy cows consumed 6.4 more kg of warm water at 39°C than cold water at 1.1°C (Cunningham et al., 1964). If water could be heated using economical passive alternative energy sources then a feasible method reducing energy expenditure by wintering cattle could become a management option. The objective of this study was to determine influences of water and air temperature on quantity and pattern of water intake.

Materials and Methods

This study was conducted at the USDA-ARS Fort Keogh Livestock and Range Research Laboratory near Miles City, MT from December through February in 2009-2010 and 2010-2011. Six paddocks (~ 320 ha) were grazed by 79 pregnant range cows. If snow cover restricted grazable forage, mature grass hay was fed to insure minimum amount of DMI. Mean daily high temperature for yr 1 and 2 was -4.6°C and -3.2°C, respectively. Mean daily low temperatures for yr 1 and 2 was -18.6°C and -14.3°C, respectively. Precipitation for December 1st to March 1st for yr 1 and 2 was 12.2 mm and 16.5 mm, respectively. Three paddocks provided cold (8.2 ± 0.4˚C) and three warm (31.1 ± 1.3˚C) stock water delivered in Ritchie waters. Warm water drinkers were heated by a Rheem outdoor tankless propane water heater. Water intake/paddock was measured daily (~ 0830) by an electronic water flow meter. Water intake from snow consumption was not measured. Bushnell Trophy Cam XLT motion activated trail cameras were set up at each water source in every paddock to determine individual

[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 authors and does not imply its approval to the exclusion of other products that may be also suitable.

drinkers, time of day they consumed water, and number of trips/d. Daily temperature was recorded onsite by a 2000 Series WatchDog Weather Station manufactured by Spectrum Technologies, Inc. Days were categorized by daily high temperature: warm (> -3º C), cool (-9.5º to -3º C), and cold (< -9.5ºC). In 2009, cows received 0.45kg hd^{-1} d^{-1} of a protein supplement while in 2010 cows received 5-6 kg of grass hay twice weekly for 76 days. Water samples were collected each year and sent to Midwest Laboratories, INC (Omaha, Nebraska) for water quality analysis. Water quality was considered adequate for yr 1 and 2 (Table 1).

Statistical Analysis

Data were analyzed as a completely randomized design with paddock as the experimental unit using the Kenward-Roger degrees of freedom method. The MIXED procedure (SAS Inst. Inc., Cary, NC) was used to test all main effects and all possible interactions. The model included fixed effects of water temperature, year, air temperature, and their interactions. All interactions remained in the model regardless of significance. Significance was determined at $P \leq 0.05$.

Results and Discussion

Water Intake

Cows in warm water paddocks consumed more water than cows provided cold water ($P < 0.001$; 27.7 and 19.5 ± 1.0 L/d for cows drinking warm and cold water, respectively; Table 2). Estimated energy required to bring average quantity of water drank from consumption temperature to body temperature were 56.9 kcal for cold and 24.9 kcal of warm water consumers. Cows in yr 2 consumed more water than cows in yr 1 ($P < 0.001$; 26.1 and 21.4 ± 1.0 L/d for cows in yr 2 and yr 1, respectively). NRC (2000) reports approximate total daily water intake for a 409 kg wintering pregnant beef cow with ambient temperature at 4.4°C is 22.7 L. Adams et al. (1995) measured daily water consumption for 3 winters at Fort Keogh in similar range and winter conditions and reported daily water intake ranged from 16.9 L to 20.5 L. Arias and Mader (2011) reported daily water intake of 17.3 L in a Nebraska feedlot in winter with average water temperature of 10°C.

Daily Drinkers

Percent of cows accessing drinkers each day was not influenced by water temperature ($P = 0.56$; 65 and 68 ± 3% for cold and warm water, respectively; Table 2). There was a trend toward more daily cow drinkers in yr 2 compared to yr 1 ($P = 0.09$; 62 and 70 ± 3.5 % for yr 1 and 2, respectively). Greater utilization of drinkers in year 2 may be an artifact of a greater number of days hay was fed in the vicinity of water. In a previous study at Fort Keogh, (Adams et al. 1995) when cows did not consume water during a day, 13.6% of them did not consume water the second day. In the present study, snow probably impacted drinking behavior of cows both years. In yr 1, snow fell mid-December and covered the ground throughout the study, although cows had sufficient access to vegetation and were able to graze winter range. In yr 2, snow was on the ground throughout the study and was deeper than yr 1, restricting accessibility to vegetation. It was determined that cows would be unable to depend on winter grazing to meet energy needs so hay was fed. A study conducted in Alberta Canada reported that pregnant cows relied on snow as their primary water source for 3 months with no detrimental effects on body mass change, water influx, calf birth weight or calculated energy requirements in Alberta, Canada (Degen and Young, 1990).

Year, Water Temperature, and Ambient Temperature Interactions

Year × water temperature × daily high temperature interactions ($P < 0.001$; Table 3 and 4) were observed for number of trips to water and time at water per day. In yr 1, cows made more trips to water on cold and cool days when provided warm water than when provided cold water. On warm days in yr 1, cows provided cold water made more trips per day than cows provided warm water. In yr 2, cows provided warm water made more trips/day on cool and warm days than cows consuming cold water, with no difference in number of trips per day on cold days. On cold days in yr 1 and warm days in yr 2, cows provided warm water came to water later in the day than cows consuming cold water. Cows came in to water the earliest on cold days in yr 2, regardless of water temperature. Cows came in to water earlier on warm days in yr 1 than warm days in yr 2. Time in to water on cool days did not differ between years. There appears to be no discernible trends when cattle accessed water as influenced by year, water temperature or the daily high temperature. At a feedlot in Nebraska in winter, maximum daily temperature and temperature humidity index were the best predictors of daily water intake (Arias and Mader, 2011).

Economically important benefits from increased water intake in beef cows supplied warm water during winter have not been established. Increased water intake has been shown to have a positive relationship to DMI. Drinking behavior in Musk Oxen (*Ovibos moschatus*) in winter in Alaska was positively related to time spent feeding whether the water was available as liquid or as snow (Crater and Barboza, 2007). Lacey et al. (1988) also found as DMI increased in yearling steers in a feedlot in summer, water intake increased. In dairy cows, daily water intake increased up to 65.2 L, mainly due to increased intake of dry matter and milk production (Woodford et al. 1984). In the present study, cows on native range in the winter with access to warm water consumed 30% more water compared to cows with cold water access.

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Table 1. Yearly water quality measurements of ground sourced water at study site

	Date	
Item	12/4/09	1/7/11
Sodium, ppm	367.00	358.00
Calcium, ppm	1.10	1.20
Magnesium, ppm	0.30	0.30
pH	9.28	8.62
Nitrate, ppm	n.d	n.d.
Sulfate, ppm	42.00	38.00
Total Dissolved Solids, ppm	1013.00	907.00
Iron, ppm	0.03	0.02
Manganese, ppm	n.d.	n.d.
Chloride, ppm	22.00	19.00
Fluoride, ppm	2.70	2.30

Table 2. Effect of water temperature on water intake and percent of cattle drinking per day

	Water Temperature			
Measurement	Cold	Hot	SEM	*P*-value
Water Intake, L	19.5	27.7	0.9	< 0.001
Daily Drinkers, %	65	68	3.2	0.56

Table 3. Effect of water temperature and daily high temperature on daily trips to water

Treatment	DH Temp	Year 1	Year 2
Cold	Cold	0.83 e	0.86 e
	Cool	0.90 de	1.09 bc
	Warm	0.99 cd	0.82 e
Hot	Cold	1.10 bc	0.85 e
	Cool	1.14 b	1.38 a
	Warm	0.87 e	1.12 b

Means across treatment followed by the same lower case letter do not differ (P<0.05)

Table 4. Effect of water temperature and daily high temperature on time of first daily drink

Treatment	DH Temp	Year 1	Year 2
Cold	Cold	12:24 c	11:12 a
	Cool	12:06 bc	12:14 bc
	Warm	11:49 b	12:21 c
Hot	Cold	1:03 d	11:15 a
	Cool	12:03 bc	12:24 c
	Warm	11:48 b	12:48 d

Means across treatment followed by the same lower case letter do not differ (P<0.05)

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RAM AND EWE REPRODUCTIVE BEHAVIOR AND SERUM TESTOSTERONE DURING THE EARLY AND MID-BREEDING SEASON

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ABSTRACT: The expression of sexual behavior is necessary for any successful sheep breeding program. Expression of behavior varies among rams and may be influenced by stage of the breeding season. Successful reproduction may depend on the expression of ewe behavior when ram sexual interest is diminished. Each sexually experienced ram (2 – 3 yr; n = 3) was exposed to 15 ewes in estrus during the early (September) and mid- (November) breeding season. Ewes were synchronized with intravaginal progesterone release devices (EAZI-BREED CIDR). Rams were introduced to ewes approximately 30 hrs following the CIDR removal and behavior was monitored. Observed behaviors included anticipatory (vocalizations, ano-genital sniffs, flehmen, fore-leg kicks, nudges) and consummatory (mount attempts, mounts, ejaculations) behavior. Purposeful seeking behavior of both ewes and rams was recorded. Blood samples for the analysis of serum concentrations of testosterone were obtained from each ram prior to and following exposure to ewes during each breeding period. Rams were removed from ewes following a one hour observation period, and reintroduced for subsequent observations. Rams were observed for five and four total hours in a 48 hour period in September and November, respectively. Data were analyzed using non-parametric analysis (NPAR1WAY, SAS). Serum concentrations of testosterone prior to exposure to estrous ewes were lower (P = 0.04) at mid- compared to early breeding season (1.7 vs 5.7 ± 1.1), but magnitude of the increase following exposure to estrous ewes remained similar (P = 0.5) at both time points. Expression of sexual behaviors in this limited number of rams did not differ (P > 0.2) with season. Although the number of times a ram sought a ewe did not change with stage of the season, the number of times ewes sought the ram tended to be increased (P = 0.09) in the mid-breeding season (4.7 vs 11.3 ± 2.1). Based on this limited data, it appears that seeking behavior in the ewe increases as serum concentrations of testosterone decrease in the ram. This behavior may help insure pregnancy when sexual interest in the ram has diminished.

KEYWORDS: Ram, Ewe, Reproductive Behavior

Introduction

In seasonally-breeding species, serum concentrations of testosterone increase with the onset of the breeding season, and decline as the season progresses with minimal concentrations of testosterone in the non-breeding season (Delgadillo et al., 2004). Expression of male sexual behavior is dependent on serum concentrations of testosterone. Although castrated males do not express male-typical reproductive behavior, minimal concentrations of testosterone are needed to display the full spectrum of behaviors in sexually experienced males (Damassa et al., 1977).

Female sexual behavior fluctuates across the estrous cycle caused by cyclic variations in estrogen and progesterone. Physical activity generally increases with onset of estrus in most species, and has been shown to peak in the ewe at the time of ovulation (Lindsay and Fletcher, 1972). This behavior may serve to locate and affiliate with a sexual partner (Margiasso et al., 2010) since tethered rams are successful in breeding ewes dispersed in a large pasture (Orihuela, 2009). The expression of ram seeking behavior becomes more important when suitable sexual partners are limited or sexual interest from the male is low.

It was hypothesized that as serum concentrations of testosterone decline over the breeding season in the ram, the expression of ewe seeking behavior and affiliation increases to insure the establishment of pregnancy.

Materials and Methods

Sexual behavior was monitored in three sexually-experienced rams (2 – 3 yr) exposed individually to a pen of estrus synchronized ewes (n = 15) during the early (September) and mid- (November) breeding season. Prior to exposure to rams, ewes were fitted with intravaginal progesterone release devices (EAZI-BREED CIDR) for 14 d and 15 d. To stagger the expression of estrus, CIDRs were removed from half of the ewes 24 hr following initial removal at 14 d. Rams were introduced to ewes 30 hr following CIDR removal when the initial expression of estrus was expected. Rams were placed into breeding pens (6 x 25 m) and were monitored for the expression of sexual behavior for one hour. Following the observation period rams were removed and reintroduced six hours later for another hour of observation. Behavior was monitored in a similar way the following day and subsequent morning for a total of five hours in September and four hours in November.

Recorded behaviors included anticipatory (vocalizations, ano-genital sniffs, flehmen, fore-leg kicks, nudges) and consummatory (mount attempts, mounts ejaculations) behavior. Purposeful seeking behavior of both rams and ewes was recorded.

Blood samples for the analysis of serum concentrations of testosterone were collected from rams prior to and immediately following exposure to ewes in September and again in November. Blood samples were allowed to clot overnight at 4o C. Serum was separated by centrifugation and frozen at -20o C until analysis. Serum concentrations of testosterone were analyzed in a single solid phase coat-a-count kit (Siemen's Healthcare Diagnostics, Deerfield, IL) using a 50 µL sample volume with 15% intra-assay coefficient of variation.

Statistical Analysis. Behavior and serum concentrations of testosterone were analyzed with month as the dependent variable by non parametric analysis (NPAR1WAY, SAS). Associated *P-values* based on a one-sided Wilcoxen Test are reported.

Results and Discussion

Serum concentrations of testosterone prior to exposure to ewes were decreased ($P = 0.04$) by the November behavior test (5.7 vs 1.7 [±1.1] for September and November, respectively). However, change in serum concentrations of testosterone following exposure to estrous ewes did not differ ($P = 0.5$) across the breeding season. This suggests that even though basal concentrations of testosterone diminish as the breeding season progresses, the magnitude of change elicited by exposure to estrous ewes remains similar. In male goats, serum concentrations of testosterone similarly decline as the breeding season progresses (Delgadillo et al., 2004). Although November is mid-breeding season (Shain et al., 1991) and day light hours are decreasing, serum concentrations of testosterone were decreased.

Expression of sexual behavior is highly variable among rams (Perkins et al., 1992). In this limited number of rams, expression of sexual behavior did not differ ($P \geq 0.19$) across the breeding season (Table 1). Two of the three rams showed more anticipatory and consummatory behavior in September than in November, but one ram dramatically increased the expression of behavior during the November test. Since the expression of sexual behavior is variable, more rams must be observed to establish the effect of diminishing testosterone on the expression of male behavior.

Expression of sexual behavior in ewes is subtle and may be regarded as purely receptive or an absence of behavior (ie. they stand to be mounted). However, proceptive behavior is expressed by the ewe and includes ram seeking behavior. In the present experiment, ewes tended ($P < 0.09$) to express more purposeful seeking behavior in the mid-breeding season than the early breeding season (Table 1). Although estrogen is known to increase motor activity in animals (Tou and Wade, 2002), ewes do not show an increase in random activity in absence of males, but express purposeful ram-seeking behavior in their presence (Lindsay and Fletcher, 1972). This purposeful seeking behavior may serve to insure pregnancy when rams are limited or when male sexual interest is diminished. Seeking behavior in the ewe may explain the acceptable pregnancy rate poor sexually performing rams obtain when breeding intensity is low (Stellflug et al., 2008).

Implications

Ewe seeking behavior increases as serum concentrations of testosterone decline across the breeding season. Although changes in expression of male sexual behavior were not evident in the current study, purposeful seeking behavior in the ewe may insure reproduction when males are limited or have diminished sexual interest.

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Table 1. Seeking behavior of ewes and rams and sexual behavior expressed by rams exposed to estrous ewes in the early- and mid-breeding season

Behavior	September	November	SEM	*P value*
Ano-genital sniff	26.6	30.7	5.0	0.50
Flehmen	2.7	11.4	5.4	0.33
Foreleg kick	25.8	28.9	6.3	0.50
Nudge	5.6	7.5	3.8	0.50
Vocalization	31.1	14.4	10.1	0.19
Mount Attempt	2.7	2.2	1.11	0.50
Mount	6.7	10.8	2.9	0.19
Ejaculation	2.7	3.1	1.7	0.33
Seeking Behavior – Ewe	4.7	11.3	2.1	0.09
Seeking Behavior - Ram	8.1	28.4	7.2	0.12

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FEED INTAKE AND EFFICIENCY OF F1 LAMBS

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ABSTRACT: Objective estimates of feed efficiency for progeny of terminal-sire breeds of sheep are needed to improve the value of market lambs. Because recent terminal-sire breed-comparison data are lacking, we determined effects of terminal-sire breed on feed efficiency of F1 lambs. Each year for 3 yr, Columbia, USMARC Composite, Suffolk, and Texel rams were mated with mature Rambouillet ewes. From weaning until harvest each year, F1 lambs (561 wethers; 548 ewes) were fed a step-up finishing diet for ad libitum intake. Pen was the experimental unit, with 1 self feeder per pen. There were 3 pens per year for each sex and each sire breed. Measured amounts of feed were delivered weekly or biweekly; feed remaining at the end of each period was removed and weighed. Days on feed varied across years (84 to 105 d). Dry matter intake, BW gain, G:F, feed conversion (FC), residual feed intake (RFI), and residual BW gain (RGN) were measured or calculated. General linear models, with sire breed, year, and sex as fixed effects, were used to analyze all traits. Year was significant in all models ($P < 0.01$). Sex affected ($P < 0.03$) DMI and FC, tended to affect G:F ($P < 0.07$), but did not affect any other traits. The DMI was greatest ($P < 0.01$; 156.9 kg) for Suffolk-sired, least (137.6 kg) for Texel-sired, and intermediate (145.7 kg) for Columbia- and Composite-sired lambs. Gain in BW was greatest ($P < 0.01$) for Suffolk-sired lambs (27.7 vs. 23.8 kg for all other sire breeds). Compared with other sire breeds, FC was least ($P < 0.03$) and G:F was greatest ($P < 0.02$) for Suffolk-sired lambs (FC, 5.8 vs. 6.2 kg of DMI/kg of gain; G:F, 0.177 vs. 0.167 kg of gain/kg of DMI). Breed did not affect RFI. The RGN for Suffolk-sired was greater ($P < 0.02$; 1.5 kg) than RGN (-0.5 kg) for Columbia- and Composite-sired lambs; RGN (-0.3 kg) for Texel-sired lambs did not differ from of any of the others. Producers can use these results to select a terminal-sire breed to improve the value of their market lambs.
Keywords: Lamb, Feed Efficiency, Feed Intake

Introduction

An estimated 85% of the income in the lamb industry is derived from carcass and meat products (LeValley et al., 2008), and feed costs amount to nearly 60% of the cost of producing market lambs (Benson, 2002). The cost of feed grain has increased during the last decade (NASS, 2010), resulting in increased cost of lamb production. Efficiency of converting nutrients into gain is under some genetic control (Cammack et al., 2005; Snowder and Van Vleck, 2003), but direct selection for improved efficiency is dependent on the ability to measure feed intake, which has not been feasible on a large scale. Indirect methods, using specialized sire and dam breeds, can result in complementarity that decreases unit cost of production due to improved growth relative to intake (Leymaster, 2002; Kress and MacNeil, 1999), and crossbreeding is expected to increase lamb survival, growth rate, and slaughter weight (Leymaster, 2002; Nitter, 1978). The profitability and viability of the US sheep industry depends on the potential for continued scientific advances to improve profitability in areas including sheep breeding and genetics (e.g., the introduction of new genetic lines or breeds; NRC, 2008), but comprehensive data are needed to develop and evaluate specialized sire and dam lines. Current research concerning breed differences for efficiency of feed utilization in US sheep is limited. Thus, a study was initiated at the USDA, ARS, US Sheep Experiment Station, to determine whether breed of sire affects various measures of feed efficiency of F1 progeny. This study was a component of an extensive investigation designed to evaluate performance of crossbred progeny from the mating of Columbia, USMARC Composite (Leymaster, 1991), Suffolk, and Texel rams to adult purebred Rambouillet ewes.

Materials and Methods

Experimental Procedures

The US Sheep Experiment Station Institutional Animal Care and Use Committee reviewed and approved all husbandry practices and experimental, transportation, and slaughter procedures used in this study.

Each year for 3 yr, Columbia, Composite, Suffolk, and Texel rams (n = 20 to 22/breed; 85 total rams) were single-sire mated for 21 d to approximately 10 adult (age = 3 to 7 yr) Rambouillet ewes per ram. One Suffolk and 3 Texel sires did not produce progeny. After mating, ewes were managed as a single contemporary group on winter range until mid- to late January when they were moved to a feedlot and fed to meet or exceed NRC requirements (NRC, 1985). Ewes lambed from mid-March through early April each year, producing 1,834 live lambs (Columbia, 465; Composite, 485; Suffolk, 451; and Texel, 433). Within 24 h after parturition, all tails were docked and rams were castrated using elastrator bands. Ewes and lambs were herded on sagebrush steppe range beginning in late April or early May and subalpine range beginning in early July (Leeds et al., 2008). Lambs were weaned in early August at approximately 130 d of age.

Each year, lambs were transported to a feedlot at the US Sheep Experiment Station, separated into 2 groups according to sex, and given simultaneous access to a roughage receiving ration in a concrete bunk and a pelleted ration in Apache self-feeders for approximately 8 d. Both

diets were provided in amounts that were adequate to ensure ad libitum intake. Lambs that appeared unhealthy or unsound, were not raised by their birth dam, or were not in the weaning contemporary group were removed from the study at this time. Lambs were then blocked within sire and assigned to one of 3 replicated pens. Final pen assignments were adjusted to approximately equalize mean body weights and representation of maternal grandsires among pens and to avoid assignments of full-sib lambs to the same pen (Leeds et al., 2008). There were 24 pens each year and a maximum of 16 lambs per pen. Pen was the experimental unit for all traits in this analysis. These procedures resulted in a subset of lambs (n = 351 in 2006, 383 in 2007, and 375 in 2008; 1,109 total: 561 wethers and 548 ewes) that were placed on feed. Each year, wethers were assigned randomly within sire to 1 of 3 harvest groups for serial assessment of carcass merit and scheduled for slaughter when the mean BW of all wether lambs reached 54.4, 61.2, or 68.0 kg (Leeds et al., 2008). In 2006, all ewes were fed until 5 d before the final group of wethers were transported to the abattoir. In 2007 and 2008, ewes were randomly assigned within sire to removal groups corresponding to wether harvest groups with the exception of the final group for each year. In 2007, the final ewe group was removed 19 d before the wethers; in 2008 the final ewe group was removed 7 d before the wethers.

Diets are described in Table 1. Lambs were transitioned to a finishing diet using a series of step-up diets with the overall objective of feeding the final diet for as long as possible while ensuring animal health during the transition phase. In 2006, all lambs were fed sequentially Diets 1a and 2 to 6 for 7, 7, 7, 14, 14, and 56 d, respectively. Wethers were fed Diet 6 for an additional 5 d. In 2007, all lambs were fed sequentially Diets 1b to 6 for 7, 7, 7, 7, 14, and 56 d, respectively. Wethers were fed Diet 6 for an additional 19 d. In 2008, all lambs were fed Diet 1b for 7 d. When transitioning to Diet 2, extra corn was inadvertently mixed into the ration, and caused severe acidosis in approximately 20% of the lambs within 24 h. Diet 2 was immediately removed, and after approximately 16-h without feed, the roughage receiving ration was fed for the remainder of the period. The overall 7-d diet composition for the first period was estimated to be approximately equal to Diet 2. After recovery, lambs were fed sequentially Diets 1b to 6 for 7, 7, 7, 7, 14, and 28 d, respectively. Wethers were fed Diet 6 for an additional 7 d. Feed remaining at the end of each period was removed from the feeders, weighed, and discarded.

Bodyweight was measured every 7 d throughout the feeding period. When a lamb was transported to slaughter or removed from the study for health reasons or death, the weight of the lamb at the last weigh date was considered its final bodyweight. Individual lamb BW and gain were averaged within each pen in each feeding period to produce a single, weighted estimate of BW and gain for each pen to coincide with the measurement of feed delivery. Cumulative BW gain (**CGN**) was defined as the summation of period BW gain from the start of the study through Period 9. Period 9 corresponded with 91, 105, and 84 d on test in 2006, 2007, and 2008, respectively.

Pen feed intake for each period was calculated as the difference between feed delivered and feed removed from the feeders at the end of each period and weighted according to the number of lambs in the pen for that period. If a lamb died or was removed from the study due to death or for health reasons on any day between scheduled weigh dates, the lamb's metabolic BW ($BW^{0.75}$; **MBW**) at the last weighing before removal and the combined MBW of all lambs in the pen for that period were used to adjust pen feed intake down in proportion to the number of days the lamb was in the pen before removal using:

$$\text{Adjusted pen FI (}\mathbf{FI}\text{)} = \text{Pen FI} \times \left[\frac{d_T \times \sum_{i=1}^{n} MBW_i - \sum_{j=1}^{k} (d_T - d_j) \times MBW_j}{d_T \times \sum_{i=1}^{n} MBW_i} \right];$$

where MBW_i is MBW for Lamb i of the n lambs in the pen at the start of the period; MBW_j is the MBW of Lamb j of the k lambs removed from the study during the period; d_T = duration of the test period in days, d_k = the number of days Lamb j was present.

Pen DMI was calculated from adjusted pen feed intake. Cumulative DMI (**CDMI**) was defined as the summation of period DMI from the start of the study through Period 9. The G:F and cumulative feed conversion (**FC**; F:G) were also calculated on dry matter bases through Period 9. The FC was included because producers generally describe animal performance using FC, rather than G:F.

Residual feed intake (**RFI**) and residual BW gain (**RGN**) were calculated using CGN, CDMI, and cumulative maintenance requirements (**CMT**). The CMT was calculated as the summation of predicted maintenance requirement from Periods 1 to 9, where period maintenance was modeled as:

$$\text{Period maintenance} = [(\text{period beginning BW} + \text{period final BW})/2]^{0.75} * \text{period duration, in days.}$$

The conventional approach for modeling RFI includes point-estimates of maintenance requirement (generally midtest $BW^{0.75}$) and BW gain (Archer et al., 1999). Conventional approaches for modeling maintenance and BW gain were not appropriate for this dataset because pen was the experimental unit, with individual animal measurements pooled within pen, and animals were removed from the study due to slaughter, sickness, or death as the study progressed. Thus, there were no direct point-estimates for pen maintenance or BW gain.

Data Analyses

Beginning BW, final BW, CDMI, CGN, G:F, and FC through Period 9 were described with a linear model using PROC GLM (SAS Inst. Inc., Cary, NC). The full

model included fixed effects of sire breed, year, sex, and all two-way interactions. All design variables and interaction terms remained in the model regardless of significance level. No interaction term was significant for all traits, but each term was significant for some trait, so all terms were included in the model for continuity across traits. The models to describe RFI and RGN were similar to those described above, but with the addition of continuous effes of CMT and CGN to the RFI model, and CMT and CDMI to the RGN model. The RFI and RGN were then calculated as the difference between each breed's effect and the grand mean of the 4 breeds.

Results and Discussion

Suffolk- had the greatest beginning BW ($P < 0.01$; 40.3 kg); Columbia- (38.9 kg) and Composite-sired (38.4 kg) were intermediate; and Texel-sired lambs had the least (36.9 kg; Table 2). Suffolk- had the greatest final BW ($P < 0.01$; 68.6 kg), with Composite- (63.8 kg) and Columbia- (63.3 kg) intermediate; Texel-sired lambs (60.1 kg) had the least final BW. Suffolk-sired lambs had the greatest CGN ($P < 0.01$). Suffolk- gained 27.7 kg, Columbia- and Composite- both gained 24.0 kg, and Texel-sired lambs gained 23.1 kg. Leymaster and Jenkins (1993) reported greater BW for Suffolk- than for Texel-sired lambs at 189 d of age. Leymaster (1991) compared Suffolk- and Composite-sired lambs and found that lambs born to mature ewes were not different for postweaning GN and final BW at 125 d of age. Leymaster and Smith (1981) found no differences between Columbia- and Suffolk-sired lambs for postweaning GN or 154 d BW. Two environmental considerations may contribute to the greater differences among the breeds in this study than were reported for the earlier studies. In the current study, lambs were approximately 230 d of age at completion and were managed in a more extensive preweaning environment than were lambs in the earlier studies. Either of these variables or the interaction between them may cause disparity in results when breeds are evaluated in different production systems. Another factor may be genetic trends within the breeds in response to selection (Hanford, et al., 2002; Notter, 1998, 2006, 2008). Columbia, Suffolk, and Texel breeders may each utilize selection and genetic evaluations differently to meet their selection objectives, resulting in differing performance among the breeds over time. The Composite sheep have been managed as a research population to minimize inbreeding without intense selection for growth after being closed to outside germplasm (Leymaster, personal communication).

Suffolk- had the greatest CDMI ($P < 0.01$; 156.9 kg); Columbia- (147.6 kg) and Composite- (143.9 kg) were intermediate; and Texel-sired lambs (137.6 kg) had the least CDMI. There were no reports in the literature of direct comparisons between any of the breeds for DMI, G:F, FC, RFI, or RGN. Suffolk-sired lambs were most efficient (G:F, $P < 0.02$; FC, $P < 0.03$). The G:F of Suffolk- was 0.177 kg GN/kg DMI, compared with 0.168 for Composite- and Texel- and 0.163 for Columbia-sired lambs. The FC of Suffolk- was 5.76 kg DMI/kg GN, followed by Texel- (6.09), Composite- (6.11), and Columbia-sired lambs (6.33). Among the 3 breeds with lesser CGN (Columbia, Composite, and Texel), Texel- consumed less DM than did Columbia- and Composite-sired lambs. Even though the CDMI was less for Texel-sired lambs, the insignificant differences in CGN among the lesser gaining breeds may have resulted in insignificant differences in G:F and FC among the 3 breeds. Even though Suffolk-sired lambs had the greatest DMI, they also gained enough to offset this DMI, resulting in the greatest G:F among the 4 sire breeds.

Differences among breeds for RFI only approached significance ($P < 0.09$). The large differences among breeds for DMI were not reflected in RFI, but the breeds ranked the same for both traits. Residual GN for Suffolk- was greater ($P < 0.02$; 1.5 kg) than for Columbia- (−0.8 kg) and Composite-sired lambs (−0.2 kg); RGN for Texel-sired lambs (−0.3) did not differ from any of the others. The nonsignificant breed differences in RFI, despite apparent numeric differences, was probably because Suffolk- and Texel-sired lambs were on opposing ends of the distributions for both CDMI and CGN. These differences caused greater standard errors in the prediction of CDMI to calculate RFI because Suffolk- and Texel- were regressed further to the mean than were Columbia- and Composite-sired lambs. A similar observation was apparent when comparing the breed means for RGN. Even though not statistically different, Texel- had numerically greater RGN than Composite-sired lambs, but also had greater standard errors (Table 2), resulting in significant differences between Suffolk- (1.5 kg) and Composite-sired (−0.2 kg) lambs, but not between Suffolk- and Texel-sired (−0.3 kg) lambs. These results indicate that RFI may not be a reliable indicator of efficiency of feed utilization in growing lambs.

Sex affected ($P < 0.03$) beginning and final BW, CDMI, and FC, tended to affect G:F ($P < 0.07$), but did not affect any other traits. Year was significant in all models ($P < 0.01$). The year × breed interaction was significant for FC ($P < 0.03$) and tended to be significant for CGN ($P > 0.06$), but was not significant for any other trait. The year × sex interaction was significant for beginning BW, CGN, RGN, F:G, and FC ($P < 0.01$ for all traits). The breed × sex interaction was significant for beginning BW ($P < 0.03$), tended to be significant for FC, G:F, and RGN ($P = 0.08$, 0.10, and 0.11, respectively), but was not significant for any other trait.

In summary, a dearth of refereed scientific publications on the effects of terminal-sire breed on feed efficiency of US lambs reduces opportunities for making genetic improvements in traits that are economically important to growers and feeders of lambs in the US (Leymaster; 1981, 1991, 1993; Lupton, 2008). The present large-scale study included a nationwide sample, except for Composite, of terminal-sire genetics and was conducted to address that need for data. Except for one trait, RFI, which did not differ among terminal-sire breed, Suffolk-sired lambs excelled for all measures of growth and feed efficiency. Columbia- and Composite-sired lambs were generally comparable and intermediate between Suffolk- and Texel-sired lambs for growth traits. All BW traits and CDMI of Texel-sired lambs were less than for all other crosses, and measures of feed efficiency of Texel- and

Composite-sired lambs were generally comparable and intermediate between Suffolk- and Columbia-sired lambs.

Implications

Sheep producers can use the data from this study to select terminal-sire breeds that will promote their production objectives for growth and efficiency of market lambs.

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Table 1. Diet composition (DM basis, %)

Diet	Whole corn	Finish pellet[1]	CSB[2]	Alfalfa pellet	ME[3], Mcal/kg
1a	35.0	8.0	1.5	55.5	2.511
1b	42.4	9.6	1.5	46.5	2.583
2	47.5	10.3	1.5	40.7	2.655
3	60.0	12.6	1.5	25.9	2.799
4	72.0	14.9	1.5	11.6	2.940
5	78.0	17.2	1.5	3.3	3.016
6	79.5	19.0	1.5	0.0	3.041

[1]Wheat middling-based pellet containing 160 g/t lasalocid, vitamins, and minerals.
[2]Concentrated separator byproduct.
[3]Weighted average based on NRC (2007).

Table 2. Sire breed least-squares means for lamb traits

	Sire breed				
Trait	Columbia	USMARC-Composite	Suffolk	Texel	*P*-value
Beginning BW, kg	38.9 ± 0.28^{b}	38.4 ± 0.28^{b}	40.3 ± 0.28^{a}	36.9 ± 0.28^{c}	< 0.01
Final BW, kg	63.3 ± 0.84^{b}	63.8 ± 0.84^{b}	68.6 ± 0.84^{a}	60.1 ± 0.84^{c}	<0.01
BW gain, kg	24.0 ± 0.35^{b}	24.0 ± 0.35^{b}	27.7 ± 0.35^{a}	23.1 ± 0.35^{b}	< 0.01
Dry matter intake, kg	147.6 ± 1.27^{b}	143.9 ± 1.27^{b}	156.9 ± 1.27^{a}	137.6 ± 1.27^{c}	< 0.01
Gain:Feed, kg/kg	0.163 ± 0.002^{b}	0.168 ± 0.002^{b}	0.177 ± 0.002^{a}	0.168 ± 0.002^{b}	< 0.02
Feed:Gain, kg/kg	6.33 ± 0.08^{b}	6.11 ± 0.08^{b}	5.76 ± 0.08^{a}	6.09 ± 0.08^{b}	< 0.03
Residual feed intake, kg	1.8 ± 0.99	-1.6 ± 0.98	1.4 ± 1.64	-2.0 ± 1.42	< 0.09
Residual BW gain, kg	-0.8 ± 0.30^{b}	-0.2 ± 0.31^{b}	1.5 ± 0.49^{a}	$-0.3 \pm 0.46^{a,b}$	< 0.01

[a,b,c] Means within a row with different superscripts differ ($P \leq 0.05$). Mean separation tests were adjusted for multiple comparisons using the Tukey-Kramer option in PROC GLM.

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CORRELATIONS BETWEEN MEASURES OF FEED EFFICIENCY AND FEEDLOT RETURNS FOR F1 LAMBS

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ABSTRACT: Objective estimates of feedlot return for progeny of terminal-sire breeds of sheep are needed to improve lamb profitability. Thus, we used recent economic data to determine the effects of terminal-sire breed on returns of F1 lambs. Annually for 3 yr, Columbia, USMARC Composite, Suffolk, and Texel rams were mated with mature Rambouillet ewes. From weaning until harvest, F1 lambs (561 wethers; 548 ewes) were fed a step-up finishing diet for ad libitum intake. Pen was the experimental unit, with 3 pens of lambs per year for each sex and sire breed and 1 self feeder per pen. Measured amounts of feed were delivered weekly or biweekly. Feed remaining at the end of each period was removed and weighed. Days on feed varied across years (84 to 105 d). Feed intake (FI), BW gain, G:F, feed conversion (FC), residual feed intake (RFI), residual BW gain (RGN), and feedlot return ($R) were determined. Return was based on 2010 Idaho feeder and slaughter lamb prices, feed costs, and other typical costs; no discounts were applied for excessively fat lambs. Year and breed, but not sex, affected ($P < 0.01$) $R. The $R for Suffolk-sired ($38.15) was greater than that for Columbia- ($28.49) and Texel-sired ($27.22), but not different from $R for Composite-sired lambs ($32.54). Pearson correlations between $R and BW gain, G:F, and FC were 0.76, 0.85, and −0.84, respectively ($P < 0.01$). Correlations were not significant between $R and RFI ($P = 0.76$), RGN ($P = 0.10$), and FI ($P = 0.39$). Even though BW gain accounted for less variation in $R than did G:F and FC, BW gain does not require measurement of FI, which is not typically measured in the industry. Selecting terminal-sire breeds with increased genetic merit for postweaning BW gain should simultaneously improve returns from feedlot lambs.
Keywords: Lamb, Feed Efficiency, Profit

Introduction

Feed costs amount to nearly 60% of the cost of producing market lambs (Benson, 2002). The cost of feed grain has increased during the last decade (NASS, 2010), resulting in increased cost of lamb production. An increase in profitability of lamb production is dependent on reducing input costs and/or increasing production output (Snowder and Van Vleck, 2003), and any reduction in feed intake or increase in feed efficiency without compromising growth rate can have a significant positive economic impact on lamb production. The profitability and viability of the US sheep industry depends on the potential for continued scientific advances to improve profitability in areas including sheep breeding and genetics (NRC, 2008). However, current research concerning breed differences for efficiency of feed utilization in US sheep is limited, and no current research reports the correlation between measures of feed efficiency and feedlot return (**$R**). Thus, a study was initiated at the USDA, ARS, US Sheep Experiment Station, to determine whether breed of sire affects various measures of feed efficiency and $R of F1 progeny. This study was a component of an extensive investigation designed to evaluate performance of crossbred progeny from the mating of Columbia, USMARC Composite (Leymaster, 1991), Suffolk, and Texel rams to adult purebred Rambouillet ewes.

Materials and Methods

Experimental Procedures

The US Sheep Experiment Station Institutional Animal Care and Use Committee reviewed and approved all husbandry practices and experimental, transportation, and slaughter procedures used in this study.

Each year for 3 yr, Columbia, Composite, Suffolk, and Texel rams (n = 20 to 22/breed; 85 total rams) were single-sire mated for 21 d to approximately 10 adult (age = 3 to 7 yr) Rambouillet ewes per ram. One Suffolk and 3 Texel sires did not produce progeny. After mating, ewes were managed as a single contemporary group on winter range until mid- to late January when they were moved to a feedlot and fed to meet or exceed NRC requirements (NRC, 1985). Ewes lambed from mid-March through early April each year, producing 1,834 live lambs (Columbia, 465; Composite, 485; Suffolk, 451; and Texel, 433). Within 24 h after parturition, all tails were docked and rams were castrated using elastrator bands. Ewes and lambs were herded on sagebrush steppe range beginning in late April or early May and subalpine range beginning in early July (Leeds et al., 2008). Lambs were weaned in early August at approximately 130 d of age.

Each year, lambs were transported to a feedlot at the US Sheep Experiment Station, separated into 2 groups according to sex, and given simultaneous access to a roughage receiving ration in a concrete bunk and a pelleted ration in Apache self-feeders for approximately 8 d. Both diets were provided in amounts that were adequate to ensure ad libitum intake. Lambs that appeared unhealthy or unsound, were not raised by their birth dam, or were not in the weaning contemporary group were removed from the study at this time. Lambs were then blocked within sire and assigned to one of 3 replicated pens. Final pen assignments were adjusted to approximately equalize mean body weights and representation of maternal grandsires among pens and to avoid assignments of full-sib lambs to the same pen (Leeds et al., 2008). There were 24 pens each year and a maximum of 16 lambs per pen. Pen was the experimental

unit for all traits in this analysis. These procedures resulted in a subset of lambs (n = 351 in 2006, 383 in 2007, and 375 in 2008; 1,109 total: 561 wethers and 548 ewes) that were placed on feed. Each year, wethers were assigned randomly within sire to 1 of 3 harvest groups for serial assessment of carcass merit and scheduled for slaughter when the mean BW of all wether lambs reached 54.4, 61.2, or 68.0 kg (Leeds et al., 2008). In 2006, all ewes were fed until 5 d before the final group of wethers were transported to the abattoir. In 2007 and 2008, ewes were randomly assigned within sire to removal groups corresponding to wether harvest group with the exception of the final group for each year. In 2007, the final ewe group was removed 19 d before the wethers; in 2008 the final ewe group was removed 7 d before the wethers.

Bodyweight was measured every 7 d throughout the feeding period. When a lamb was transported to slaughter or removed from the study for health reasons or death, the weight of the lamb at the last weigh date was considered its final bodyweight. Individual lamb BW and gain were averaged within each pen in each feeding period to produce a single, weighted estimate of BW and gain for each pen to coincide with the measurement of feed delivery. Cumulative BW gain (**CGN**) was defined as the summation of period BW gain from the start of the study through Period 9. Period 9 corresponded with 91, 105, and 84 d on test in 2006, 2007, and 2008, respectively.

Pen feed intake for each period was calculated as the difference between feed delivered and feed removed from the feeders at the end of each period and weighted according to the number of lambs in the pen for that period. If a lamb died or was removed from the study due to death or for health reasons on any day between scheduled weigh dates, the lamb's metabolic BW ($BW^{0.75}$; **MBW**) at the last weighing before removal and the combined MBW of all lambs in the pen for that period were used to adjust pen feed intake down in proportion to the number of days the lamb was in the pen before removal using:

$$\text{Adjusted pen FI (\textbf{FI}) =}$$

$$\text{Pen FI} \times \left[\frac{d_T \times \sum_{i=1}^{n} MBW_i - \sum_{j=1}^{k} (d_T - d_j) \times MBW_j}{d_T \times \sum_{i=1}^{n} MBW_i} \right];$$

where MBW_i is MBW for Lamb i of the n lambs in the pen at the start of the period; MBW_j is the MBW of Lamb j of the k lambs removed from the study during the period; d_T = duration of the test period in days, d_k = the number of days Lamb j was present.

Pen DMI was calculated from adjusted pen feed intake. Cumulative DMI (**CDMI**) was defined as the summation of period DMI from the start of the study through Period 9. The G:F and cumulative feed conversion (**FC**; F:G) were also calculated on dry matter bases through Period 9. The FC was included because producers generally describe animal performance using FC, rather than G:F.

Residual feed intake (**RFI**) and residual BW gain (**RGN**) were calculated using CGN, CDMI, and cumulative maintenance requirement (**CMT**). The CMT was calculated as the summation of predicted maintenance requirement from Periods 1 to 9, where period maintenance was modeled as:

$$\text{Period maintenance =}$$

$$[(\text{period beginning BW} + \text{period final BW})/2]^{0.75} * \text{period duration, in days.}$$

The conventional approach for modeling RFI includes point-estimates of maintenance requirement (generally midtest $BW^{0.75}$) and BW gain (Archer et al., 1999). Conventional approaches for modeling maintenance and BW gain were not appropriate for this dataset because pen was the experimental unit, with individual animal measurements pooled within pen, and animals were removed from the study due to slaughter, sickness, or death as the study progressed. Thus, there were no point-estimates for pen maintenance or BW gain.

The value of lambs was determined using beginning and final BW and 2010 USDA Weekly National Lamb Market Summary (AMS, 2010a,b) reports to reflect current industry lamb prices in the Intermountain region, even though the lambs were fed in 2006 to 2008. Prices were not adjusted for breed, perceived differences in feeder lamb quality, or final lamb fatness. All breed means were within a 36.3- to 40.9-kg weight range for feeder lambs and a 56.8- to 77.2-kg range for slaughter lambs and lamb prices were thus not adjusted for differences in BW among the breeds. The value of the lambs at the beginning of the study was \$2.90/kg and final value was \$3.39/kg. The increase in value per kilogram for slaughter vs. feeder lambs is unusual, but may reflect the current volatility of the US lamb market. Feed costs (**\$F**) were estimated based on August 2010 prices of feed delivered to the US Sheep Experiment Station and were \$0.24/kg for the study period. Feedlot yardage was calculated as \$0.08 per lamb per day. Combined deworming, vaccination, and shearing costs were estimated to be \$2.36/lamb. Interest cost for feed and lamb purchase prices were calculated at the rate of 5%. Final BW was reduced by 4% to simulate BW shrink during transportation to market, and trucking costs were estimated at \$1.61/lamb. Interest costs for yardage, deworming, vaccination and shearing were expected to be minimal and were not included in the analysis. The \$R was calculated as: final lamb value – (beginning lamb value + estimated costs).

Data Analyses

The \$F and \$R through Period 9 were described with a linear model using PROC GLM (SAS Inst. Inc., Cary, NC). The full model included fixed effects of sire breed, year, sex, and all two-way interactions. All design variables and interaction terms remained in the model regardless of significance level. Pearson correlation coefficients were estimated between \$R and beginning BW, final BW, FI, CGN, RFI, RGN, G:F, and FC.

Results and Discussion

The $R for Suffolk- ($38.15) was greater than that for Columbia- ($28.49) and Texel-sired ($27.22), but not different from $R for Composite-sired lambs ($32.54; SE ± $2.35 for all breeds). Suffolk- had the greatest $F ($P < 0.01$; $43.33); Columbia- ($40.75) and Composite-sired ($39.74) were intermediate; and Texel-sired lambs had the least ($37.99: SE ± $0.35 for all breeds). Year and breed, but not sex, affected ($P < 0.01$) $R; all three variables affected $F ($P < 0.01$). Even though Suffolk- had the greatest $F, Suffolk- and Composite-sired lambs had the greatest $R. Texel-sired lambs had the least $F, but also had the least $R.

Pearson correlations (Table 1) between $R and BW gain, G:F, and FC were 0.76, 0.85, and −0.84, respectively ($P < 0.01$). Correlations were not significant between $R and RFI ($P = 0.76$), RGN ($P = 0.10$), and FI ($P = 0.39$). The G:F and FC accounted for the largest portion of the variation in $R. Even though BW gain accounted for less variation in $R than did G:F and FC, BW gain does not require measurement of FI, which is not typically measured in the industry. Selecting terminal-sire breeds with increased genetic merit for postweaning BW gain should simultaneously improve returns from feedlot lambs. Selection of a terminal-sire breed based upon merit for RFI or RGN should not be expected to result in improvement in $R.

In summary, a growing body of evidence indicates that efficiency of feed utilization is under genetic control in sheep (Cammack et al., 2005; Snowder and Van Vleck, 2003), but a dearth of refereed scientific publications on the effects of terminal-sire breed on feedlot return of US lambs reduces opportunities for making improvements in profit for growers and feeders of lambs in the US. The present large-scale study included a nationwide sample, except for Composite, of terminal-sire genetics and was conducted to address that need for data. Suffolk-sired lambs excelled for feedlot return, and Columbia- and Texel-sired lambs had lower feedlot returns.

Implications

Sheep producers can use the data from this study to select terminal-sire breeds that will promote their production objectives for feedlot return of market lambs.

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Table 1. Pearson correlation coefficients between feedlot return and performance traits

Trait	$R	*P*-value
Beginning BW, kg	−0.50	< 0.01
Final BW, kg	0.55	<0.01
BW gain[1], kg	0.76	< 0.01
Feed Intake[2], kg	−0.10	= 0.39
Gain:feed, kg/kg	0.85	< 0.02
Feed:gain, kg/kg	−0.84	< 0.03
Residual feed intake, kg	−0.03	= 0.76
Residual BW gain, kg	0.20	= 0.10

[1]Cumulative BW gain
[2]Cumulative feed intake, dry-matter basis

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GENETIC ASSOCIATIONS BETWEEN BOVINE RESPIRATORY DISEASE AND CARCASS TRAITS IN FEEDLOT STEERS

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ABSTRACT: Bovine respiratory disease (**BRD**) is one of the most prevalent and economically burdening diseases facing the beef cattle industry. The economic impact of the disease makes it a primary candidate for research to improve health and profitability of feedlot cattle. Therefore, the primary objectives of this study were to estimate variance components for BRD in feedlot cattle using feedlot treatment records (**Trt**) and to evaluate genetic and environmental correlations of the disease with HCW, LM area, marbling score (**MS**) and subcutaneous backfat thickness (**FAT**). Data included health and carcass records on 2,870 crossbred steers managed in a commercial feedlot in Southeast Colorado over a two year period. Two multivariate models were fitted to estimate direct genetic effects and associated correlations between Trt and carcass traits where lean and fat traits were evaluated independently. Heritability estimates were 0.17 ± 0.06, 0.30 ± 0.05, 0.39 ± 0.05, 0.62 ± 0.04, and 0.24 ± 0.04 for Trt, HCW, LM area, MS and FAT, respectively. Genetic correlations of Trt with carcass traits were 0.19 ± 0.30 with HCW, 0.03 ± 0.25 with LM area, -0.30 ± 0.21 with MS, -0.004 ± .26 with FAT. Environmental correlations were low and favorably correlated with estimates of -0.05 ± 0.02, -0.01 ± 0.02, -0.06 ± 0.02, and -0.05 ± 0.02, between Trt and HCW, LM area, MS, and FAT, respectively. Results indicate that genetic improvement for a lower probability of being treated for BRD is possible through selection over time. Estimates of genetic correlations were generally low with high standard errors with respect to the estimates indicating no major antagonisms between higher carcass merit and Trt. Though low, the environmental correlations suggest a benefit from well implemented preventative protocols for bovine respiratory disease in the feedlot.

Key words: beef cattle, bovine respiratory disease, carcass trait, health

Introduction

Bovine respiratory disease is the single most costly disease facing the beef cattle industry (USDA NAHMS, 2000). Economic losses from post-weaning incidence of BRD have been estimated to range from $13.90 to $92.26 per head for treated animals (McNeill et al., 1996; Faber et al., 1999; Snowder et al., 2006; Schneider et al., 2009). Griffin et al. (1997) estimated annual losses to the US cattle industry to be near $1 billion, while $3 billion is spent on prevention and treatment costs.

As the pathology is further characterized for the microbial causative agents, there is potential for treatment and prevention protocols to be developed to dramatically reduce incidence of BRD. Though improvements in treatment and prevention protocols may reduce indirect costs associated with production loss, it would have marginal to no impact on cost already associated with treatment and prevention (McNeill et al., 1996; Faber et al., 1999; Snowder et al., 2006; Schneider et al., 2009). Beef consumers have become increasingly aware of animal well being and the environmental conditions in which they are raised. The consumer base also expects red meat products to be free from antimicrobial and therapeutic drug residues administered in the treatment of infected animals (Issanchou, 1996). Public concern is increasing regarding the potential overuse of antimicrobials in animal agriculture, and the implications that improper use may have on the development of antibiotic resistant organisms in human medicine.

Selection for disease resistance is an alternative method to reduce economic losses while addressing public and consumer concern for animal welfare and healthfulness of the beef products. Before selection schemes can be implemented it is important to understand the genetic variation within populations and any potential genetic antagonisms with other economically relevant traits (Golden et al., 2000). Therefore, the primary objectives of this study were to estimate variance components for BRD in feedlot cattle using feedlot treatment records (**Trt**) and to evaluate genetic and environmental correlations of the disease with HCW, LM area, marbling score (**MS**) and subcutaneous backfat thickness (**FAT**).

Materials and Methods

Crossbred steer calves were obtained from a single ranch source in western Nebraska originating from one of three management units in November of 2007 and 2008 (n = 1,551 and 1,319; respectively) and shipped to a commercial feedlot in Southeastern Colorado. Cattle were

in transit approximately seven hours over a distance of 536 kilometers. Each year, steers were received over three days and housed overnight in feedlot receiving pens with unlimited access to grass hay and water. Processing occurred the following day and cattle were allocated to their respective pens. In some cases, initial processing did not occur until two days post arrival due to limitations related to individual animal processing time. Once processed, steers were housed in pens averaging 260 head per pen (6 pens in 2007 and 5 pens in 2008) with a minimum of 99 and a maximum of 310 head per pen.

Calves in the study were a result of multi-sire breeding pastures. Sire identification was performed through DNA sampling of both sire and progeny through a commercial lab. Eighty one percent of calves were successfully sire identified (n = 2,331). A five generation sire pedigree (n = 3,255) was constructed for the purpose of variance component estimation. The pedigree consisted of 386 sires with 309 sires having direct ties to progeny with data. Of the sires with progeny data, 270 (87%) had more than two progeny with a mean of 8 progeny per sire (SD = 6.9) and a minimum of 2 and maximum 60 progeny with data.

Treatment records were used as the phenotype for susceptibility to BRD, which was defined as an animal within the population with a higher risk of becoming afflicted with BRD or is less likely to moderate the microbial lifecycle and remain clinically healthy when exposed to causative agents. Thus, Trt was a binary observation where steers showing clinical signs of BRD and subsequently treated were coded as a 1 while untreated animals were coded as 0. During year one a total of 698 steers (45.0%) were treated for BRD, 94 steers (7.0%) in year two, and 792 total (27.6) over the combined years.

Steers were humanely harvested in Dumas, TX and Greeley, CO in years one and two, respectively. Relevant carcass traits were collected by trained personnel. Carcass traits included HCW, LM area, marbling score (**MS**), and subcutaneous backfat thickness (**Fat**). Not all animals had carcass observations due to mortality during the feeding phase and loss of identification through the harvesting process. Counts of valid carcass observations and summary statistics for treated verses not treated steers are given in Table 1.

Two multivariate sire models were used to estimate direct genetic and residual (co)variance parameters between Trt and relevant carcass traits. Thus, Trt was evaluated with lean traits (HCW and LM area) and fat traits (MS and FAT) separately. Contemporary group was fitted as the only fixed effect in both models. Contemporary groups were defined as a combination of year, ranch unit of origin, and feedlot pen for analysis of Trt during the feeding period, while an additional level of harvest date was included for carcass traits. The random effect of sire was included to estimate additive genetic effects, which were assumed to be continuous for all carcass traits, and a probit threshold link function was applied to the binary dependant variable, Trt, to convert binary observations to the underlying scale (Gianola and Foulley, 1983; Harville and Mee, 1984). The linear model used can be described in matrix notation as,

$$\begin{bmatrix} 1 \\ 2 \\ 3 \end{bmatrix} \begin{bmatrix} & & \\ & & \\ & & \end{bmatrix} \begin{bmatrix} \\ \\ _3 \end{bmatrix}$$

$$\begin{bmatrix} 1 & & \\ & 2 & \\ & & _3 \end{bmatrix} \begin{bmatrix} 1 \\ 2 \\ 3 \end{bmatrix} \begin{bmatrix} 1 \\ 2 \\ 3 \end{bmatrix}$$

where known incidence matrices **X** and **Z** relate unknown fixed ($\mathbf{b_i}$), and direct genetic ($\mathbf{u_i}$) effects, respectively, to observations in $\mathbf{y_i}$. Observations in $\mathbf{y_i}$ associated with Trt are pseudo continuous observations on the underlying scale. The $\mathbf{e_i}$ is vector a random residual terms specific to animals with records for trait i.

The first and second moments of the model were assumed to be

$$\mathbf{E}\begin{bmatrix}\mathbf{y}\\ \mathbf{u}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{Xb}\\ \mathbf{0}\\ \mathbf{0}\end{bmatrix} \text{ and}$$

$$\mathbf{var}\begin{bmatrix}\mathbf{u}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\frac{\mathbf{1}}{\mathbf{4}}\mathbf{G_0} \otimes \mathbf{A} & \mathbf{0}\\ \mathbf{0} & \mathbf{R_n}\end{bmatrix},$$

where **u** and **e** are vectors of additive direct genetic and residual variance, respectively for each trait i. **A** is the Wright's numerator relationship matrix $\otimes$ is the Kronecker product operator, $\mathbf{G_0}$ is the additive genetic (co)variance matrix and $\mathbf{R_n}$ is a matrix of residuals such that with only trait 1, trait 2, or trait 3 measured, $^2_{e_1}$, $^2_{e_2}$, $^2_{e_3}$ will be on the diagonal with subscripts defined above. With 2 traits measured, $^2_{e_i}$ will be on the diagonal and $_{e_i,e_j}$ will be on the corresponding off-diagonal, where $^2_{e_i}$ is the variance due to residual effects for trait i, and $_{e_i,e_j}$ is the residual covariance for i^{th} and j^{th} traits measured on the same animal with $i \neq j$. Due to mortality and loss of records all traits were not measured on the same animal, and therefore some residual covariances are, by definition, zero, such as feedlot treatment records with carcass traits for animals that died during the feeding period.

The genetic parameters for all traits and their standard errors were estimated using ASREML (Ver. 3.0, VSN International, Ltd., Hemel Hempstead, UK) which employs an average information REML algorithm. Additive genetic variance was calculated as four times sire variance estimated through ASREML.

Results and Discussion

Heritability of Trt on the underlying scale was estimated to be 0.17 ± 0.06 (Table 2). This estimate is higher than previous estimates (Table 2) of 0.07 reported by Schneider et al. (2010), and the observed estimates of 0.04 and 0.08 reported by Snowder et al. (2006 and 2007), respectively. However, when converted to the underlying scale the estimate reported by Snowder et al., (2006) increased to 0.18 which is similar to the current estimate.

The current estimate is also larger than those reported for calves during the pre-weaning phase by Muggli-Crockett et al., (1992, 0.10 ± 0.02), Snowder et al., (2005, 0.07 ± 0.01 to 0.19 ± 0.01), and Schneider et al., (2010, 0.11 ± 0.06) (Table 2). The higher heritability estimate supports the findings proposed by Snowder et al., (2006) that heritability increases with increased disease prevalence. Prevalence rates used to estimate heritabilities of Schneider et al. (2010) and Snowder et al. (2006 and 2007) were reported at 9.43% and 17%, respectively, compared to the prevalence 27.6% in the current study. Similarly Bishop et al. (2010) proposed that estimates of heritability for disease resistance were underestimated due to prevalence, imperfect sensitivity and specificity, or the ability to accurately classify truly diseased animals. The current heritability estimate for BRD resistance, utilizing the phenotype of Trt, indicates that with intensive selection programs genetic progress can be made to improve resistance to BRD in the finishing phase.

Heritabilities for all carcass traits were moderate ranging from 0.24 to 0.62. When compared to other studies that also investigated the genetic associations between BRD and carcass measures, heritability of HCW (0.30 ± 0.05) was estimated to be lower than the 0.44 ± 0.08 and 0.71 ± 0.10 reported by Snowder et al. (2007) and Schneider et al. (2010), respectively. However, LM area (0.39 ± 0.05) and MS (0.62 ± 0.04) estimates were similar to those reported by Snowder et al. (2007).

Genetic and residual correlations between Trt and carcass traits are presented in Table 3. Genetic covariances between HCW and LM area and MS and FAT were moderate and favorable at 0.34 ± 0.14 and 0.38 ± 0.13, respectively. The genetic correlation estimate for Trt with MS was -0.30 ± 0.21. This estimate is in agreement with that reported by Schneider et al., (2010), who estimated genetic correlations between MS and Trt to be -0.43 ± 0.20. However, the current estimate is higher than the 0.09 ± 0.13 reported by Snowder et al. (2007) between Trt and MS. Estimates for genetic correlations between Trt and HCW was unfavorable (Table 3) and not in agreement with the favorable estimate reported by Schneider et al., (2010) of -0.22 ± 0.22 between Trt and HCW. Snowder et al., (2007) reported a slight but positive genetic correlation of 0.04 ± 0.14 between Trt and HCW. Genetic correlations for Trt with FAT were low and consistent with previous results (Snowder et al., 2007; Schneider et al.,2010).

Table 3. Estimates of genetic (r_g ± SE) and environmental (r_e ± SE) correlations of bovine respiratory disease with carcass traits[1]

	Trt	
	r_g	r_e
HCW	0.19 ± 0.30	-0.05 ± 0.02
LM area	0.03 ± 0.25	-0.01 ± 0.02
MS	-0.30 ± 0.21	-0.06 ± 0.02
Fat	-0.004 ± 0.26	-0.05 ± 0.02

[1] Trt = Binary treatment records (Yes=1), HCW = Hot carcass weight (kg), LM area = Longissimus muscle area (cm2), MS = Marbling score (Small 00 = 400), Fat = Subcutaneous fat thickness (mm)

Environmental correlations were near zero with small SE compared to genetic correlations (Table 3). These results in the current study would agree with the conclusion of Snowder et al. (2007), that environmental approaches including management and preventative therapies to decrease BRD incidence would improve overall carcass quality.

Implications

Heritability of Trt in the current study for resistance to BRD was low to moderate and would suggest that with highly intensive selection programs genetic improvement could be made over time to reduce the probability of being treated for BRD during the finishing phase. It is important to take into consideration the genetic relationship between BRD susceptibility and carcass traits when developing successful breeding programs to improve the health of steers, while maintaining optimal economic outputs. This study illustrated favorable genetic correlations between BRD susceptibility both MS and FAT. Though the standard error was high in relation to the estimate, there was a genetic antagonism estimated between HCW and BRD susceptibility. Further research is warranted to investigate the genetic correlations which were all estimated with high SE.

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Table 1. Summary statistics for evaluated carcass traits[1] of treated and non-treated feedlot steers

	No. Records	Mean	SD	Minimum	Maximum
Treated					
HCW	632	355.7	35	172.8	454.1
LM area[1]	632	83.3	8.7	51.7	115.6
MS[2]	632	402.9	55.7	250	670
FAT[3]	632	12.6	4.1	0.8	33.5
Not Treated					
HCW	1893	353.9	32.2	154.7	474
LM area	1886	81.9	8.2	50.7	115.8
MS	1892	403.3	68	250	750
FAT	1884	13.2	4.2	1	36.6

[1]HCW = Hot carcass weight (kg), LM area = Loin muscle area (cm^2), MS = Marbling score (Small00 = 400), FAT = Subcutaneous fat thickness (mm)

Table 2. Literature estimates of direct heritabilities on the observed scale for pre and post-weaning incidence of BRD

	h^2_d[1]		
Breed	Pre-weaning	Post-weaning	Source
Multi-Breed	-	0.17 ± 0.06	Current study
Multi-Breed	0.10 ± 0.02	0.06 ± 0.07	Muggli-Cocket et al. (1992)
Multi-Breed	0.10 ± 0.01	-	Snowder et al. (2005)
Multi-Breed	-	0.08 ± 0.01	Snowder et al. (2006)
Multi-Breed	-	0.08 ± 0.01	Snowder et al. (2007)
Angus Cross	0.12 ± 0.06	0.07 ± 0.04	Schneider et al. (2008)[2]

[1] h^2_d = direct heritability estimate
[2] estimates on the underlying scale

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RANDOM REGRESSION METHODOLOGIES USED FOR A DAYS TO WEIGHT GENETIC PREDICTION IN BEEF CATTLE

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ABSTRACT: The idea of reducing the number of days required for livestock to reach their desired endpoint is not new, with its economic importance first discussed in 1957. Given this economic relevance, genetic evaluation research for reducing these required days has received very little attention throughout the pertinent literature with the exception of the swine industry. There are many different production scenarios in today's beef industry, and a prediction for the required number of days to reach a single weight endpoint does not lend itself well to these diverse production systems. Random regression is an attractive alternative to calculate days to weight (**DTW**) genetic predictions due to its inherent properties. The methodology results in the ability to calculate regression curves for growth, which allow EBV to be calculated for any age or number of days on feed. The objective of this study was to develop a linear random regression model for the prediction of the required number of DTW. Data were obtained from the Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta, consisting of pedigree and weight observations on 1,324 cattle spanning the years 1999 – 2007. Individual animals averaged 5.77 weight observations with weights and ages ranging from 293 kg to 863 kg and 276 to 519 days, respectively. A linear random regression model, with Legendre polynomials as its base function, was used to regress age of the individual animals in days on weight. Fixed effects in the model included an overall fixed regression of age on weight to account for the mean relationship between age and weight as well as a contemporary group effect where contemporary group contained breed of the animal, feedlot pen and year of measure. Heritability estimates for DTW ranged from 0.56 ± 0.09 for the number of days to reach 293 kg all the way to 0.93 ± 0.01 for the number of days to reach 863 kg. These high heritability estimates indicate that placing selection pressure in an effort to reduce the number of days to reach a finish weight endpoint can result in relatively rapid genetic trend.

Key Words: Beef Cattle, Days to Finish, Heritability, Random Regression

Introduction

Reducing the number of required days for livestock to reach a specific weight endpoint has received very little attention throughout literature. With the exception of the swine industry this research has been almost non-existent, with only a handful of studies pertaining to beef cattle having been published going back to 1957. In summary, this research reported an average phenotypic correlation of -0.46 between the number of days to reach a perceived quality grade and net income per 45.4 kg of slaughter weight (Lindholm and Stonaker, 1957). More recently, Kuehn (2000) determined it feasible to obtain accurate variance component estimates for a linear random regression of days to finish weight using simulated data while Jubileu (2003) looked at differences between more traditional approaches such as multivariate models versus random regression techniques using Simmental weight data. Both Kuehn and Jubileu stressed the advantages of using random regression methodologies in the calculation of days to finish EPD.

Random regression allows the calculation of EPD along any given point of the regression line which is attractive for days to finish because each individual producer's "target" endpoint may be different. Random regression models have been implemented in many instances in various livestock industries for the genetic evaluation of test day records in dairy cattle (Ptak and Schaeffer, 1993; Guo and Swalve, 1997; Brotherstone et al., 2000) and growth data in pigs (Andersen and Pedersen, 1996) and beef cattle (Meyer, 1999; Legarra et al. 2004) just to name a few.

The lack of published research in this area of beef cattle genetic improvement is puzzling given the nature of "days to finish" as one of the economically relevant traits described by Golden et al. (2000). The objective of this study was to explore the feasibility of creating a days to weight (**DTW**) genetic prediction from a field data set using random regression methodologies.

Materials and Methods

Repeatedly measured age and weight observations (n = 7,633) recorded approximately every two to four weeks depending on test year, representing 1,324 individual animals spanning the years 1999 – 2007, were obtained from the Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta, Canada.

For the linear random regression of age on weight, the data set was initially filtered with the requirement for individual animals to possess 2 or more data points--a necessity to fit a linear regression line. After this initial requirement, contemporary groups were formed using a combination of year of the feeding period (each of eight years), feedlot pen (each of four feedlot pens), and breed type (Angus, Charolais and Charolais cross). Formation of

contemporary groups in this manner resulted in 62 unique groups with an average size of 21.3 animals per group. The minimum and maximum group size was 3 and 42 animals, respectively.

Editing the data in this manner resulted in a final data file consisting of 7,632 records, with only one animal removed for the data point restriction. For the purpose of estimating variance components, a 3-generation pedigree was built for the 1,323 individuals in this final data file. The final pedigree consisted of 5,414 individual animals, along with 1,386 unique sires and 2,705 unique dams. The average inbreeding for the individuals in this pedigree was 1.5% with minimum and maximum inbreeding levels of 0% and 25%, respectively.

Random Regression Model

A linear random regression model with Legendre polynomials as the base function was used to regress age on weight for the trait days to weight. The general form for a random regression model is represented in matrix form below.

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Qu} + \mathbf{Zpe} + \mathbf{e}$$

In the above equation, **y** represents a vector of age observations recorded on individual animals, **X** is an incidence matrix relating age observations in **y** to contemporary group and fixed regression coefficients containing weight observations for the regression of age on weight to their solutions in **b**, **Q** is an incidence matrix consisting of standardized weight observations relating the age observations in **y** to the random additive genetic Legendre polynomial regression coefficients in **u**, **Z** is an incidence matrix relating the age observations in **y** to the permanent environmental effects in **pe**, **e** is a vector of random residuals which include the temporary environmental effects for each observation. The random effects in the model are assumed to have means of zero and variances represented by

$$\text{var}\begin{bmatrix}\mathbf{u}\\ \mathbf{pe}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{A}\otimes\mathbf{G} & 0 & 0\\ 0 & \mathbf{I}\otimes\sigma^2_{pe} & 0\\ 0 & 0 & \mathbf{I}\sigma^2_e\end{bmatrix}.$$

Above, **A** was Wright's numerator relationship matrix and **I** was an identity matrix with an order equal to the total number of observations in **y**. The (co)variance matrix **G** is a 2 x 2 matrix consisting of the additive genetic variance for the random regression intercept and linear terms as well as the genetic covariance between the intercept and linear terms. The values σ^2_{pe} and σ^2_e represent the permanent environmental and residual variances, respectively.

Following Jamrozik et al. (1997), the structure of the residual variance was modified to allow for different residual variances for increasing weight using the equation

$$\text{var}[\mathbf{e}] = diag\{\sigma^2_{e_k}\},$$

where k is equal to the number of different residual variance sub-classes. The number of different sub-classes was equal to 4 representing each of the four quartiles for the distribution of weight observations.

Genetic variance estimates obtained from the random regression of age on weight were converted back to the observed scale using the formula

$$G_0 = \Phi G_{rr} \Phi^T$$

where $\mathbf{G_0}$ is the observed genetic (co)variance matrix between orthogonalized weights in Φ. $\mathbf{G_{rr}}$ is the random regression genetic (co)variance matrix as described by Schaeffer (2003).

The linear random regression model described above was implemented using the statistical software package ASREML (Gilmour et al., 2009).

Results and Discussion

Age and weight summary statistics obtained from the final data file are shown below in Table 1. Animals in this final file possessed an average of 5.77 observations with minimum and maximum observations of 2 and 9 observations per animal, respectively.

Table 1. Age and weight summary statistics for the data used in the linear random regression of age on weight for the calculation of the days to weight genetic prediction.

	Age[a]	Weight
N	7,632	7,632
Average	395.9	513.0
Variance	2,036.5	5,978.9
Minimum	276	293
Maximum	519	863

[a]Age is reported in days.

Estimates of genetic (co)variance between the intercept and linear terms for the Legendre polynomial random regression as well as the permanent environmental and residual variances are reported below in Table 2. These variances are then transformed back to the observed scale, shown in Figure 1, for selected weights within the range of weights reported in Table 1.

In Figure 1, genetic variance is very similar to phenotypic variance due to the rather small estimates of residual variances (table 2). Heritability estimates for the trait days to weight range anywhere from 0.56 ± 0.09 for the number of days to reach 293 kg to 0.93 ± 0.01 for the number of days to reach 863 kg. When compared to heritability estimates for other commonly evaluated traits, these are quite high.

Given the absence of literature heritability estimates for the number of days to reach a weight endpoint in beef cattle, in order to make comparisons to these

estimates obtained by random regression, a subsequent model was implemented using repeated measures methodology. The repeated measures model is endpoint indifferent, meaning no matter how the observations are adjusted; the resulting heritability estimate will be the same. The variable days was adjusted to reflect the number of days to reach 500 kg, a point near the middle of the weight distribution of this population. Since the ages were adjusted to a weight endpoint, the fixed regression of age on weight was removed from the evaluation resulting in contemporary group remaining as the sole fixed effect. This repeated measures model also contained the same residual variance classes as the random regression model.

The resulting estimates of genetic variance and heritability for days to weight using this repeated measures model was 460 ± 73.4 days^2 and 0.66 ± 0.09, respectively. This heritability estimate was higher than the 293 kg heritability estimate, but lower than the 863 kg heritability estimate obtained from the random regression model.

To make a direct comparison between the repeated measures model and random regression model for the number of days to reach 500 kg, genetic variance and heritability were calculated from the random regression model for the number of days to 500kg. The estimates of genetic variance and heritability from the random regression model were 420 ± 46.4 days^2 and 0.68 ± 0.06, respectively. Comparing these values to those obtained from the repeated measures model, we can see that both sets of estimates are well within the standard errors of one another, which leads us to believe the random regression model is estimating the same trait. Differences between the two models are seen in the data extremes where the data density is low (Figure 2).

Figure 2 contains the distribution of weight observations. In this data set, there are very few observations above 700 kg and considering that the maximum weight observation is 863 kg, these observations may perhaps be influencing the random regression model, thereby pulling heritability upward. Even so, the heritability estimates for the random regression model should be higher than the estimates obtained from the repeated measures model due to the fact that the random regression model is properly accounting for the changing covariance structure between successive weight endpoints.

Even with the elevated heritability estimates, the random regression model is a more attractive alternative for the calculation of days to finish. The use of this model allows individual producers to specify their own finish endpoint, which in and of itself is reasoning why these this methodology should be used for the "days to finish" traits.

Implications

Analysis of the weight and age observations show that selection efforts aimed at improving the number of days to reach a given weight endpoint can result in relatively rapid genetic change. The random regression model used will allow individual producers to set their own finish endpoints, allowing them to make more profitable selection decisions for their own operations.

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Table 2. Days to weight Legendre polynomial variance estimates (SE) obtained from the linear random regression of age on weight.

	Intercept[1]	Linear[1]	PE[2]	R11[3]	R22[3]	R33[3]	R44[3]
Intercept	1323 (107.4)	605.1 (34.2)					
Linear	0.83 (0.03)	406.6 (22.5)					
PE			278.7 (65.8)				
R11				33.42 (1.46)			
R22					43.62 (1.78)		
R33						54.14 (2.16)	
R44							42.82 (1.83)

[1]Intercept and linear genetic (co)-variaince. Genetic covariance is above the diagonal while the genetic correlation is below the diagonal.
[2]Permanent environmental variance
[3]Residual variances where R11, R22, R33, and R44 represent the first, second, third and fourth quartiles from the distribution of weight observations.

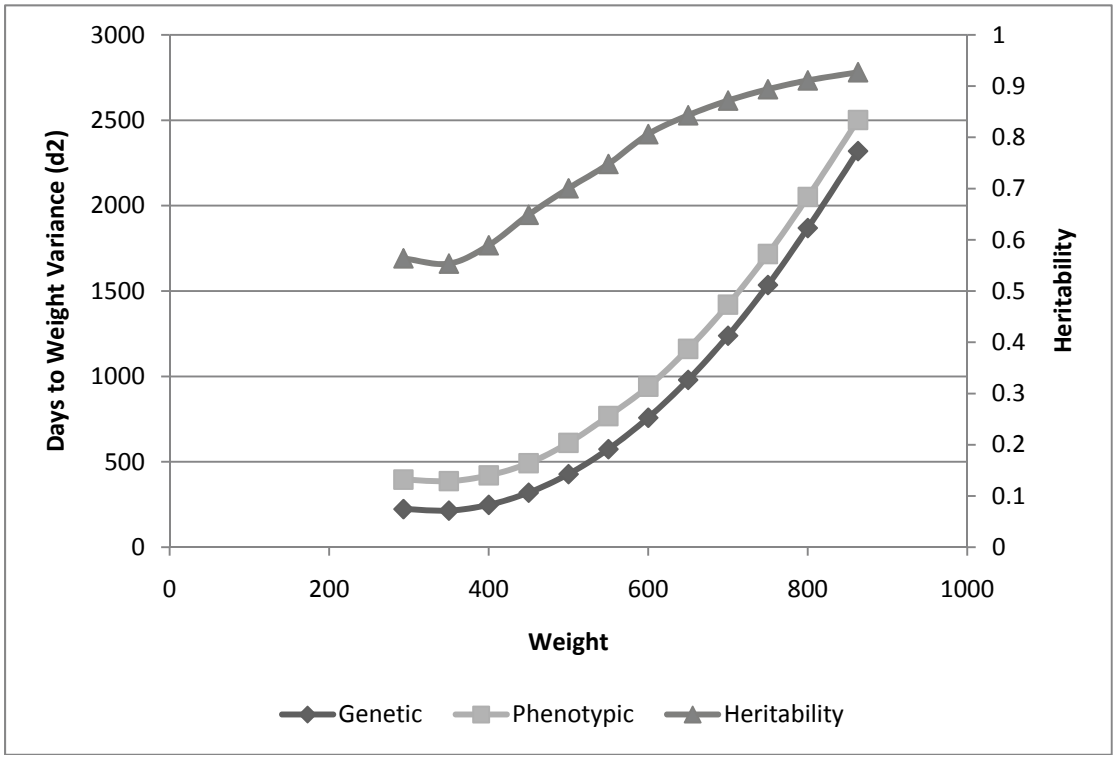

Figure 1. Observed days to weight genetic and phenotypic variance as well as heritability for selected weights across the range of weight observations.

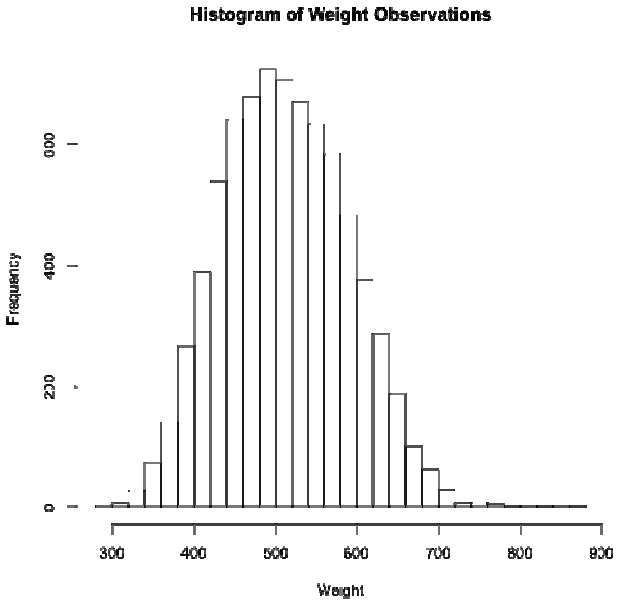

Figure 2. Histogram of weight observations used in the linear random regression of the days to weight genetic prediction.

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GENETIC PARAMETERS FOR ULTRASOUND MEASUREMENT IN BRANGUS CATTLE

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ABSRTACT: The use of ultrasound as an indicator of carcass performance in live animals can be used in an economically efficient and timely manner to make breeding and/or terminal decisions that influence genetic improvement in carcass traits. Given the utility of ultrasound and that ultimately ultrasound should be combined with actual carcass data to calculate expected progeny differences (EPD), the objective of this study was to estimate genetic parameters for ultrasound and carcass characteristics in Brangus Cattle as a step in the development of a more accurate carcass evaluation. Traits included ultrasound measures of 12th rib fat thickness (UBF), longissimus muscle area (UREA), and intramuscular fat percentage (UIMF). There were 64,058 Brangus bulls and heifers with observations in the International Brangus Breeders Association data base. Also obtained was historical pedigree information on 1,257,211 animals. These observations were taken between 300 and 430 days of age per Beef Improvement Federation guidelines (BIF, 2010). Of the 25,232 Brangus cattle with at least one ultrasound observation, 25,101 had UBF data, 25,119 had UREA data, and 20,783 had UIMF data. The data were analyzed using a three trait animal model with ASREML in order to estimate heritability and genetic correlations. The fixed effects of the model included contemporary group (defined as animals of the same sex, same weaning contemporary group, and same yearling management group), age of dam (a categorical fixed effect per BIF guidelines (BIF, 2010)), and age at ultrasound scanning as a linear covariate. The heritability estimates for UREA, UIMF, and UBF were 0.29 ± 0.01, 0.34 ± 0.02, and 0.30 ± 0.01 respectively. Genetic correlations between UREA and UIMF, UIMF and UBF, and UREA and UBF were -0.01 ± 0.04, and 0.36 ± 0.04 and 0.10 ± 0.04 respectively. These parameters will serve as a basis for use in genetic evaluation of carcass characteristics with the next step in that development being estimation of genetic correlations with actual carcass measures.

Introduction

Carcass characteristics of live animals can be estimated in an economically efficient manner when using ultrasound to help make genetic improvement decisions. The use of real-time ultrasound equipment is a relatively inexpensive way of estimating carcass composition in a way that is practical and nondestructive (Johnson et al., 1993). Carcass traits are generally considered highly heritable in order to improve carcass merit. Beef breed associations and breeders are collecting yearling ultrasound measurements for fat and muscle for both bulls and heifers and developing substantial data resources. With this potential for improvement many authors have estimated genetic parameters for ultrasound measurements of live yearling animals, while a few authors have estimated the genetic parameters of yearling Brangus cattle for fat deposits and longissimuss muscle area (Johnson et al., 1993; Moser et al., 1998; Stelzleni et al., 2002).

While ultrasound is a way to estimate carcass composition in live animals, heritabilities and correlations must be estimated before ultrasound data can be effectively utilized (Stelzleni et al., 2002). The objective of this study was therefore to estimate genetic parameters for 12th-rib fat thickness, longissimus muscle area, and percent intramuscular fat of yearling Brangus bulls and heifers utilizing ultrasound technology.

Materials and Methods

There were a total of 25,232 animals with ultrasound observations that were obtained from the International Brangus Breeders Association (**IBBA**). The traits included in this analysis were 12th rib fat thickness **(UBF)**, longissimus muscle area **(UREA)**,

and percent intramuscular fat **(UIMF)**. Before analysis observations from animals who were less than 300 days old or greater than 430 days old at the time of ultrasounding were removed per Beef Improvement Federation Guidelines (BIF, 2010). Animals that were one of a set of twins or were the result of embryo transfer also had their observations removed.

Contemporary groups for ultrasound were formed based on combinations of sex, weaning contemporary group and yearling management group (as defined by IBBA). Using this strategy, resulted in 1,420 unique groups each containing at least ten animals. Any contemporary group of less than ten animals was removed from the analysis.

In order to estimate variance components a 3-generation pedigree was built for the animals in the final data set. The pedigree consisted of 64,058 individual animals, with 7,428 unique sires, and 34,510 unique dams.

A three trait animal model was used to estimate heritability and genetic correlations among UREA, UIMF, and UBF. The fixed effects of the model included contemporary group, age of dam (a categorical fixed effect per BIF guidelines (BIF, 2010)) and age at ultrasound scanning as a linear covariate. The model used is represented in matrix notation as,

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \\ \boldsymbol{y_3} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X_1} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{X_2} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{X_3} \end{bmatrix} \begin{bmatrix} \boldsymbol{b_1} \\ \boldsymbol{b_2} \\ \boldsymbol{b_3} \end{bmatrix}$$

$$+ \begin{bmatrix} \boldsymbol{Z_1} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{Z_2} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{Z_3} \end{bmatrix} \begin{bmatrix} \boldsymbol{u_1} \\ \boldsymbol{u_2} \\ \boldsymbol{u_3} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \\ \boldsymbol{e_3} \end{bmatrix},$$

where matrices X and Z are known incidence matrices that relate unknown fixed **($\mathbf{b_i}$)**, and direct genetic **($\mathbf{u_i}$)** effects, respectively, to observations $\mathbf{y_i}$. $\mathbf{e_i}$ is a vector of random residual terms that are specific to animals with records for trait i.

For the model, the first and second moments are assumed to be

$$\mathbf{E}\begin{bmatrix} \mathbf{y} \\ \mathbf{u} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{Xb} \\ \mathbf{0} \\ \mathbf{0} \end{bmatrix} \text{ and}$$

$$\mathbf{var}\begin{bmatrix} \mathbf{u} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{G_0} \otimes \mathbf{A} & \mathbf{0} \\ \mathbf{0} & \mathbf{R_n} \end{bmatrix},$$

where **u** and **e** are vectors of additive direct genetic and residual variance, respectively for each trait. **A** is Wright's numerator relationship matrix, while $\otimes$ is the Kronecker product operator, $\mathbf{G_0}$ is the additive genetic (co)variance matrix and $\mathbf{R_n}$ is the residual matrix where if only trait 1, trait 2, or trait 3 is measured, σ^2_{e1}, σ^2_{e2}, σ^2_{e3} are on the diagonal. When two traits are measured, σ^2_{ei} will be on the diagonal and is the variance due to residual effects for trait i. The $\sigma_{ei,ej}$ will be on the off-diagonal and is the residual covariance for ith and jth traits measured on the same animal. Not all animals have records for each ultrasound measurement, therefore some residual covariances are zero by definition.

For all traits the genetic parameters and their corresponding standard errors were estimated using ASREML (Ver. 3.0, VSN International, Ltd., Hemel Hempstead, UK) which uses an average information REML algorithm.

Results and Discussion

In table 1 the summary statistics along with the heritabilities for the ultrasound data are reported.

Table 1. Summary statistics for each trait and heritabilities ± SE.[1]

Trait	N	Mean	SD	Heritability
UBF	25,101	0.202	0.088	0.304±0.017
UREA	25,119	10.86	2.16	0.298±0.019
UIMF	20,783	3.35	0.939	0.343±0.020

[1]UBF=Ultrasound Back Fat (cm); UREA= Ultrasound Rib Eye Area (cm^2); UIMF= Ultrasound Percent Intramuscular Fat (%)

With a moderate heritability estimate of 0.298±0.019, the UREA estimate supports the previous literature also using Brangus cattle (Moser et al., 1998; Johnson et al., 1993; and Stelzleni et al., 2002). The estimated heritability for UBF was 0.304±0.017. There are many varying estimates for UBF, in Robinson et al. (1993) the estimates ranged from 0.15 to 0.42 with a mean of 0.30 using five data sets of Polled Hereford, Hereford, and Angus cattle. While there is not information on the heritability of UIMF in the literature for Brangus cattle, there were estimates on carcass marbling scores and the correlation of ultrasound images of UIMF and

carcass marbling score. Heritability estimates for marbling score ranged from 0.26 to 0.47 (Arnold et al., 1991; Wilson et al., 1993), while Herring et al. (1998) reported a correlation between predicted or UIMF and actual percent of IMF of 0.61.

Table 2. Phenotypic and genetic correlations ± SE[1]

Trait	UREA[2]	UIMF[2]	UBF[2]
UREA		-0.006±0.05	0.097±0.04
UIMF	0.003±0.02		0.362±0.04
UBF	0.284±0.01	0.179±0.02	

[1]Genetic correlations above the diagonal, phenotypic correlations below diagonal.
[2]UBF=Ultrasound Back Fat (cm); UREA= Ultrasound Rib Eye Area (cm^2); UIMF= Ultrasound Percent Intramuscular Fat (%)

The phenotypic and genetic correlations for the three traits are shown in Table 2. In this study the genetic correlations for the ultrasound traits are low to moderate. The correlation between UIMF and UREA is almost zero, suggesting that one trait has no relationship to the other. UREA and UBF also have a low genetic correlation (0.097±0.04). From the low correlations between UREA and the other two traits it can be inferred that changing UREA would not cause a change in UBF or UIMF. A genetic correlation of 0.13 between UREA and UBF was reported by Moser et al. (1998). There was no information to compare the correlation between UREA and UIMF. The correlation between UIMF and UBF is a moderate correlation that is consistent with the 0.36 correlation reported by Stelzleni et al. (2002).

Implications

With the heritabilities and correlations found in this study it can be seen that selection for UREA would have a very small impact on the of fat deposition traits and should be useful for producers in their selection program. These parameters will serve as a basis for use in genetic evaluation of carcass characteristics with the next step in that development being estimation of genetic correlations with actual carcass measures.

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EVALUATION OF OVSYNCH AND TARGETED BREEDING EFFECT ON GESTATION AND DAYS OPEN IN DAIRY CATTLE

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ABSTRACT. In order to evaluate the effect of two hormonal protocols on pregnancy rates, days open, body condition (BC) and dairy cattle, the study was conducted in the dairy the Unit FMVZ-UJED (August, 2009 to September 2010), which used 56 Holstein cows calving. After calving were randomized to two groups: Group I (n = 30) received IM PGF_2 α on 28, 42 (detected estrus and AI) and 56 days postpartum (AI was detected estrus and cows that were not AI after 42 days (Targeted Breeding) Group II (n = 26) after 45 days postpartum and to determine the uterine involution was applied Ovsynch (GnRH + GnRH + PGF_2 α + Fixed Time AI). The body condition was assessed at calving, 25 and 50 days after delivery was also determined calving difficulty and retention of fetal membranes (RFM). The body condition was similar (3.0 ± .05 at delivery, 2.5 ± .06-25 and 2.6 ± .08 at 50 d, respectively) between groups (P> 0.05). We obtained 7.14% of dystocia and 15.7% retained fetal membranes (RFM). The Group I, 2.7 required doses of PGF2 α to first service. In Group II, treatment was initiated to 52.5 ± 5.4 days postpartum. The first service postpartum in Group I and II was to 64.03 ± 2.9 and 62.5 ± 5.8 days (P> 0.05). Pregnancy rate to first service was 76.9 vs 30% and service seconds was 95.9 vs 44% for Group I and II, 77.24 ± 3.9 and 110 ± 19.7 (P <0.05) days open, respectively. The conception services were better in Group I (1.25 ± .10) than in group II (1.6 ± .12). The cows of Group I showed better results on the reproductive parameters assessed although this would be strengthened if an economic study be carried out short, medium and long term.

Key Words: Dairy cattle, Postpartum period, Synchronization of oestrus

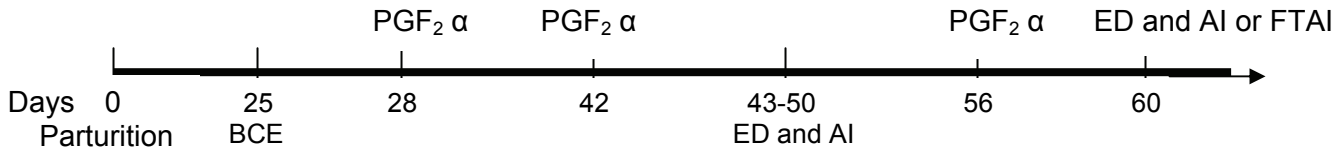
Introduction

The postpartum period (pp), milk production, nutrient consumption, body condition, caloric stress and handling determine the reproductive efficiency of dairy cattle (Butler, 2000). Hormonal treatments at pp increase gestation indices under various handling systems. Hormonal protocols synchronize estrous and ovulation, controlling follicular maturation in anestrous animals or regression of the corpus luteum in cyclic animals (Lucy *et al*., 2004). In the first 30 pp days a cow will initiate organic and physiological reestablishment, and restart of follicular maturation (Opsomer *et al*., 2000). After uterine involution any estrus induction or synchronization protocol can be initiated, which combined with artificial insemination at a fixed time improve the pregnancy rate in dairy cows (Melendez *et al*., 2006). Targeted Breeding includes up to three prostaglandin applications (PGF_2 α) starting at 28 days pp and continued for 14 days in cows that have not been serviced (Murugavel *et al*., 2003). On the other hand, Ovsynch is applied independently of the estrous stage in reproductively healthy cows eliminating the detection of estrous and it includes the intramuscular application of GnRH and PGF_2 α (Pursley *et al*., 1995). The objective of this study was to evaluate the effect of two hormonal protocols in pp, on the pregnancy indices and days open in dairy cows.

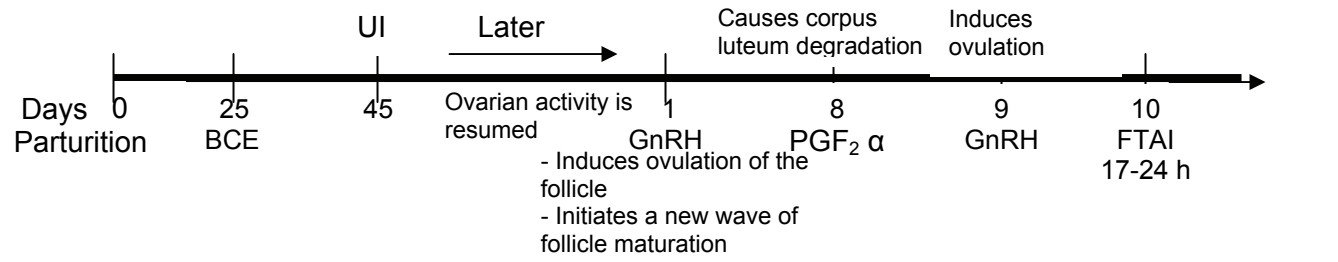
Materials and Methods

The work was carried out from August 2009 to September 2010, the study was conducted in the dairy the Unit FMVZ-UJED, in Durango México, in an area with semidry-temperate BS^1(w) climate (Köppen, 1936, as referenced by Aguilar, 2001). A total of 56 multiparous Holstein-Friesian cows were used under the following handling procedure: green forage was provided twice a day (irrigated pasture), dry forage (oats and alfalfa) or corn silage and 4 to 6 Kg of concentrated feed (17% CP) per animal, as well as free access to a mineral mixture containing 12% Ca and 12% P and trace minerals. Milking was carried out every 12 hrs. Animals were annually immunized against clostridial and viral respiratory diseases, and leptospirosis, dewormed and had an application of vitamins. A dry period of 45 to 60 days was allowed; the antepartum, partum and expulsion of fetal membranes (FM) were monitored as well as the course of uterine involution (UI) during the first 20 days pp. Body condition (BC) was evaluated in a 1-5 scale (Keown, 2002). Animals were randomly selected to receive one of the two hormonal protocols as follows: (BCE = Body Condition Evaluation, ED = Estrous Detection, AI = Artificial Insemination and FTAI = Fixed Time Artificial Insemination).

Protocol 1. Treatment of Group I (n = 30)



Protocol 2. Treatment of Group II (n = 26).



At the end of each protocol, estrous was detected twice a day (am-pm) for 30 minutes each time in order to verify if serviced cows returned to estrous. Pregnancy diagnosis was carried out after the last servicing via rectal palpation. Data was statistically analyzed using ANOVA and differences between means were established using Student's T test (SPSS, 2006).

Results and Discussion

Body condition of animals was similar between groups at the time of parturition 3.0 ± 0.05, at 25 days 2.5 ± 0.06 and 50 days 2.6 ± 0.08 (all P > 0.05). In general, up to 7.14% of 1st degree dystocia parturitions occurred and 15.7% of FM retention; the latter were animals different from those with dystocia. Fetal membranes were expelled on average after 4.4 ± 0.32 hrs (range 1 to 9 hrs). In Group I cows required an average of 2.7 doses of PGF_2 α until first servicing with an average interval between the first and second servicing of 39.3 ± 2.12 days. On the other hand, in Group II treatment was started on average at 52.5 ± 5.4 days pp. In general on average 1.6 ± 0.09 services were needed per conception. Table 1 shows the results for the variables measured in this study.

Taking into consideration the optimal BC at parturition (3.5) the animals throughout this study had poor BC, therefore we assume that this variable had negligible effect on the reproductive variables measured in this study (Keown, 2002; Rutter, 2002). Pedron *et al*., (1993) found that BC at parturition had no effect on milk production, the parturition to first service interval, parturition to conception interval and services per conception. Dystocia and FM retention are within normal parameters. Manspeaker (2005) reported up to 15% dystocia parturitions deriving between 5 to 10% FM retention, and a similar percentage of later endometritis and metritis. The fact that the first to second service interval does not coincide with the estrous cycle timing could mean that pregnancy was not maintained due to early corpus luteum degradation or embryo absorption leading to anestrous. These could be due to heat stress (temperatures reached 35 ºC), poor BC or low post-service progesterone concentration. Pregnancy loss due to various causes has been reported to reach between 9 and 21% (Olson, 2002). In Group II the interval between services, in which no evident sign of estrous was detected, was greater than the time equivalent to one estrous cycle. The results obtained for the variables measured in this study were better in Group I, which provides evidence that the use of PGF_2 α in an established management protocol is adequate for this type of cattle when compared to Ovsynch. Days open in both groups are within limits recommended for obtaining an interval between parturitions of 360 days. Considering these results it is determined that an integral strict management protocol helps increase fertility of dairy cattle.

Conclusions

The pregnancy index (PI) is measured in time units, thus the main advantage of a postpartum hormonal protocol together with a fixed time insemination resides in that 100% of the cows are treated and serviced before 70 days pp and, even though PI is low at first service, it will increase after estrous is detected. The Targeted Breeding protocol resulted in less days open and an improved PI even without modifying the general handling conditions of the herd.

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Table 1. Postpartum reproductive behavior in multiparous Holstein-Friesian cows

Variables	Group I Mean ± SE	Group II Mean ± SE
First postpartum service (d)	64.03 ± 2.9a	62.5 ± 5.8a
Interval between 1st and 2nd service (d)	24.33 ± 1.52a	39.3 ± 2.12b
Fertility at 1st service (%)	76.9	30
Fertility at 2nd service (%)	19	14
Services per conception (Num)	1.25 ± .10a	1.6 ± .12b
Days open (d)	77.24 ± 3.9a	110 ± 19.7

*Different letters among rows mean statistical difference at 0.05 confidence level

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GENETIC EVALUATION OF POSTPARTUM INTERVAL IN CHAROLAIS COWS

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ABSTRACT: Shorter postpartum intervals have been known to be associated with higher reproductive efficiency. The objective of this research was to estimate heritability, repeatability and EBV using postpartum interval data (PPI; from the calving date to the next breeding date), totaling 25,568 PPI records from 13,256 Charolais cows owned by 3,941 breeders in the Canadian Charolais Association (CCA). The AI date was recorded as breeding date in the data and was subsequently used to calculate unreasonable PPI records whereby females with PPI > 108 d and < 30 d were eliminated from the data. The mean PPI was 78.6 d (SD = 14.99). One PPI record was calculated for 13,256 cows, whereas 6,305 cows had more than one record. Postpartum interval records were divided into those recorded from cows giving birth in spring (from January to June, n = 24,673), and those recorded from cows calving in fall (from July to December, n = 894). Genetic parameters were estimated using a repeated records maternal animal model with REML. The model contained the random effect of breeding value and fixed factors of breeder, age, calving year and parity. A low heritability (0.038 ± 0.008) was estimated from this population and the estimated repeatability was 0.209. By applying regression analysis, all factors had significant effects (P<0.05) upon PPI based on a simple model that included the linear effect of age of cow. Similar significance level for age was calculated when a quadratic term for age of cow was included in the model. The low heritability of PPI in Canadian Charolais suggests that selection for reduced PPI would require high data density to be effective.

Keywords: PPI, Heritability, Genetic

Introduction

Reproductive traits play an important role in breeding programs, because there is a direct relationship between high rates of reproduction and the profitability of beef herds. Postpartum infertility and anestrus were first recognized as problems which effect reproductive ability over 80 years ago (Hammond, 1927). A shorter postpartum interval (PPI) results in a higher probability of pregnancy in the next restricted breeding season, which will result in higher profitability of the cows over their lifetime (Short et al., 1990).

The postpartum interval of dairy cows illustrated by previous studies (Darwash et al., 1997, Chapman and Casida, 1935) was from 25 to 108 days, the heritability was from 0.13 to 0.26 and the repeatability was from 0.2 to 0.28. Several studies (Mialon et al., 2000, Warnick, 1955, Brown, 1954) in the beef industry have reported that postpartum anoestrus interval played a critical role in achieving the goal of one calf per year. However, fewer studies on beef cows' genetic variability for postpartum interval have been reported. Mialon et al. (2000) reported that the mean interval from calving to first anestrus or Charolais cows was 69±25 days, heritability was 0.35 and repeatability was 0.6.

In Charolais, the postpartum interval is also a major component of the biological control of reproductive ability. The present study aimed to estimate genetic variability. We were also interested in the effects of several fixed factors on postpartum interval.

Method and Materials

*Data and Anima*l

The data used in the presented study were from the Canadian Charolais Association (CCA). The 25,568 PPI records used in the study were from 13,256 Charolais cows

owned by 3,941 breeders. AI date was recorded as breeding date of the cow to calculate PPI. Females with PPI > 108 d and < 30 d were eliminated from original data. There were 24,673 records from cows calving spring (from January to June), while 894 records from those giving birth in fall (from July to December). The number of postpartum interval (PPI) records was variable for individual cows ranging from 1 to 11. There were 47.56% cows with more than one PPI record.

Genetic Parameters and Statistical Analysis

Genetic parameters were estimated using a repeated records animal model with REML. The model contained the random effect of breeding value and fixed factors of breeder, age, calving year and parity. Thus the linear model was:

$Y_{ijklm}= \alpha_i+\beta_j+\gamma_k+\delta_l+a_m+\varepsilon_{ijklm}$

α_i= fixed effect of breeder i (3941 levels)

β_j= fixed effect of calving year j (27 levels)

γ_k= fixed effect of age of the cow k (16 levels)

δ_l= fixed effect of parity of the cow l (11 levels)

a_m= random additive genetic effects(EBV) of animal

ε_{ijklm}= random residual effects

Based on the model, the variance and covariance component of the data were estimated using DMUAI (Madsen and Jensen, 2000). These variance components σ_a^2 (additive genetic), σ_c^2 (permanent environment) and σ_p^2 (phenotype) are used to get h^2 (heritability) and r^2 (repeatability) as follows:

$$h^2 = \sigma_a^2 / \sigma_p^2 \quad \text{and} \quad r^2 = (\sigma_a^2+\sigma_c^2)/ \sigma_p^2$$

The GLM procedure of SAS was used to evaluate the potential fixed factors ignoring parity effect based on simple linear model (Model 1) and quadratic model (Model 2) with quadratic term for age of cow.

Result and Discussion

Genetic Parameters

The PPI from calving day to AI day of Charolais cows based on the data had a mean of 78.6±14.99 days. Figure1 shows the distribution of all the data. The result was longer than the postpartum interval (69±25 days) on Charolais cows reported by Mialon et al.(2000). This difference could be due to the method to calculate PPI, the heat detection success, or environmental factors. The detection of heat may be one of the most critical factors influencing the calculated PPI. There are many methods for detection, and the ability of individuals is variable (Brown, 1954). However, the difference was not significant between herds in Warnick (1955).

The heritability (h^2) of PPI estimated in the study was 0.038 ± 0.008, which was less than 0.12 of Charolais cows reported by Brown (1954) and 0.13 of dairy cows reported by Darwash(1997). The test for a null hypothesis: $h^2=0$ was not significant ($P<0.132$), which means there was a relatively small genetic effect on PPI. So selection for reduced PPI would require high data density to be effective. One previous study (Brown, 1954) had shown a heritability ($h^2= 0.008$ and $h^2=0.012$) of calving interval of beef cattle, which was lower than that for PPI in this study. The estimated repeatability is 0.209 which was similar with those two previous articles. The moderate repeatability reflected a biological effect on PPI.

Table 1. Heritability of PPI on data groups

Group	Variance estimates		h^2
	σ_A^2	σ_p^2	
Whole data	7.88346	203.0166	0.038
Parity 1	4.319539	158.419	0.027
Parity 2	4.582168	195.4628	0.023

σ^2_A:additive genetic variance

σ^2_P : phenotype variance

h^2: heritability

Effect of Calving Season

Table 2 shows the statistical comparison of PPI between two calving seasons. The average mean of PPI in spring is 1.35 days shorter than that in fall. This result corresponded with study of Hanson (Hanson et al. 1983), however it was on the opposite side of the study by Darwash (1997). Both the breed of the cattle and the method to divide the season may have led to different results. After all, every study cited above showed an effect of season on PPI. The effect of season on PPI may due to the ration of light and dark, the energy balance of feed or

the temperature.

Table 2. Statistical summary on PPI of two seasons

season	Number of observations	mean	SD
spring	24673	78.64	14.93
fall	894	79.99	14.25

Statistical Analysis

Table 3 shows the comparison of statistical results between Model 1 and Model 2. All the factors within model 1 are statistically significant ($P < 0.05$) indicating a linear relationship between PPI and fixed effects. The breeder ($P<0.218$) and calving year ($P<0.6592$) effects become insignificant in quadratic model (Model 2). Age is the most significant term in Model 1 and Model 2 .The negative value of age term indicated that PPI tended to be shorter, with age increasing, which was similar to the conclusion made by Torell and Ben (1998). Besides effects discussed in this study, the body condition, season and nutrition may have influence on PPI.

Table 3. Summary of statistical results o Model 1 and Model 2

Terms	Model			
	1		2	
	Estimate	p-value	Estimate	p-value
Breeder	0.0002	0.005[ab]	0.0001	0.218
Calving year	-0.1339	0.023[a]	-0.0258	0.659
Age	-0.8581	<0.001[ab]	-3.3927	<0.001[ab]
Age*Age			0.2542	<0.001[ab]

[a] $p<0.05$

[b] $p<0.01$

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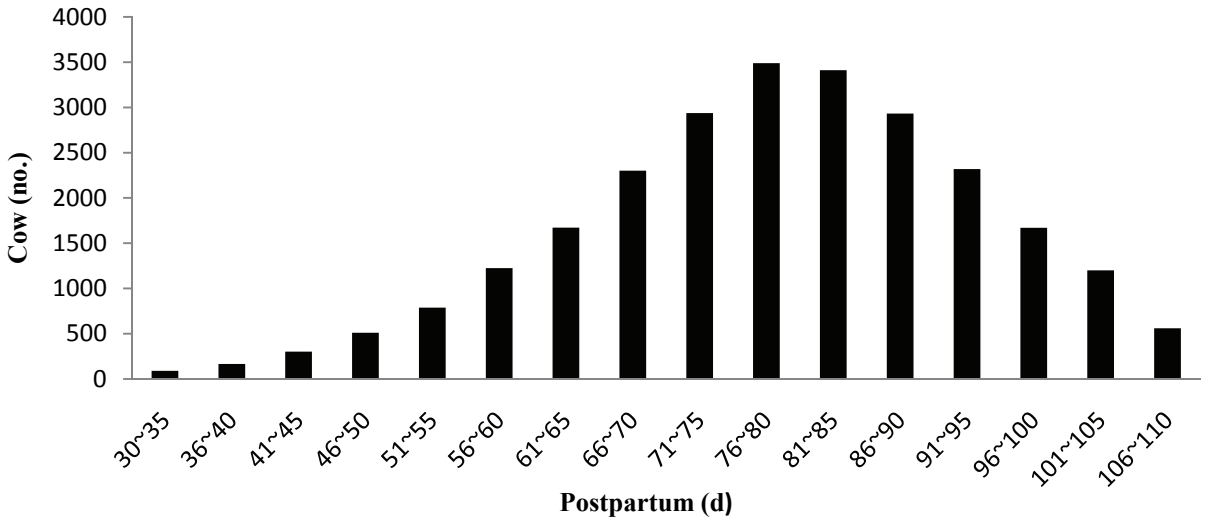

Figure 1. The frequency distribution of postpartum interval. The postpartum interval had been divided to 16 levels, depending on the days.

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GENETIC AND PHENOTYPIC PARAMETERS FOR CARCASS AND ULTRASOUND TRAITS OF AMERICAN SHORTHORN BEEF CATTLE

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ABSTRACT: Assessing the genetic relationship between carcass and ultrasound traits plays an important role in increasing accuracy of selection for carcass traits. The objective of this study was to estimate (co)variance components for fat-related carcass and ultrasound traits of Shorthorn cattle. Data were obtained from the American Shorthorn Association (ASA) for all available carcass and ultrasound traits. The data included 4,222 observations on 12–13th-rib fat thickness (**FAT**) and marbling score (**MS**). For live animal measurements 1,614 observations on ultrasound 12–13th-rib fat thickness (**UFAT**) and ultrasound intramuscular fat (**IMF**) were available. Also, historical pedigree information on 714,903 animals was obtained from ASA. From this pedigree, a reduced, 3 generation ancestral pedigree (21,272) was constructed beginning with animals that had either a carcass or ultrasound record. Traits were analyzed with a multivariate animal model and average information REML procedures to estimate heritabilities, genetic, and phenotypic correlations among traits. Fixed effects included contemporary group and the linear effect of age at measurement for all traits. For ultrasound traits, the linear effect of percent Shorthorn breed was included as another fixed effect. Heritability estimates for FAT, MS, UFAT, and IMF were 0.46 ± 0.06, 0.51 ± 0.06, 0.36 ± 0.07, and 0.24 ± 0.06, respectively. The estimated genetic correlation between FAT and MS was 0.46 ± 0.09. Further, MS had a moderately positive genetic correlation with IMF (0.55 ± 0.26). Also, UFAT had a moderately positive genetic correlation with IMF (0.43 ± 0.17). All other estimates of genetic correlations between traits were not significantly different from zero. The phenotypic correlation between MS and FAT (0.24 ± 0.01) was low. Including ultrasound data in genetic evaluation models increased the average accuracy of carcass traits 2.8 and 2.5 % for FAT and MS, respectively. These results indicate that genetic improvement in carcass traits can be achieved through including ultrasound traits as indicator traits which makes the genetic prediction more accurate.

Key Words: Shorthorn, Beef cattle, Carcass traits.

Introduction

Real-time ultrasound is used in animal science to take measurements on live animals with the goal of evaluating carcass traits that otherwise require slaughtering animals. Live animal measurements on indicator traits are widely used in the genetic evaluation of economically relevant carcass traits. Genetic evaluations for carcass traits based on ultrasound measurements of yearling cattle have the potential to reduce the expense and time associated with progeny testing and increase the rate of genetic progress (Devitt and Wilton, 2001). Carcass traits are increasingly important to the beef cattle industry, especially with the introduction of more detailed carcass specification systems and the payment of premiums for products satisfying the requirements of specific markets (Robinson et al., 1993). Previous research shows that there is a genetic association between carcass measurements and real-time ultrasound measurements on live animals (Crews and Kemp, 2001; Nkrumah et al., 2007; MacNeil and Northcutt, 2008; MacNeil et al., 2010). The accuracy of breeding value estimates for carcass traits can be increased by including live animal measurements with actual carcass data on relatives (Crews and Kemp, 2002). The goal of this study was to estimate genetic parameters for fat-related carcass traits and their RTU indicator traits in American Shorthorn cattle.

Materials and Methods

Data were obtained from the American Shorthorn Association (ASA) for all available fat-related carcass and ultrasound traits to estimate genetic and phenotypic parameters. Studied traits were 12–13th-rib fat thickness (FAT), marbling score (MS), ultrasound 12–13th-rib fat thickness (UFAT) and ultrasound intramuscular fat (IMF).

Data were edited to keep only records that ranged between 0.0245-3.048 cm, 1-10, and 1-9% for both FAT and UFAT, MS, and IMF, respectively. Contemporary groups (CG) for FAT and MS were constructed by combining sex, season of birth, year of birth, harvest group and date. Contemporary groups for UFAT and IMF included sex, birth year, birth season, weaning management group, ultrasound management group, and measurement date. Contemporary groups that contained only one record were excluded. This process resulted in 5,836 records as a final data set which included 4,222 observations on FAT and MS with 793 unique contemporary groups. For live animal measurements 1,614 observations on UFAT and IMF were presented in 216 unique CG.

Historical pedigree information on 714,903 animals was obtained from the ASA. From this pedigree, a reduced, 3 generation ancestral pedigree (21,272) was constructed beginning with animals that had either a carcass or ultrasound measure.

Two models were fitted to the data. The first model (model C) was used to analyze only the carcass data and the second model (model F) was fitted for all data. Using

ASREML (Ver. 3.0, VSN International, Ltd., Hemel Hempstead, HP1 1ES, UK), traits were analyzed with a multivariate animal model and average information REML procedures to estimate heritabilities, genetic, and phenotypic correlations among traits. Fixed effects included contemporary group and the linear effect of age at measurement for all traits. For ultrasound traits, the linear effect of percent Shorthorn breed was included as another fixed effect. The linear model used to estimate genetic and environmental (co)variances for all data (F) can be described as

$$\begin{bmatrix} Y_1 \\ Y_2 \\ Y_3 \\ Y_4 \end{bmatrix} = \begin{bmatrix} X_1\beta_1 \\ X_2\beta_2 \\ X_3\beta_3 \\ X_4\beta_4 \end{bmatrix} + \begin{bmatrix} Z_1u_1 \\ Z_2u_2 \\ Z_3u_3 \\ Z_4u_4 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \\ e_3 \\ e_4 \end{bmatrix},$$

Where

Y_i are vectors of observations with subscripts 1, 2, 3, and 4 denoting FAT, MS, UFAT, and IMF, respectively; X_i and Z_i are design matrices relating the data to their respective fixed effects (β_i) and random animal effects (u_i), respectively; and (e_i) random residual effects.

The first and second moments of the model are

$E[Y] = X\beta$ and $E[u] = E[e] = 0$

$$\text{var}\begin{bmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \\ \mathbf{u}_3 \\ \mathbf{u}_4 \end{bmatrix} = \begin{bmatrix} \mathbf{A}\sigma^2_{u_1} & \mathbf{A}\sigma_{u_1,u_2} & \mathbf{A}\sigma_{u_1,u_3} & \mathbf{A}\sigma_{u_1,u_4} \\ \mathbf{A}\sigma_{u_2,u_1} & \mathbf{A}\sigma^2_{u_2} & \mathbf{A}\sigma_{u_2,u_3} & \mathbf{A}\sigma_{u_2,u_4} \\ \mathbf{A}\sigma_{u_3,u_1} & \mathbf{A}\sigma_{u_3,u_2} & \mathbf{A}\sigma^2_{u_3} & \mathbf{A}\sigma_{u_3,u_4} \\ \mathbf{A}\sigma_{u_4,u_1} & \mathbf{A}\sigma_{u_4,u_2} & \mathbf{A}\sigma_{u_4,u_3} & \mathbf{A}\sigma^2_{u_4} \end{bmatrix}$$

and

$$\text{var}\begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \\ \mathbf{e}_3 \\ \mathbf{e}_4 \end{bmatrix} = \begin{bmatrix} \mathbf{I}\sigma^2_{e_1} & \mathbf{I}\sigma_{e_1,e_2} & 0 & 0 \\ \mathbf{I}\sigma_{e_2,e_1} & \mathbf{I}\sigma^2_{e_2} & 0 & 0 \\ 0 & 0 & \mathbf{I}\sigma^2_{e_3} & \mathbf{I}\sigma_{e_3,e_4} \\ 0 & 0 & \mathbf{I}\sigma_{e_4,e_3} & \mathbf{I}\sigma^2_{e_4} \end{bmatrix}$$

where **A** is the additive numerator relationship matrix, **I** is an identity matrix of order appropriate to the numbers of observations. The **A** matrix was constructed for 21,272 animals which include 8,122 inbred animals. Residual covariances between carcass and ultrasound traits were constrained to be zero because no animal had both carcass and ultrasound measurements.

Results and Discussion

Summary statistics describing the data set are presented in Table 1. The average estimates of FAT (1.09 cm) and MS (5.31) were similar to the estimates of 1.0 and 5.3, respectively, reported by Pariacote et al. (1998) for Shorthorn cattle. Kemp et al. (2002) and MacNeil and Northcutt (2008) reported slightly higher FAT and MS averages for Angus cattle, whereas, Crews et al. (2003) reported slightly lower estimates for Simmental cattle. The average estimate of UFAT (0.50 cm) was lower than (0.933 cm) reported by Nkrumah et al. (2007) for animals sired by Angus and Charolais bulls. This average of UFAT was intermediate to the 0.407 and 0.562 cm UFAT reported by Crews and Kemp (2002) for composite bulls and heifers, respectively. Mean IMF (3.31%) was lower than the mean IMF (3.91%) for Angus cattle reported by MacNeil et al. (2010). Newman et al. (2002) reported similar estimate of 3.24% for crossbred animals sired by Shorthorn bulls.

Table 1. Summary statistics for12–13th-rib fat thickness (FAT, cm), marbling score (MS), ultrasound 12–13th-rib fat thickness (UFAT, cm), and ultrasound intramuscular fat (IMF, %) in American Shorthorn cattle.

	n	Mean	SD	Min.[a]	Max.[a]
FAT	4,222	1.09	0.39	0.10	2.84
MS	4,222	5.31	0.97	1.50	9.90
UFAT	1,614	0.50	0.20	0.12	1.47
IMF	1,614	3.31	0.90	1.29	8.54

[a]Min = Minimum; Max = Maximum.

Estimates of heritability (h^2 ± SE), genetic covariance, and genetic correlation (rg ± SE) for carcass and ultrasound traits are represented in table 2. The heritability estimate for FAT (0.46 ± 0.06) was similar to the estimates of 0.46 reported by Pariacote et al. (1998) for Shorthorn cattle. This estimate is slightly higher than the estimates reported in a review paper by Koots et al. (1994a). Heritability estimate for MS of 0.51 ± 0.06 was higher than those of Marshall (1994), Koots et al. (1994a), Kemp et al. (2002), Veseth et al. (1993), Wilson et al. (1993), and MacNeil et al. (2010). Pariacote et al. (1998) reported a higher estimate for MS (0.88) than the current heritability estimate. The heritability estimate for UFAT of 0.36 ± 0.07 was lower than those reported by Crews and Kemp (2001) who reported estimates of 0.5 and 0.44 for composite bulls and heifers, respectively. The IMF heritability estimate (0.24 ± 0.06) was lower than the estimate of 0.31 reported by MacNeil et al. (2010) and Kemp et al. (2002).

Table 2. Estimates[a] of heritability (h^2 ± SE), genetic covariance, and genetic correlation (rg ± SE) for carcass and ultrasound traits[b].

	FAT	MS	UFAT	IMF
FAT	**0.46 ± 0.06**	0.060	0.007	-0.023
MS	0.46 ± 0.09	**0.51 ± 0.06**	0.013	0.096
UFAT	0.33 ± 0.26	0.26 ± 0.24	**0.36 ± 0.07**	0.011
IMF	-0.33± 0.28	0.55 ± 0.26	0.43 ± 0.17	**0.24 ± 0.06**

[a]Estimates of genetic correlation are below the diagonal, and genetic covariance are above the diagonal. On the diagonal, heritability estimates (indicated by boldface).
[b]FAT = 12–13th-rib fat thickness; MS = marbling score; UFAT = ultrasound 12–13th-rib fat thickness; and IMF = ultrasound intramuscular fat.

The estimated genetic correlation between FAT and MS was 0.46 ± 0.09. The reported estimate seems to be in the range of estimates reported in a review paper by Marshall (1994). Pariacote et al. (1998) reported that fat thickness was genetically correlated (0.26) with marbling score. The genetic correlation estimate of FAT with UFAT (0.33 ± 0.26) and IMF (-0.33 ± 0.28) were not significantly different from zero. Also, MS had a moderately positive genetic correlation with IMF (0.55 ± 0.26) which is similar to the estimate of MacNeil et al. (2010), who reported 0.56. The current estimate of genetic correlation between MS and IMF tends to be toward the lower end of the range of estimates reported by MacNeil and Northcutt (2008) for Angus cattle. Further, UFAT had a moderately positive genetic correlation with IMF (0.43 ± 0.17). The current estimate is higher than those of Devitt and Wilton (2001) and Kemp et al. (2002), who reported estimates of 0.28 and 0.38, respectively.

Table 3 summarizes estimates of phenotypic (co)variances and phenotypic correlations. Residual covariances between carcass and live animal measurements were constrained to be zero because no animal had both carcass and ultrasound data. The phenotypic correlation between MS and FAT (0.24 ± 0.01) was low. This result is in agreement with Pariacote et al. (1998) who reported a phenotypic correlation estimate of 0.20 for Shorthorn cattle. Koots et al. (1994b) in a review paper reported an unweighted average of 0.22. This result in the range of estimates (0.12-0.38) cited by Marshall (1994). UFAT had a moderately positive phenotypic correlation with IMF (0.23 ± 0.02). This result is higher than the estimate (0.13) obtained by Devitt and Wilton (2001).

Table 3. Estimates[a] of phenotypic variance, phenotypic covariance, and phenotypic correlation (rp ± SE) for carcass and ultrasound traits[b]

	FAT	MS	UFAT	IMF
FAT	**0.115**	0.066	-	-
MS	0.24 ± 0.01	**0.639**	-	-
UFAT	-	-	**0.022**	0.021
IMF	-	-	0.23 ± 0.02	**0.381**

[a]Estimates of phenotypic correlation are below the diagonal, and phenotypic covariance are above the diagonal. On the diagonal, phenotypic variance estimates (indicated by boldface).
[b]FAT = 12–13th-rib fat thickness; MS = marbling score; UFAT = ultrasound 12–13th-rib fat thickness; and IMF = ultrasound intramuscular fat.

Table 4 summarizes statistics for EBV and accuracies for carcass traits using two different models. Including ultrasound data to evaluation models increased the average accuracy of carcass traits 2.8 and 2.5 % for FAT and MS, respectively. Crews and Kemp (2002) pointed out that the addition of live animal date to the genetic evaluation of carcass traits increased the EBV average accuracy and reduced the range.

Table 4. Summary statistics for EBV and accuracies[a] for carcass traits[b]

	Model C[c]		Model F[d]	
	FAT	MS	FAT	MS
Minimum EBV	-0.51	-1.64	-0.51	-1.65
Maximum EBV	0.70	1.89	0.67	1.87
Mean accuracy, %	10.18	10.79	10.47	11.07
Min.[f] accuracy, %	-0.01	-0.008	0.02	0.0007
Max.[f] accuracy, %	61.66	63.07	61.73	63.17

[a]BIF accuracy
[b]FAT = 12–13th-rib fat thickness; MS = marbling score.
[c]Included only carcass traits.
[d]Included both carcass and ultrasound traits.
[f] Min. = Minimum; Max. = Maximum.

Implications

The moderate and positive genetic correlation between marbling score and intramuscular fat suggests that increasing the genetic potential for intramuscular fat will increase the genetic potential for marbling score. However, there was no genetic association between back fat and ultrasound back fat in this study. The average EBV accuracies for carcass traits were increased by the addition of ultrasound measurements to the evaluation model. These results indicate that genetic improvement in carcass traits can be achieved through including ultrasound traits as indicator traits which makes the genetic prediction more accurate.

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Differential gene expression combined with phenotypic data for animal genetic evaluation

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Abstract

Gene expression data were typically used for identification of candidate genes for economically important traits. In the Bayesian framework, differential expression data can be utilized to construct the prior distribution for breeding value estimation. Expression levels were normalized before analysis. Normalized data of identified genes were fitted to the phenotypic measures in a principle component model to reduce the multicollinearity among variables. Genetic contribution to the particular trait was predicted with the expression level of identified genes and corresponding regression coefficient estimates. Predicted genetic values for each animal then were implemented in a linear mixed model as the mean of the prior distribution of breeding value. The breeding value was obtained from the fully conditional posterior distribution. Microarray data associated to the drip loss were used for testing the model. Phenotypic measures for drip loss for the 74 animals were simulated within the range from 0.87% to 3.32% based on the correlation coefficients between drip loss and expression levels of identified genes. The method developed here is an improvement of BLUP method. It has additional benefit in estimation of breeding value as the weighted average of expression levels and phenotypic measures. This method has the advantage of giving more reliable estimate for young sire without many performance records from progeny.

Keywords

Gene expression
Breeding value
Bayesian

Introduction

Microarrays examine expression level of genomic sequences simultaneously, which is as a powerful tool for detecting potential genes. However, differential expressions between the high and low lines of individuals may be obscured by the complication of genetic network. One way to analyze the expression change is for QTL mapping offering candidates for marker assisted selection (Walsh and Henderson, 2004). Use of array data can speed up the finding of new QTLs and the pathway analysis can help to disclosure the genetic network of complex traits. For the improvement in selective breeding, gene expression have to properly related to the phenotypic and marker data. Many methods have been developed for the prediction of genomic breeding values using single nucleotide polymorphism maker data (Zhang et al, 2010). Where individuals were genotyped and the breeding values were estimated by

adding up each marker effects. Alternatively, continuous measure of expression data of genes can be used for breeding value prediction. The objective of this study is to develop an approach for applying gene expression data in animal genetic evaluation.

Material and Method

Animal breeding values were estimated in few steps. For microarray array data, measures of expression abundance may slightly change from chip to chip for different individuals. The expression data were normalized before analysis. Normalized data of all identified genes then were fitted to the phenotypic measures. The identified genes are usually related to each other in a metabolic pathway for some biological function. Dependence among genes was taken into account in a principle component model to reduce the multicollinarity. Genetic contribution to the particular trait was predicted by using the expression levels of identified genes and their corresponding regression coefficient estimates. In the second step, the predicted genetic values for each animal then were implemented as the means of prior distributions of breeding value in a linear mixed model under Bayesian context. The breeding value estimate then was obtained from fully conditional posterior distribution.

Microarray data associated to the drip loss by Ponsuksili and others (2008) were used for testing the model. Expression data for the 74 animals were obtained from Gene Expression Omnibus public repository (GEO accession number: GSE10204). Data were processed with BioConductor (Gentleman et al, 2004.) to extract the expression data. Phenotypic measures for drip loss and pedigrees for the full-sib families were not in the repository. Therefore, the phenotypic values were simulated within the range from 0.87% to 3.32% as noted in the figure of the article. Assuming a simple linear regression model, phenotypic data were simulated using the correlation coefficients between the expression level and the drip loss. The mean and variance of drip loss were estimated based on the range. The highest correlated identified gene (r=0.563) were used for the estimation of drip loss.

Result

For the current data set, with no contemporary effect and assuming all individuals were unrelated, the breeding value estimate was dominated by the prior value. The heritability of the drip loss depends on how much of variation that can be explained by the identified genes. The increase of number of identified genes for prediction tends to have lager portion of phenotypic variance explained.

Discussion

The method developed here is considered as an improvement of BLUP method. It has additional benefit in estimation of breeding value as the weighted average of expression levels and phenotypic measures. This method has the advantage of giving more reliable estimate, especially for young sire without many performance records from their progeny.

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COMPARISON OF FEEDING DRY DISTILLERS GRAINS IN A BUNK OR ON THE GROUND TO CATTLE GRAZING SUBIRRIGATED MEADOW

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ABSTRACT: The objective of this study was to compare feeding dry distillers grains with solubles (DDGS) in a bunk or on the ground to cattle grazing subirrigated meadow. One hundred fourteen, March-born steers (279 ± 29 kg BW) were assigned to one of two feeding treatments: DDGS fed in a bunk or on the ground. Six pastures were used and pasture served as the experimental unit. Steers were fed the daily equivalent of 0.9 kg/steer (DMB) and supplement was delivered 3 d/wk. Dry distillers grains with solubles was in meal form as received directly from the ethanol plant. The experiment was conducted during a 72 d period at the University of Nebraska, Gudmundsen Sandhills Laboratory near Whitman, NE from March 10 to May 20, 2010. Steers continuously grazed the same pasture throughout the experiment. For bunk fed steers, bunks were not moved for the duration of the study. Steers fed on the ground received supplement in a different location within the pasture at each feeding. Steer BW was recorded on two consecutive days at the initiation and completion of the feeding period. Steers were not limit fed prior to weighing. After completion of the feeding period, soil samples were collected from three sites where DDGS was fed on the ground and three control sites. At each site, six samples were collected and composited into one. Samples were analyzed for pH, OM, nitrate, phosphorous, sulfate, and potassium. No significant differences were seen in soil components between DDGS and control sites ($P > 0.3$). Steers fed in a bunk had greater ADG than steers fed on the ground (0.53 vs. 0.42 kg; $P < 0.001$). The NRC (1996) was used to retrospectively calculate the DDGS intake difference between treatments. For steers fed in a bunk, a reduction in DDGS intake between 0.36 and 0.41 kg/day would have resulted in a 0.11 kg/day reduction in ADG. This is the equivalent of 36-41% waste. At $200 (DMB) per ton for DDGS, the cost of the wasted distillers grains was between $0.08 and $0.09 per day. An advantage in animal performance to feeding DDGS in a bunk versus on the ground was seen in this study.

KEYWORDS: Dry distillers grains, supplementation, bunk, grazing

Introduction

Growth of the ethanol industry in Nebraska and surrounding states has increased the availability of distillers co-products for livestock feed. Distillers grains plus solubles is high in protein, energy and phosphorous, making it an excellent supplement in many grazing situations (Gustad, 2006). In a summary of 14 grazing trials, Griffin et al. (2009) reported supplementation of dried distillers grains with solubles (DDGS) increased final BW and ADG quadratically. In addition, DDGS supplementation decreased forage intake quadratically, however total intake for supplemented cattle increased quadratically with increased DDGS levels (Griffin et al., 2009).

Feeding DDGS on the ground may result in higher waste levels when compared to feeding it in a bunk, but may increase its use in practical grazing situations and increase profitability. Therefore, the objective of this study was to compare feeding DDGS in a bunk or on the ground to grazing cattle.

Materials and Methods

One hundred fourteen, March-born steer calves (279 ± 29 kg BW) were assigned to one of two feeding treatments: DDGS fed in a bunk or on the ground. Six pastures were used and pasture served as the experimental unit. Steers were fed the daily equivalent of 0.9 kg/steer (DMB) and supplement was delivered 3 d/wk.

The experiment was conducted at the University of Nebraska Gudmundsen Sandhills Laboratory (GSL) near Whitman, NE according to protocol approved by the University of Nebraska-Lincoln Animal Care and Use Committee. Calves grazed subirrigated meadow dominated by cool-season grasses, sedges, and rushes. The most common grasses were slender

wheat grass [*Elymus trachycaulus* (Link) Matte], red top bent (*Agrostis stolenifera* L.) and timothy (*Phleum pretense* L.). Other grasses present included Kentucky bluegrass (*Poa pratensis* L.) and smooth bromegrass (*Bromus inermus* Leyss.). Wooly sedge (*Carex lanuginose* Michx.) and several spike rush species (*Eleocharis* spp.) were also abundant. The primary legumes included white clover (*Trifolium repens* L.) and alsike clover (*Trifolium hybridum* L.) and red clover (*Trifolium Pratense* L.). Warm-season grasses present in minor amounts were prairie cordgrass (*Spartina pectinata* L.) and big bluestem (*Andropogon gerardii* Vitman) (Volesky et al., 2004). The study site had been hayed the previous summer so cattle grazed re-growth.

The experiment was conducted for 72 days from March 10 to May 20, 2010. Steers continuously grazed the same pasture throughout the experiment. Steer BW was recorded on two consecutive days at the initiation and completion of the feeding period. Steers were not limit fed prior to weighing.

After completion of the feeding period, soil samples were collected from three sites where DDGS was fed on the ground and three control sites. Sample cores were collected from a 0-20 cm depth increment. At each site, six samples were collected and composited into one. Samples were analyzed for pH, OM, nitrate, phosphorous, sulfate, and potassium.

All data were analyzed as an unstructured treatment arrangement in a completely randomized design using MIXED procedures (SAS Inst. Inc., Cary, NC). The model included the effect of feeding method. Pasture was used as the experimental unit. Differences were considered significant when *P*-values were < 0.10.

Results

No significant differences were seen in soil components between DDGS and control sites ($P > 0.3$). A visible difference between fed and control areas was apparent. Grass was slightly greener in fed areas compared to control areas. Since samples included soil from a depth of 20 cm, this may have diluted the soil components compared to those present at a shallower depth.

Steers fed in a bunk had greater ADG than steers fed on the ground (0.53 vs. 0.42 kg; $P < 0.001$). The NRC (1996) was used to retrospectively calculate the DDGS intake difference between treatments. For steers fed in a bunk, a reduction in DDGS intake between 0.36 and 0.41 kg/day would have resulted in a 0.11 kg/day reduction in ADG. This is the equivalent of 36-41% waste. At $200 (DMB) per ton for DDGS, the cost of the wasted DDGS was between $0.08 and $0.09/d. In comparison, steers fed WDGS on the ground were reported to have a 13 % waste over those fed in a bunk (Musgrave et al., 2009). Part of this difference might be explained through ground conditions. The WDGS were fed on upland range from October to December whereas the current study was conducted subirrigated meadow from March to May. Subirrigated meadow is characterized by dense plant growth, possibly allowing the DDGS to go deep within the grass and become unavailable to the animal.

The most profitable choice of DDGS feeding method depends on the production goal of the feeding period. If ownership of the cattle will be retained beyond the term of the DDGS feeding period and least cost to acheive a specified rate of gain is the production goal, then feeding on the ground would have been the most profitable choice. In our experiement we estimated the cost associated with feeding in a bunk, which includes bunk purchase and delivery and a three year bunk life span, to be $\$0.16/\text{steer}\cdot\text{d}^{-1}$. The value of the wasted DDGS was about $0.09, so if about 40% additional DDGS was fed on the ground, the cost to gain 0.5 kg/d would be $0.07 less than feeding in a bunk. This strategy would be appropriate if a set rate of gain was desired and BW gain above that rate was of no value. On the other hand, if the goal was to maximze profitability of the DDGS feeding period and ownership of the cattle will not be retained beyond that period then feeding in a bunk would be the most profitable provided the cost of gain is less than the value of the gain, even with the price slide. Whenever the cost of gain is less than the sale price, profitability is maximized when gain is maximized. If additional DDGS is fed, less waste would occur if fed in a bunk, therefore more weight would be gained by the animal and so long as the cost of feeding in a bunk ($0.16/d) doesn't increase the cost of gain above the sale price profitability at any given level of DDGS feeding would be greater if fed in a bunk. In this experiment, the cost of gain when DDGS was fed in a bunk was less than the sale price of the steers and therefore profit was greater in steers fed in a bunk.

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Table 1. Soil nutrient characteristics (0-20 cm) on sites following feeding of DDGS and on adjacent control sites.

	Ground	*Bunk*	*SE*	*P-value*
pH	7.6	7.7	0.3	0.82
OM	3.0	3.1	0.2	0.86
Nitrate-N (ppm)	5.2	3.5	1.3	0.41
Nitrate-N (kg/ha)	13.8	9.8	3.5	0.45
P Bicarb (ppm)	7.0	5.7	0.8	0.33
P Bicarb (kg/ha)	15.7	12.7	1.9	0.33
Sulfate-S (ppm)	23.3	24.0	7.6	0.95
K (ppm)	87.7	83.3	8.7	0.74

Table 2. Performance of steers fed DDGS on the ground or in a bunk.

	Bunk	Ground	SE	P-value
Initial BW (kg)	279	279	3.6	0.89
Final BW (kg)	318	309	4.1	0.12
ADG (kg/d)	0.5	0.4	0.02	<0.001

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CONCEPTION RATES AND SERUM PROGESTERONE PROFILES IN RAMBOUILLET EWES TREATED WITH INTRAVAGINAL PROGESTERONE AND PROSTAGLANDIN $F_{2\alpha}$ INJECTIONS

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ABSTRACT: Eighty-one Rambouillet ewes (64.5 ± 1.0 kg BW) were used to examine serum concentrations of progesterone (P4) and conception rates of ewes treated with a P4-containing intravaginal insert (CIDR, 0.3 g of P4) and $PGF_{2\alpha}$ (10 mg; i.m., Lutalyse) during a fall breeding season. Ewes were kept in an outdoor pen (10 x 30 m^2) and fed alfalfa hay (1.6 kg/ewe daily). Twenty-seven ewes were randomly assigned to serve as either controls (CIDR plus saline injection), PG1 (CIDR plus 10 mg $PGF_{2\alpha}$ injected at CIDR removal) or PG2 (CIDR plus 5-mg $PGF_{2\alpha}$ injected at CIDR removal and 4 h later). The CIDR inserts remained in place for 14 d. Four Rambouillet rams were joined with ewes 24 h after CIDR removal for a period of 6 d, removed for 6 d, and reintroduced for 6 d. Blood was collected on alternate days beginning on d 0 (CIDR insertion) until d 14 (CIDR removal) after which sampling continued daily until d 21. On d 14, blood was collected before CIDR removal and 4 h later before the second $PGF_{2\alpha}$ injection. During the 14-d period the CIDR was in place, serum P4 differed among days ($P < 0.001$; 8.0 ± 0.2 ng/mL 48 h after insertion, 2 ng/mL immediately before CIDR removal). Serum P4 before CIDR removal and 4 and 24 h later did not differ ($P > 0.25$) among treatment groups. Progesterone declined ($P < 0.02$) in all 3 treatment groups 4 h after CIDR removal with both $PGF_{2\alpha}$-treated groups declining further ($P < 0.008$) by 24 h after CIDR removal. Progesterone values from 24 h to 7 d after CIDR removal were similar among treatment groups ($P = 0.26$). Pregnancy rates of ewes lambing to two 6-d service periods were 55.6, 85.2, and 77.8 % respectively for control, PG1, and PG2 ewes ($P = 0.04$). Number of lambs born per ewe did not differ among treatment groups when considering only ewes lambing ($P = 0.16$). Offspring ADG tended to be heavier for lambs born to control ewes ($P = 0.09$). Treatment with CIDR inserts for 14 d and $PGF_{2\alpha}$ after CIDR removal resulted in an increased lambing percentage compared with control ewes.

Key words: CIDR, $PGF_{2\alpha}$, sheep

INTRODUCTION

Reproductive efficiency is a key component of livestock industries, in particular the sheep industry. Many estrus synchronization methods have been explored to achieve increased conception and lambing rates, concentrate labor costs, create uniform lamb crops, and supply a seasonal market endeavoring to increase overall profits. Synchronized estrus is achieved by inhibiting estrus, causing CL regression, inducing ovulation, or any combination of the three (Kridli et al., 2003; Lauderdale, 2010). Progesterone or other progestogens inhibit estrus. Natural progesterone can be administered using controlled internal drug releasing (CIDR) devices intravaginally (Knights et al., 2003), progestogens in the form of orally active melengestrol acetate (Knights et al., 2003; Lauderdale, 2010), subcutaneous implants impregnated with norgestomet (Syncro-mate-B; Knights et al., 2003; Lauderdale, 2010) or intravaginal sponges impregnated with medroxyprogesterone acetate or flurogestone acetate (Schoenemann and Hallford, 1982; Knights et al., 2003). Luteolytic compounds like $PGF_{2\alpha}$ and its analogues cause CL regression and are usually administered as i.m. injections (Lauderdale, 2010). Progesterone-based treatments can be effective during any stage of the estrous cycle and manipulation of the estrous cycle can result in varied effects on reproductive traits such as estrus responses, pregnancy rates, and lambing rates. Therefore, the objective of this study was to evaluate the effect of $PGF_{2\alpha}$ injections in conjunction with CIDR treatment on conception rates of Rambouillet ewes during the normal fall breeding season.

MATERIALS AND METHODS

All procedures involving animals were approved by the New Mexico State University Institutional Animal Care and Use Committee.

Animals and Treatments. Eighty-one mature Rambouillet ewes (64.5 ± 1.0 kg BW) were used to examine serum concentrations of progesterone and pregnancy rates as influenced by CIDR inserts with or without $PGF_{2\alpha}$ during the normal fall breeding season. All animals were maintained in a single pen (10 x 30 m^2) under ambient conditions. Ewes were provided alfalfa hay at 1.6 kg daily with ad libitum access to water, salt, and shade. Before initiation of the study ewes were vaccinated against *Chlamydia psittaci bacterin* (Colorado Serum Company, Denver, CO) and *Campylobacter fetus jejuni bacterin* (Colorado Serum Company, Denver, CO). Initial and final ewe weights were recorded.

Ewes were stratified by age and BW and randomly assigned to 1 of 3 treatments. Control ewes (n = 27) received an intravaginal CIDR insert (0.3 g progesterone; Pharmacia and Upjohn Pty Limited, Rydalmere, NSW) plus one 2-mL injection of saline (i.m., 0.9% NaCl solution) at CIDR removal. A second group of 27 ewes received a CIDR insert plus one 2-mL injection of $PGF_{2\alpha}$ (10 mg; i.m., Lutalyse, Pharmacia and Upjohn Company, Division of

Pfizer Inc., New York, NY) at insert removal. The third group of 27 ewes received a CIDR insert plus a 1-mL injection of $PGF_{2\alpha}$ at CIDR removal and a second 1-mL injection of $PGF_{2\alpha}$ 4 h after insert removal (10 mg $PGF_{2\alpha}$ total; i.m). All CIDR inserts remained in place for 14 d. Four 18-month-old Rambouillet rams were joined with ewes 24 h after CIDR removal. Rams remained with ewes for 6 d, were removed for 6 d, and then were reintroduced for another 6 d.

Blood Collection and Hormone Analysis. Blood samples were obtained (jugular venipuncture) every other day before feeding beginning on d 0 (day of CIDR insertion) until d 14 (day of CIDR removal) after which sampling continued daily until d 21. On d 14, blood samples were collected just before CIDR removal and 4 h after CIDR removal but before the second injection of $PGF_{2\alpha}$. Blood was collected into 9-mL sterile serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO) and allowed to clot at room temperature for approximately 30 min before centrifugation for 15 min at 1,500 x g and 4°C. Serum was stored frozen (-20°C) in plastic vials until assayed.

Serum progesterone concentrations were quantified by solid phase RIA using components of a commercial kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA; Coat-A-Count) and methods described by Schneider and Hallford (1996). Within assay CV was 5.5% and the average between assay CV was 3.0% over 9 assays.

Gestation and Lamb Management. During gestation, ewes were fed alfalfa hay with cracked corn (0.45 kg/animal daily) at amounts appropriate for stage of gestation. Approximately 30 d before lambing, ewes were vaccinated against *Clostridium perfringens* types C and D and *Clostridium tetani* (Bar Vac CD/T; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO).

On the day of birth, lambs were individually identified and birth weight, sex, and type of birth were recorded. The day after birth, each lamb received an i.m. injection containing 1 mg selenium and 68 USP units of vitamin E (BO-SE, Schering-Plough Animal Health, Union, NJ) and tails were removed. Creep feeding was introduced at an average lamb age of 10 d and continued until weaning. Lambs were provided ad libitum access to alfalfa hay and water with a limited amount of cracked corn. Male lambs were castrated using elastrator bands and all lambs were vaccinated against *Clostridium perfringens* types C and D and *Clostridium tetani* at approximately 30 d of age. Once an average age of 60 d was achieved, lambs were weaned and weighed and received a second clostridial vaccination.

Statistical Analysis. Ewe (initial and final) and lamb (birth, weaning, and gain) weights were subjected to analysis of variance for a completely random design. Because lamb weights were likely to be influenced by type of birth (single, multiple) and (or) sex, these effects were examined along with maternal PG treatment using a 2 x 3 factorial arrangement. Analyses were computed using the general linear models procedure of SAS (SAS Inst. Inc., Cary, NC).

Effects of PG treatment on serum progesterone profiles were analyzed by ANOVA for a split-plot design having repeated measures. Effects of ewe treatment were included in the main plot and tested using animal within treatment as the error term. Day of sampling and treatment by sampling day interaction were included in the sub-plot and tested with the residual error. If sampling day by ewe treatment interactions were detected, treatment effects were examined within day. Analyses were computed using the mixed procedure of SAS with compound symmetry determined to be the appropriate covariance structure. If treatment effects were detected, means were separated using pair-wise comparisons and computed using the predicted difference option of SAS. Orthogonal contrasts were used to further evaluate effect of day. Treatment effects on ewe reproductive performance (pregnancy rate, prolificacy) were evaluated by chi-square analysis using the frequency procedure of SAS.

RESULTS AND DISCUSSION

E*we Body Weights.* Before the experiment began, weights of ewes placed in the 3 treatments were essentially identical (64.5 ± 1.0 kg; $P = 0.99$). Likewise, ewe BW at the end of the treatment period were similar ($P = 0.96$) among groups (66.6, 66.1, and 66.7 ± 1.7 for control, PG1, and PG2, respectively) indicating that use of CIDR for synchronizing estrus in conjunction with $PGF_{2\alpha}$ at CIDR removal had no adverse effect on ewe BW responses. Similar results have been reported by Iroz et al. (2009) after CIDR treatment on different days of the estrous cycle.

Progesterone Profiles During CIDR Treatment. Figure 1 presents the progesterone profile observed during the 14-d period in which the CIDR were in place. As expected, a difference among sampling days was detected ($P < 0.001$). At the time of CIDR insertion, progesterone averaged 3.8 ± 0.23 ng/mL for all ewes. At 48 h, progesterone rose to 8.0 ± 0.23 ng/mL and this value was greater than the d 0 concentration ($P < 0.001$). Progesterone on d 4, 6, 8, and 10 were also elevated ($P < 0.009$) above the d 0 value while progesterone determined on d 12 was similar to d 0 ($P = 0.30$) and the d 14 concentration was less than the d 0 value ($P < 0.001$). Gifford et al. (2003) reported progesterone concentrations in ovariectomized ewes to be 6.3 ± 0.6 ng/mL 2 h after CIDR insertion. Serum progesterone concentrations then decreased gradually from d 2 to d 14 after insertion to reach a final concentration of approximately 2 ng/mL just before the CIDR was removed. Van Cleeff et al. (1998), Duffey et al. (2003), and Gifford et al. (2003) reported similar progesterone concentrations just before CIDR removal. Hamra et al. (1986) and Wheaton et al. (1993) observed progesterone levels to follow a similar trend, increasing to peak levels by 3 to 4 d after insertion then gradually decreasing until insert removal from ovariectomized ewes after a 14-d period.

Progesterone Profiles During 24 Hours After CIDR Removal. Progesterone was also quantified in samples collected 4 and 24 h after CIDR removal. The values determined on d 14 before CIDR removal and 4 h later along with the d 15 sample (24 h after CIDR removal) were examined as a separate data set using split-plot analysis. The $PGF_{2\alpha}$ treatments were administered during

this period as well. Serum progesterone values during this 24-h period are presented in Figure 2 ($PGF_{2\alpha}$ by day, P = 0.013). Treatment responses did not differ ($P > 0.25$) at any of the 3 sampling times. Progesterone declined ($P < 0.020$) in all 3 treatment groups 4 h after CIDR removal. Values in control ewes were similar ($P = 0.79$) 4 and 24 h after CIDR removal. However, both $PGF_{2\alpha}$-treated groups had lower progesterone concentrations ($P < 0.008$) 24 h after CIDR removal than at 4 h after removal.

Van Cleeff et al. (1998) reported progesterone concentrations very similar in ewes treated for 8 d with one CIDR 10 d after a 10-mg injection of $PGF_{2\alpha}$. The observation that average serum progesterone in control ewes did not continue to decline 24 h after CIDR removal may be related to the fact that 2 of the control ewes maintained elevated levels of progesterone after CIDR removal. Van Cleeff et al. (1998) also observed that 1 ewe maintained serum progesterone concentration of 4.9 ng/mL 24 h after CIDR removal. The failure of serum progesterone to decrease in these ewes suggests the presence of a functional or abnormal CL that failed to regress. The possibility exists that CIDR treatment may induce a persistent CL in some ewes. Yates et al. (2009) reported 12 mature ewes failed to respond to CIDR removal as evidenced by estrus response. Likewise, Stapp et al. (2009) observed that 45% of 18-month-old nulliparous ewes failed to respond to CIDR withdrawal after a 14-d CIDR treatment as determined by continued elevated serum progesterone. Also, Wheaton et al. (1992) stated that 1 ewe did not exhibit estrus after a 12-d CIDR treatment.

Progesterone Profiles From 24 Hours to 7 Days After CIDR Removal. Serum progesterone values 24 h to 7 d after CIDR removal (d 15 to 21) were not influenced by a $PGF_{2\alpha}$ treatment by sampling day interaction ($P = 0.83$). Examination of effects of $PGF_{2\alpha}$ treatment pooled across sampling days revealed similar progesterone concentrations among treatment groups for all sampling days (1.6, 1.1, and 1.1 ± 0.27 ng/mL for control, PG1, and PG2 ewes, respectively; $P = 0.26$). These data indicate that the progesterone profiles for the 7-d period beginning 24 h after CIDR removal were not altered by either $PGF_{2\alpha}$ treatment at or near the time of CIDR removal and confirm that ewes in all 3 groups developed functional CL during the 7 d following CIDR removal.

Reproductive Performance. Pregnancy rates as determined by ewes lambing to two 6-d service periods were 15 (55.6%), 23 (85.2%), and 21 (77.8%) of 27 ewes, respectively for control, PG1, and PG2 ewes ($P = 0.04$). These data suggest that using $PGF_{2\alpha}$ in conjunction with a 14-d CIDR treatment increases pregnancy rates. Conception rates of 91 and 95% were exhibited by ewes treated with only a CIDR-G insert for 12 d after a 30-d breeding period (Wheaton et al., 1992, 1993). Dixon et al. (2006) reported similar data with pregnancy rates of 79.5 and 84.8% in ewes receiving a CIDR for a 5-d period with two 5-mg injections of $PGF_{2\alpha}$ given at a 3-h interval either the day before or day of CIDR removal.

Number of control ewes producing 0, 1, or 2 lambs were 12, 11, and 4, respectively, compared to 4, 11, and 12 in the PG1 group and 6, 13, and 8 in the PG2 group, respectively. Number of lambs born to each ewe tended to be greater in $PGF_{2\alpha}$-treated ewes when all ewes were examined ($P = 0.06$); however, no difference in number of lambs per treatment group was observed when only ewes lambing were evaluated ($P = 0.16$). Therefore, adding $PGF_{2\alpha}$ treatment to a 14-d CIDR protocol does not seem to affect prolificacy. Dixon et al. (2006) reached the same conclusion in 2 experiments when adding $PGF_{2\alpha}$ to a 5-d CIDR regimen. Greyling et al. (1979) found that administering $PGF_{2\alpha}$ at 3 different time points relative to removal of intravaginal progestagen sponges containing 60 mg medroxyprogesterone acetate did not cause significant differences in conception rates or number of lambs born per ewe.

Offspring Performance. Lambs born to control, PG1, and PG2 ewes weighed 5.3, 5.0, and 5.2 ± 0.18 kg, respectively at birth ($P = 0.65$). Weaning weights obtained at an average age of 60 d were 20.7, 19.8, and 19.9 ± 1.22 kg for the 3 respective maternal treatment groups (P = 0.82). Actual weaning weight was adjusted to a 60-d, single ewe lamb, mature ewe basis (Scott, 1977), and were 23.4, 22.1, and 21.6 ± 1.12 kg, respectively for offspring of control, PG1, and PG2 ewes ($P = 0.46$). Although no effect of maternal treatment was noted for lamb birth weight, actual weaning weight, or adjusted weaning weight, preweaning ADG tended to be slightly reduced in lambs from $PGF_{2\alpha}$-treated ewes (0.29, 0.26, and 0.25 ± 0.02 kg/d for control, PG1, and PG2 offspring, respectively; P = 0.09). This tendency may be due to the fact that several more $PGF_{2\alpha}$-treated ewes produced twin lambs. When lamb performance was examined by birth type, single-born lambs had heavier birth weights, weaning weights, and preweaning ADG than did multiple-born lambs ($P < 0.01$).

In conclusion, addition of $PGF_{2\alpha}$ to CIDR treatment for 14 d may increase ewe responsiveness to CIDR treatment. Likewise, $PGF_{2\alpha}$-treated ewes had increased pregnancy and lambing percentage compared to control ewes with no adverse effect on lamb numbers or performance.

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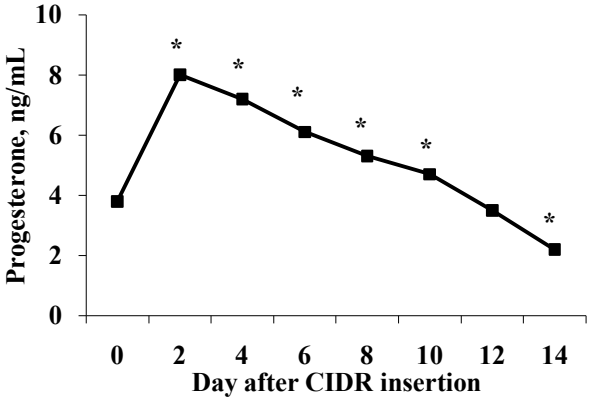

Figure 1. Serum progesterone concentrations in Rambouillet ewes treated with a progesterone-containing intravaginal insert (CIDR; d 0). The CIDR were removed on d 14. The d 0 sample was collected before CIDR insertion and the d 14 sample was collected before CIDR removal. All serum samples were obtained before morning feeding. Days indicated by * differ from d 0 ($P < 0.009$). The pooled SE for sampling days was 0.23 ng/mL.

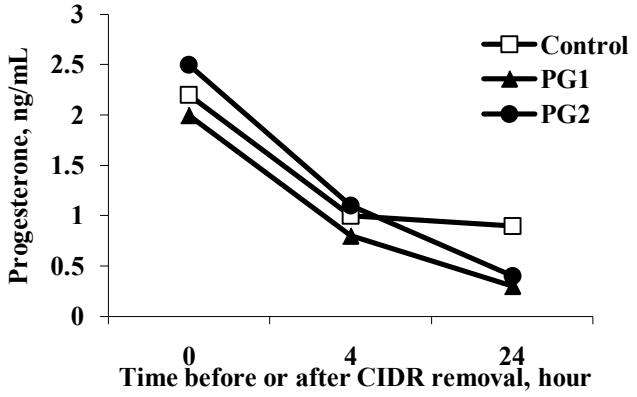

Figure 2. Serum progesterone concentrations in Rambouillet ewes treated with a progesterone-containing intravaginal insert (CIDR; d 0) plus 2 mL saline at CIDR removal (□), one 10-mg injection of $PGF_{2\alpha}$ at CIDR removal (PG1, ▲), or two 5-mg injections of $PGF_{2\alpha}$ (at CIDR removal and 4 h later, PG2, ●). All $PGF_{2\alpha}$ injections were administered intramuscularly. All samples were collected before $PGF_{2\alpha}$ treatment. The SE ranged from 0.13 to 0.35 ng/mL.

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REPRODUCTIVE CYCLICITY AND PROGESTERONE PROFILES IN POSTPARTUM RAMBOUILLET EWES TREATED WITH A PROGESTERONE CONTAINING INTRAVAGINAL INSERT AND PMSG

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ABSTRACT: Twenty-four mature fall-bred Rambouillet ewes (69.8 ± 1.7 kg) were used to study effects of a progesterone (P4) containing intravaginal insert (CIDR; 0.3 g P4) in combination with PMSG on serum P4 profiles and induction of estrus cyclicity in postpartum ewes. Ewes lambed between March 18 and 24 and each produced a single lamb. At 30 d (range 26 to 32 d) after lambing, ewes were randomly assigned to 1 of 3 treatments (n = 8/group): control (no CIDR, no PMSG), CIDR only, and CIDR+PMSG. All CIDR were inserted 30 ± 0.3 d after lambing and were removed after 5 d. At CIDR removal (d 0), ewes in the CIDR+PMSG group received an i.m. injection containing 500 IU of PMSG (Prospec-Tany TechnoGene Ltd., Rehovot, Israel). Ewes were kept in an outdoor pen (8 x 12 m) and fed alfalfa hay and cracked corn at levels appropriate for the lactation period. Jugular blood samples were collected daily beginning at CIDR insertion and continuing for 30 d and P4 was determined by RIA. On the day after CIDR removal and PMSG injection, 2 vasectomized rams with marking paint were joined with ewes and marks were observed 4 times daily. The CIDR-treated ewes had elevated ($P < 0.01$) serum P4 over controls throughout the 5-d period that the insert was in place. From d 1 through 4 after PMSG treatment, serum P4 was similar ($P > 0.15$) among groups. However, P4 in CIDR+PMSG-treated ewes rose from 2.1 ± 0.3 ng/mL on d 5 to a peak value of 16 ± 1.2 ng/mL on d 10 and then declined to 2.4 ± 0.8 ng/mL on d 19. During this same 19-d period, P4 values in control and CIDR-treated ewes remained less than 1.0 ng/mL. In the 8 CIDR+PMSG-treated females, time from PMSG treatment until P4 returned to baseline averaged 18.1 ± 0.4 d. All CIDR+PMSG-treated ewes were marked by the vasectomized rams within 2 d of treatment compared to none of the control or CIDR-treated ewes ($P = 0.001$). Data indicate that use of a CIDR in combination with 500 IU of PMSG effectively induced out-of-season cyclicity at 30 d postpartum in anestrous Rambouillet ewes.

Key words: anestrus, CIDR, sheep

INTRODUCTION

Improving reproductive efficiency is a major concern of the livestock industry. Decreasing age at puberty, reducing embryonic and postnatal mortality, and improving conception rates are ways this can be accomplished. Because gestation length of ewes is 5 mo, the possibility of 2 lamb crops in a single year exists if seasonal anestrus can be overcome. Researchers were unable to induce postpartum cyclic activity in lactating ewes by administration of melatonin (Turner and Hallford, 1993), growth hormone (Holcombe et al., 1989), GnRH (Campbell et al., 1994), insulin (Pope and Hallford, 1991), cyproheptadine (serotonin receptor antagonist; Kridli et al., 1997), or propylthiouracil (Gifford et al., 2007). However, research at many locations has demonstrated that various types of progestogens can be used successfully (intravaginal, ear implants, feeding) to induce estrus during the seasonal anestrous period in non-lactating ewes (Daniel et al., 2001; Dixon el al., 2006; Dogan and Nur, 2006). Likewise lactating ewes have been induced to exhibit estrus by administering PMSG at progestogen withdrawal during the anestrous period although fertility is usually reduced (Cognie et al., 1975). Presence of nursing lambs can increase the length of the postpartum anestrous period in ewes (Pope et al., 1989) but Hoefler and Hallford (1987) found that weaning lambs at 2 d of age did not shorten the maternal postpartum interval compared to those observed in spring-lambing ewes nursing offspring. In a study reported by deNicolo et al. (2006), lactating anestrous Romney ewes received an intravaginal progesterone insert for 14 d beginning approximately 55 d after lambing. At insert removal (approximately 69 d postpartum), ewes were treated with 800 IU of PMSG. Lambs were weaned from half the ewes at the time of insert removal/PMSG treatment and 21 d later from the remainder of the ewes. This procedure resulted in 90 and 91% of ewes with early and late-weaned lambs, respectively, being marked by rams with 33 and 36% actually becoming pregnant. Benavidez et al. (2007) further evaluated effects of intravaginal progesterone in ewes nursing lambs beginning 10 d after lambing. The progesterone insert was removed after 5 or 10 d but treatments failed to induce estrus. We hypothesized that administration of PMSG at the time of removal of the progesterone-containing insert at d 35 postpartum would aid in disrupting the anestrous state. The objective of our study was to examine serum progesterone profiles and estrus responses of early postpartum, lactating, anestrous ewes treated with an intravaginal progesterone insert and PMSG at insert removal.

MATERIALS AND METHODS

All procedures in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at New Mexico State University.

Animal Management

Twenty-four mature lactating Rambouillet ewes with single lambs were kept in a single 8 x 12 m outdoor enclosure. Animals were fed on average 0.45 kg of cracked

corn and 2.7 kg of alfalfa hay daily after blood collection. Water, salt and shade were supplied ad libitum. At parturition ewes were separated from the herd and the lambs were weighed and identified by an ear tag. On d 2 after birth, the lambs were treated i.m. with 1 mg of Se and 68 USP of vitamin E (BO-SE, Schering-Plough Animal Health, Union, NJ) and tails were removed. Starting 10 d after birth, lambs were given ad libitum access to alfalfa hay and access to limited amounts of cracked corn. Before the study started ewes and lambs were weighed and randomly sorted into 1 of 3 treatment groups. At the end of the study (60 d after lambing), the ewes and lambs were weighed again and then separated for weaning. All lambs were vaccinated against *Clostridium perfringens* type C and D and *Clostridium tetani* (BarVAC CD/T, Boehringer Ingelheim Vetmedica, Inc. St. Joseph, MO) and males were castrated using elastrator bands on d 26 (range 22 to 28 d) after lambing and at weaning.

Treatments

Ewes were stratified by age and BW and randomly assigned to 1 of 3 treatment groups (n = 8/group). Ewes in the control group did not receive an intravaginal insert and were treated with 3 mL of physiological saline (0.9% sodium choloride; Hospira, Inc. Lake Forest, IL 60045) by i.m. injection. Sixteen ewes received a progesterone-containing intravaginal insert (CIDR; 0.3 g progesterone, Pfizer Animal Health, West Ryde, NSW) on d 30 (range 26 to 32) after lambing. The CIDR was removed 5 d later. Eight of the CIDR-treated ewes also received 3 mL of physiological saline by i.m. injection immediately after insert removal. The remaining 8 ewes were injected with 500 IU of PMSG (Prospec-Tany TechnoGene Ltd., Rehovot, Israel) after CIDR removal. The day of CIDR removal and saline/PMSG treatment was designated as d 0. On d 1, 2 vasectomized rams with marking paint were joined with the ewes. Ewes were then observed 4 times daily for signs of having been mounted by the rams.

Blood Collection and Assay

Daily blood samples were collected from ewes starting the day of CIDR insertion until 25 d after CIDR were removed. Blood samples were collected using jugular venipuncture into vacuum tubes (CORVAC, Kendall Health Care, St. Louis, MO). After blood samples were collected, they were allowed to clot at room temperature for 30 min before being separated by centrifugation at 1,500 x *g* for 15 min. Serum was then stored at -20°C in plastic vials until assays could be conducted. Serum progesterone concentrations were quantified by RIA using components of a solid phase kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA; Coat-A-Count) as reported by Schneider and Hallford (1996).

Statistical Analysis

Serum concentrations of progesterone were examined by ANOVA appropriate for a split-plot design with repeated measures. The main plot included the treatment effect which was tested using animal within treatment as the error term. Effects of day and the treatment by day interaction were included in the subplot and tested using the residual error. Effects of treatment were examined within day because a significant treatment by day interaction was detected. Treatment means were separated by pair-wise comparisons using the predicted difference method. Analysis was computed using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC) with compound symmetry as the covariance structure. Weight responses were subjected to ANOVA for a completely random design and the analysis was computed using GLM procedure of SAS. Effects of treatment on the number of ewes exhibiting estrus were examined by Chi-square analysis using the frequency procedure of SAS.

RESULTS AND DISCUSSION

Animal Weight Responses

As mentioned previously, CIDR were inserted on an average of 30 d after lambing (range 26 to 32 d). Both ewes and lambs were weighed before CIDR insertion and ewes averaged 69.8 ± 1.7 kg and lambs weighed 13.7 ± 0.5 kg at approximately 30 d of age. Animal weights were also obtained at the end of the study at approximately 60 d after lambing (range 57 to 63 d) at which time ewe BW were 67.3, 69.3, and 66.9 ± 3.3 kg for controls, CIDR, and CIDR+PMSG-treated ewes, respectively (P = 0.86). Likewise, the 60-d adjusted weaning weight of lambs were 25.2, 25.5, and 22.4 ± 1.2 kg for offspring of dams in the 3 respective maternal treatment groups (P = 0.16). These data indicate that a 5-d CIDR treatment period did not impact lamb weaning weights at 60 d of age. In a previous study in our laboratory (Benavidez et al., 2007), lambs from control ewes weighed more at weaning than did those produced by ewes receiving a CIDR for 5 or 12 d beginning 10 d after lambing. Miller et al. (1996) demonstrated decreased milk production by ewes that received a progestagen-impregnated ear implant for 14 d beginning 4 d after lambing. Results of our study may suggest that adverse effects on milk production of offspring weights may be avoided by delaying the progesterone treatment until 30 d postpartum.

Serum Progesterone Profiles

Serum progesterone values quantified during the 30-d experimental period are represented in Figure 1. During the 5-d period in which the CIDR were in place, CIDR-treated ewes had elevated (P < 0.01) serum progesterone compared with controls. By 1 d after CIDR removal (day of removal considered d 0), serum progesterone concentrations were 0.6, 0.2, and 0.2 (± 0.2) ng/mL in control, CIDR, and CIDR+PMSG ewes, respectively (P = 0.38). Likewise, progesterone values were also similar (P > 0.15) among the 3 treatment groups on d 2, 3, and 4 (all values < 1.0 ng/mL). However on d 5, serum progesterone was elevated (P < 0.001) in the CIDR+PMSG-treated ewes compared with control and CIDR-treated dams (2.1 > 0.6 = 0.2 ± 0.3 ng/mL,

respectively). Ewes treated with a CIDR and PMSG had a peak progesterone concentration of 16.0 ± 1.1 ng/mL 10 d after CIDR removal and PMSG administration. Their value then declined to 2.4 ± 0.8 ng/mL on d 19 and 1.0 ± 0.4 ng/mL on d 20. During this same 20-d period, serum progesterone in control and CIDR-treated ewes remained less than 1.0 ng/mL. In the 8 CIDR+PMSG-treated females, time from PMSG treatment until serum progesterone returned to baseline averaged 18.1 ± 0.4 d. These data provide substantial evidence that CIDR+PMSG-treated ewes experienced ovulation and development of corpora lutea that produced abundant progesterone prior to their regression at a sequence similar to that observed in an ovine estrous cycle during a regular breeding season.

Estrus Response

Estrus response of ewes was determined by joining 2 marker-paint equipped vasectomized rams with ewes on the day after CIDR removal and saline/PMSG injection. All CIDR+PMSG-treated ewes were marked by rams within 2 d after PMSG treatment. None of the CIDR-treated or control ewes were marked by rams ($P < 0.001$) during the 30-d observation period after treatment.

In conclusion, the ram receptivity data supports the progesterone profiles and suggests that estrus, ovulation, and CL development were induced in the CIDR+PMSG-treated ewes. These results imply that administering a progesterone-impregnated intravaginal insert for 5 d beginning 30 d after lambing and then treating with 500 IU of PMSG at CIDR removal may be a method to successfully induce estrus and ovulation in lactating seasonally anestrous Rambouillet ewes. Additional studies are needed to determine if ewes can successfully conceive and produce live offspring after application of this treatment regimen.

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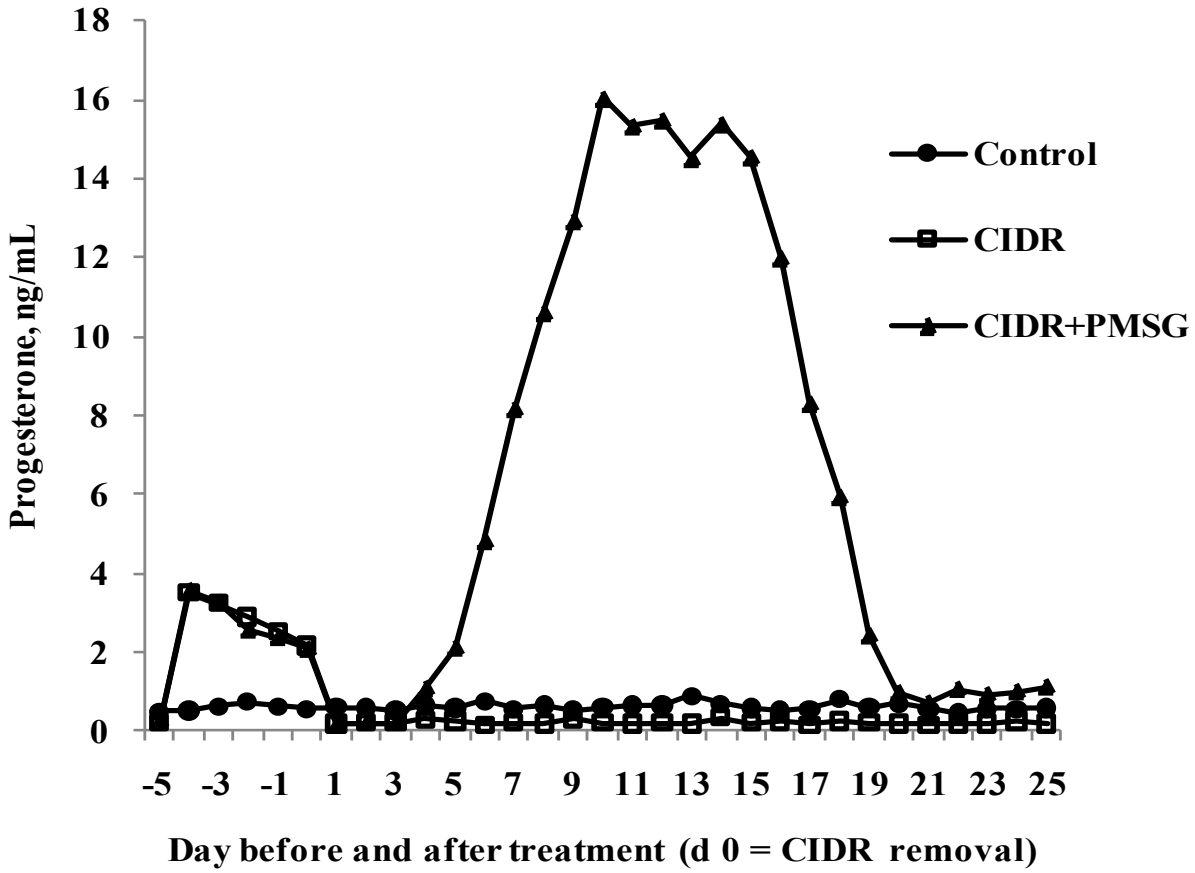

Figure 1. Serum progesterone concentrations in lactating anestrous Rambouillet ewes (single lambs). Eight ewes served as untreated controls. A second group of 8 ewes received a progesterone-containing intravaginal insert (CIDR) while a third group of 8 ewes received a CIDR and 500 IU of PMSG i. m. at CIDR removal (CIDR+PMSG). All CIDR were inserted on d 30 (range 26 to 32 d) after lambing and were removed 5 d later. During the first 5 d while the CIDR was in place, CIDR-treated ewes had elevated serum progesterone when compared to control ewes ($P < 0.001$). Serum progesterone concentrations were similar for all treatments on d 1, 2, 3, and 4 ($P > 0.16$). Progesterone values were greater ($P < 0.001$) in CIDR+PMSG-treated ewes than those in the other 2 groups from d 5 through 19.

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RESPONSE OF SUCKLING CALVES TO BRD VACCINATION AND TREATMENT WITH VITAMIN E

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ABSTRACT: Timing of vaccinations is often dictated by management practices. Vaccination at the wrong time, however, may have little if any measurable effect. Trials conducted over 3 yr determined antibody titers to bovine respiratory disease (BRD) pathogens and effects of a MLV BRD vaccine in suckled beef calves. In yr 1, 3 to 4-month-old calves were given a BRD vaccine intramuscularly (IM; n = 80), intranasally (IN; n = 80), or were not vaccinated (NV; n = 80). Serum was collected from a sub-sample (n = 15) of each group before vaccination and at the end of the summer grazing season to determine titers for bovine virus diarrhea (BVD), infectious rhinotracheitis (IBR), respiratory syncytial virus (BSRV), and parainfluenza-3 (PI-3). Morbidity rates of 5, 5, and 0 % occurred in IM, IN, and NV calves, respectively. Weight gains (76.0 ± 1.6 kg) over the 80 d grazing season did not differ ($P > 0.05$) between groups. Titers to all viruses varied but were typically higher in pre-vaccination sera compared to samples collected 80-d later. In yr 2, no calves (n ≈ 300) were vaccinated. As in yr 1, titers to all viruses were typically higher in sera (n = 40) of 3 to 4-month-old calves than at the end of the grazing season. During yr 2, no mortalities or signs of morbidity were noted. In yr 3, suckled calves from a different herd were vaccinated IM (n = 189) or IM plus 1800 IU Vitamin E (vitE; n = 134). During the 113-d grazing season, calves gained 101 ± 1.7 kg and illness was observed in only 5 IM and 2 vitE calves. Titers during yr 3 were comparable to yr 1 and 2 and were not influenced by vitamin E. Residual maternal antibodies against BRD in calves 3 to 4-months-of-age may interfere with the ability of BRD MLV vaccine to elicit an antibody response. These results do not imply vaccine failure because a cell-mediated response was not assessed. Evaluating management practices and timing of vaccinations are important on a herd-by-herd basis.

Key Words: BRD, Vaccination, Vitamin E

Introduction

The benefits of vaccines are two-fold, to protect the vaccinated animal against the potentially devastating effects of livestock diseases and, in the case of cow-calf operations, to improve the quality of colostrum available to newborn calves. Due to management practices, the timing of vaccine administration is often necessitated by whenever cattle are assembled and worked, especially at branding, movement to summer pastures, weaning, shipping, and calving. Vaccination at the wrong time, however, may have little if any measurable benefit.

Bovine respiratory disease (BRD) can be a major problem for cattle producers and is often associated with mixing of cattle and environmental stressors. Even though ranchers in northern Wyoming frequently vaccinate their suckling calves against BRD prior to movement to summer pastures in the Big Horn Mountains, anecdotal reports question the efficacy of those vaccinations. Additionally, the added stress of weaning may result in decreased performance and health (Swecker et al., 2008; Carter et al., 2005). The potential use of antioxidants, especially vitamin E, may have positive effects on the immune system due to its effects on humoral and cell-mediated immunity (Peplowski et al., 1981; Reddy et al., 1986; Nemec, et al., 1994). Parenteral supplementation of vitamin E to weaned calves may allow for greater accuracy in actual dosage received by the calf compared with feed supplementation, and when administered as an adjuvant, vitamin E may have an increased effect on the immune system (Tengerdy, 1989).

Therefore, a three yr project was conducted to determine antibody titers to BRD pathogens and effects of a modified-live BRD complex five-way vaccine in suckled beef calves at the time of movement to summer pasture. The potential effects of vitamin E on antibody titers were evaluated in yr 3.

Materials and Methods

In yr 1, 3 to 4-month-old calves were given a BRD vaccine intramuscularly (IM; n = 80), intranasally (IN; n = 80), or were not vaccinated (NV; n = 80). Serum was collected from a subsample (n = 15) of each group before vaccination and at the end of the summer grazing season to determine titers for bovine virus diarrhea (BVD), infectious rhinotracheitis (IBR), respiratory syncytial virus (BSRV), and parainfluenza-3 (PI-3). Calf weight gains and incidences of mortality and morbidity during the 80 d season were recorded for each group.

In yr 2, calves (n ≈ 300) from the same herd were not vaccinated, however, antibody titers to all viruses were determined in a sub-sample (n = 40) of the 3-4-month-old suckling calves immediately prior to, and at the end of the grazing season. Incidences of mortality and morbidity were recorded; however, the ability to obtain weight gains was precluded by adverse weather conditions in the fall.

In yr 3, suckled calves from a different herd were vaccinated IM against BRD (BRD-0; n = 189) or concurrently received the vaccination plus 1800 IU Vitamin E IM (vitE; n = 134). Antibody titers were determined in 22 BRD-0 and 21 vitE calves prior to treatment and at the

end of the 113 d grazing season. Calf weight gains and incidences of mortality and morbidity were recorded.

Antibody titers were normalized by conversion to $\log_{10}$ values to determine effects of treatment and time on titers. All data were analyzed by GLM procedures (SAS software version 9.1).

Results and Discussion

Dams and calves used in this study were provided by one producer during yrs 1 and 2 and a separate producer during yr 3. The herds were maintained separately at all times but grazed the same summer allotment. Cows in both herds were de-wormed and vaccinated with a BRD complex vaccine at the time of pregnancy testing in the fall.

In yr 1, weight gain over the 80 d grazing season did not differ $P = 0.93$) among groups and averaged 76.2 ± 1.4 kg over the 80 d grazing season. Weight of calves was not obtained in yr 2 because of an early winter storm. In yr 3, calve weight gain was not influenced ($P = 0.25$) by treatment and averaged 100.9 ± 1.8 kg over the 113 d grazing season.

Morbidity rates of 5, 5, and 0% occurred in IM, IN, and NV calves, respectively, during yr 1. In yr 2, calves were not vaccinated and no mortalities or signs of morbidity were detected. In yr 3, morbidity was observed in only 5 IM and 2 vitE calves.

Antibody titers varied by time ($P \leq 0.001$) and were higher ($P \leq 0.001$) in pre-vaccination blood samples collected when the calves were 3 to 4 months of age than at the end of the breeding season (Figure 1). Antibody titers similarly declined ($P < 0.001$) during yr 2 when all calves were NV. Antibody titers to BRD, IBR, IRSV and IP3 did not differ ($P > 0.05$) between vaccinated (IM or IN) and NV groups at the end of the grazing season.

Calves from a separate herd were used in yr 3 to determine if the decline in titers to the BRD viruses was unique to the first herd and to determine if treatment with vitamin E influenced antibody titers. Vitamin E is an antioxidant and has been purported to improve animal immunity. In finishing beef cattle treatment with vitamin E numerically decreased numbers of morbid cattle requiring re-treatment (Rivera et al., 2002). Titers in this herd were higher ($P < 0.01$) overall compared to the other herd but titers were not influenced by treatment with vitamin E ($P \geq 0.15$).

Potential effects of vitamin E require further investigation to evaluate effects of varying doses and timing of treatments. The results, however, illustrate two important time-honored concepts. First, the high titers prior to vaccination in these 3 to 4 month-old-calves most likely reflect antibodies derived from the dam's colostrums. The subsequent drop in antibody titers reflects a time-related decay in colostral immunity. Bovine maternal antibodies can persist for 4 to 6 months of age (Coria and McClurkin, 1978) especially in calves where the cow-herd is well-vaccinated. Second, titers in blood samples at the end of the grazing season indicate no appreciable antibody response to the modified live virus vaccine administered by either route, a finding attributable to interference or neutralization of the vaccine virus by the colostrums-derived antibodies present in the calves' blood at the time of vaccination. Maternally derived antibodies have been reported to block the induction a vaccine-induced response to BVD (Ellis et al., 2001) and rotavirus (Parreno et al., 2004) in calves.

In summary, the presence of maternally transferred antibodies against BRD in calves at 3 to 4 months of age may inhibit the ability of BRD MLV vaccine to elicit an antibody response. These results, however, do not invariably imply total vaccine failure because there are two arms of the immune response stimulated by vaccination; cell-mediated and humoral (antibody)-mediated immunity. Blood titers measure only antibody-mediated responses. A cell-mediated response was not assessed in this study. Since there was apparently no serious exposure to virulent viral pathogens on summer pasture resulting in death in these calves, a true measure of protection verses susceptibility was not possible.

Implicaitons

This study reinforces the idea that management practices and timing of vaccinations should be evaluated on a herd-by-herd basis. Calves in these herds may be vulnerable to future challenge from BRD viruses unless re-vaccinated. Vaccination at inappropriate times is costly and may provide a false sense of security to producers.

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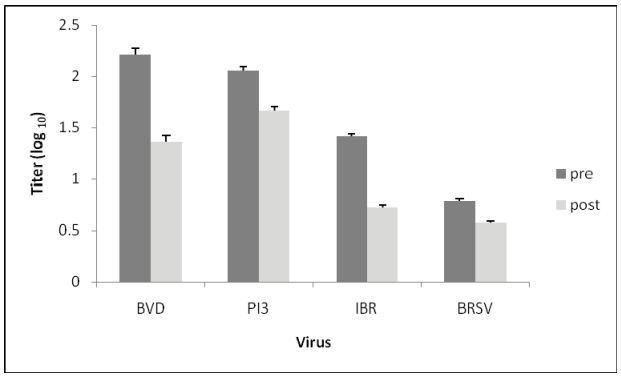

Figure 1. Antibody titers to bovine virus diarrhea (BVD), infectious rhinotracheitis (IBR), respiratory syncytial virus (BSRV) and parainfluenza-3 (PI3) at the beginning (Pre) and end (Post) of the grazing season. Values expressed as titer to $\log_{10} \pm$ SEM

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EVALUATING GLYCERIN SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE OF SHEEP

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ABSTRACT: This 3-year study evaluated the effect of glycerin supplementation on ewe reproductive efficiency, blood glucose and insulin concentrations. Mature Polypay and Rambouillet ewes (n = 225) were orally dosed with glycerin following estrus synchronization at rates of 0, 50, 100, 200 or 300 g/hd. In year 3 an additional 16 ewes were supplemented (SUP) with 0.57 kg of range cake for 21 days prior to breeding. Blood samples were collected (n = 25 ewes) for 10 hours post drenching. Blood was analyzed for insulin and glucose concentrations. Ewes were exposed to rams for 35 days. Pregnancy was determined by ultrasonography evaluation. Number of lambs born per ewe exposed was not different ($P > 0.10$) between treatments (TRT) in year 1 and 2. Pregnancy rates were not different ($P = 0.55$) by TRT in year 3. In yr 1, a TRT x time interaction ($P < 0.01$) for insulin ($ng \bullet ml^{-1}$) concentrations was observed, with 200 g glycerin having lower insulin than 100 g glycerin. In yr 1, glucose exhibited a TRT x time interaction ($P < 0.01$); 50 g glycerin had lower glucose ($mg \bullet dl^{-1}$) than 0 g glycerin and 200 g glycerin having lower levels than 100 g glycerin. Glucose concentrations exhibited a quadratic response with a peak at hr 1 and returned to baseline by hr 7. In yr 2, insulin peaked at hr 4 and declined to hr 10. Glucose was higher ($P < 0.05$) for 200 and 300 g glycerin than 0, 50 and 100 g glycerin in yr 2. Glucose exhibited a quadratic response ($P < 0.01$) with a peak at hr 2. Glucose increased linearly in year 3, ($P < 0.01$; 135.8, 176.44, 163.12, 175.12, 195.63, and 161.44 $mg \bullet dl^{-1}$ for 0, 50, 100, 200, 300 g of glycerin and SUP, respectively). Glucose had a quadratic response ($P < 0.01$); glucose peaked at hr 1 and return to baseline by hr 7. In yr 3, insulin had a treatment by time interaction ($P < 0.05$); insulin peaked at hr 2, 50 and 100 g glycerin returned to baseline by hr 7; however, 200 and 300 g glycerin did not. Insulin ($P < 0.01$) increased with increasing levels of glycerin. Glycerin changed blood glucose and insulin concentrations; however, it did not influence reproductive performance.

Key Words: Sheep, glycerin

Introduction

Sheep producers expect a high percentage of multiple births to improve their profitability. Research has reported that flushing ewes increased the number of offspring produced per ewe (Hulet et al., 1962). The general purpose of flushing is to increase the number of growing follicles, fertilized and developing oocytes, and ultimately increase the number of offspring. Nutrition has been shown to influence several reproductive functions, including hormone production, oocyte competence, uterine environment, fertilization, and early embryonic development. Researchers have looked at the influence of nutrition on reproduction in sheep through flushing for several years. Numerous researchers (Torell et al., 1972; Hulet et al.,1962; and Botkin and Lang, 1978) have shown that flushing increased the number of lambs born per ewe. Dietary supplements containing high energy and protein have been reported to increase ovulation rates in ewes (Downing et al, 1995a). Similarly, increased ovulation rates were reported when glucose was infused intravenously (Downing et al, 1995b; Williams et al, 1997).

Gutierrez and Co-workers (2007) reported that sheep drenched with a minimum of 50 ml of glycerol solution increased the number of large follicles observed on day 0 of estrus. Rizos et al (2008) reported that feeding propylene glycol (oral dose of 500 ml) to dairy cows positively altered systemic concentrations of a number of metabolic variables (insulin, non-esterified fatty acids, beta-hydroxybutyrate and glucose), which have been related to fertility. However, they did not observe an effect of propylene glycol treatment on follicular dynamics or the length of the postpartum interval. The objective of this research project was to determine a lowest dose of glycerin that would improve the number of offspring in sheep.

Material and Methods

Animal Management and Treatments. All experimental protocols were approved by the South Dakota and North Dakota State Universities Animal Care and Use Committees. Two hundred twenty-five mature crossbred Polypay (year 1, n=75) and Rambouillet (years 2, n=75 & 3, n=75) May lambing ewes were stratified by weight.

Within stratification, ewes were randomly assigned to one of five glycerin treatments (0, 50, 100, 200 or 300 g/hd). Ewes all received the same amount of fluids (350 ml) by drenching in yr 1 and stomach tubed in yrs 2 and 3. In year 3 an additional 16 ewes were individually supplemented (SUP) with 0.57 kg of range cake (14% CP) for 21 days prior to breeding. Ewes had ad-libitum access to dormant range and fresh water.

Experimental Sampling Procedures. Ewes were synchronized using a Controlled Internal Drug Releasing (CIDR) devise and given 10 mg of $PGF_{2\alpha}$ following CIDR removal. Ewes were dosed with glycerin on day 1 of the breeding season. Blood samples were collected from a subset of ewes (5 ewes/treatment) at 0, 1, 2, 3, 4, 5, 7, and 10 h post-drenching via venipuncture of the jugular vein into 10-ml evacuated tubes (Fisher Scientific, Pittsburgh, PA). Blood samples were centrifuged at 3,000 x g for 15 min at 4°C and plasma was collected and stored at -20°C until glucose and insulin analyses were performed. Plasma samples for glucose were frozen in liquid nitrogen. Circulating concentrations of glucose were determined by a colorimetric assay (Stanbio Glucose LiquiColor). Intra- and inter-assay coefficients of variation for glucose assays were 8.4% and 7.0%, respectively. Circulating concentrations of insulin were determined by an Insulin Coat-A-Count RIA (Siemans). All insulin samples were run in one assay with an intra-assay coefficients of variation being 1.4%. Eight rams (black-faced and Rambouillet rams, year 1; Rambouillet rams in years 2 & 3) were placed with ewes for 35 days. Pregnancy was determined by ultrasonography evaluation at 45 and 86 d post ram exposure.

Statistical analysis. Each year was analyzed as a separate experiment because of changes in the ewe flock and number of treatments among years. Pregnancy status in January or February and lambing rate response to level of supplemental glycerin were analyzed in a randomized complete block design using the MIXED procedure of SAS (PROC MIXED, SAS Institute, Cary, NC). Ewes were stratified into blocks by initial BW and randomly assigned within each block to glycerin treatments. Block was considered a random effect. Least squares means were calculated and linear and quadratic polynomial contrasts were constructed to evaluate the influence of increasing levels of glycerin. For year 3, the model was executed twice. The first execution was with only the glycerin treatments (not including the positive control) and the polynomial contrasts were included. The second execution included the positive control treatment, and instead of polynomial contrasts, protected LSD was used to pairwise compare the positive control to each glycerin level.

Because the subset of ewes for plasma glucose and insulin evaluation were chosen at random from the experimental flock, these data were analyzed in a completely randomized design with ewe considered the experimental unit and designated as a random effect. The treatment structure was a 5 glycerin level × 8 sampling time factorial. Sampling time was considered a repeated measure. Least squares means were calculated and linear and quadratic polynomial contrasts were constructed for treatment and time to evaluate the influence of increasing levels of glycerin and the response over time. Year 3 responses were analyzed in 2 models as described above.

Results and Discussion

Number of lambs born per ewe exposed is reported in Table 1. Number of lambs born per ewe exposed and pregnancy rates were not different ($P \geq 0.17$) by glycerin treatment. Butler et al. (2006) reported no difference in proportion of first postpartum dominant follicles that became ovulatory in Holstein cows given 500 ml of propylene glycol. Our results are contrary to Knight et al. (1975) who reported an increase of 30% in the number of twin ovulations when ewes were supplemented with lupin grain. Gutierrez et al (2007) reported an increase in the number of large follicles with 50 ml of glycerol solution drenched. The lack of improvement in pregnancy and number of lambs born might be explained by the ewe's good body condition score. Smith et al. (1981) reported that protein intakes of around 200 g/day produced a maximum increase in ovulation rate with no additional response to further increases in either energy or protein intake. They indicated that this result could be due to ewes in good body condition. The lower pregnancy rates and number of lambs born per ewe exposed in year 2 were likely the result of external environmental factors not caused by the experimental treatments.

Circulating glucose and insulin concentrations for all 3 years are reported in Table 2 and 3. A treatment x time interaction ($P < 0.01$) for circulating glucose concentrations was found in year 1; 50 g of glycerin had lower glucose concentrations (181.70 mg/dl) compared to 0 g of glycerin (213.93 mg/dl) and 200 g of glycerin (231.30 mg/dl) had decreased concentrations compared to 100 g of glycerin (250.34 mg/dl). A treatment x time interaction ($P < 0.01$) for circulating glucose concentrations were found in year 1 because glucose concentrations rose to different levels at hr 1 in proportion to the dosage of glycerin. Circulating glucose concentrations exhibited a quadratic time response ($P < 0.01$, Table 3) with the highest concentrations at hr 1 (304.74 mg/dl) and concentrations returned to pre-glycerin drenching by hr 7 (154.0 mg/dl). In yr 1, a treatment x time interaction ($P < 0.01$) for circulating insulin (ng/ml) concentrations was observed; 200 g of glycerin (0.43 ng/ml) had lower insulin concentrations compared to 100 g of glycerin (0.62 ng/ml). A treatment x time interaction ($P < 0.01$) for circulating insulin concentration were found in yr 1 because insulin concentrations rose to different levels at hr 1 in proportion to the dosage of glycerin. In yr 2, insulin concentrations peaked at hr 4 (1.97 ng/ml) and declined to hr 10 (0.88 ng/ml). Glucose concentrations were greater ($P < 0.05$) for 200 and 300 g of glycerin than for 0, 50 and 100 g of glycerin. Glucose concentrations showed a quadratic time response ($P < 0.01$) with concentrations peaked at hr 2 than decreased. Circulating glucose concentrations increased linearly in both yr 2 and 3, ($P < 0.01$; Table 2). Glucose concentrations had a quadratic response to time ($P < 0.01$); glucose concentrations peaked at hr 1 and return to pre-drenching levels by hr 7. In yr 3,

insulin concentrations had a treatment by time interaction ($P < 0.05$); insulin concentrations peaked at hr 2, and ewes in the 50 and 100 g of glycerin treatment returned to pre-drenching levels by hr 7. However, concentrations of insulin in ewes treated with 200 and 300 g of glycerin peaked at hr 3 but did not turn to pre-dosage concentrations by hr 10. Insulin concentrations ($P < 0.01$) increased with increasing levels of glycerin.

The results of increased circulating glucose concentrations would be expected with the administration of glycerin. We expected a linear increase in concentrations of glucose with increased levels of glycerin treatment; however, the glucose increase did not reflect the level of glycerin given. In yr 1, circulating glucose for the 200 g treatment was lower compared to the 100 and 300 g treatments. It would be expected that the 200 g of glycerin would be decreased concentrations of glucose compared to the 300 g of glycerin treatment. However, why concentrations were decreased in 200 g of glycerin ewes compared to 100 g of glycerin is unknown. In yr 2, treatments containing 200 and 300 g of glycerin were not different. According to Sano and Co-workers (1990) infusion of glucose into sheep and reported a 3-fold increase in blood glucose within 60 min and concentrations peaked at 160 mg/dl. The greater concentrations of glucose reported in this project could be due to the level of glucose used, or by differences between drenching and infusing. However, Sano and Co-workers (1990) reported similar insulin concentrations as those reported in this study. Grummer et al. (1994) reported a linear increase in circulating blood glucose and insulin concentrations with increasing levels of propylene glycol. They measured circulating plasma glucose and serum insulin for 6 hrs post-drenching; an initial peak in both glucose and insulin was followed by decreasing concentrations to pre-drenching levels. Furthermore, Butler et al. (2006) reported higher blood glucose and insulin concentrations with propylene glycol (500 ml/dl) than the control Holstein cows.

Implications

The results of this trial do not support the use of glycerin as a method to increase the number of lambs born per ewe exposed. However, the incorporation of glycerin into a flushing supplement might be of value with ewes in poor body condition. Future research is needed to determine if a longer duration of glycerin supplementation would be beneficial. Changes in blood metabolites indicate that drenching ewes was able to increase circulating concentrations of glucose and insulin. However these changes were of short duration and did not appear to impact reproductive performance of the ewes.

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Table 1. Effect of glycerin treatment on pregnancy rate and number of lambs born per ewe exposed

	Glycerin Treatment (g/hd)						
	0	50	100	200	300	SUP[a]	*P*-value[b]
Number of lambs born/ewe exposed							
Year 1	1.67	1.66	1.76	1.47	1.72		.8991
Year 2	0.67	0.73	0.57	0.43	.053		.7506
Pregnancy Rate, %							
January							
Year 1	0.80	0.87	0.84	0.79	0.88		.9414
Year 2	1.00	0.93	0.86	1.00	1.00		.2207
Year 3	1.00	0.94	0.94	1.00	1.00	1.00	.5526
February							
Year 1	1.00	1.00	1.00	0.86	1.00		.0908
Year 2	0.93	0.87	0.57	0.79	0.73		.1710
Year 3	0.94	0.94	1.00	1.00	1.00	1.00	.5526

[a] SUP = Supplemented with 0.57 kg of range cake (14% CP)

[b] *P*-value for F-tests of treatment effect.

Table 2. Effect of glycerin treatment on insulin and glucose concentrations

	Glycerin Treatment (g/hd)							
	0	50	100	200	300	SUP[a]	Linear	Quadratic
Glucose, mg/dl								
Year 1[c]	213.93	181.70	250.34	231.30	265.99		.0018	.3827
Year 2	170.92	175.20	194.06	222.68	214.61		.0033	.7146
Year 3	135.80	176.44	163.12	175.12	195.63	161.44	<.0001	<.0001
Insulin, ng/ml								
Year 1[c]	0.14	0.22	0.62	0.43	Non-Est			
Year 2	0.76	1.43	1.27	1.30	1.35		.2989	.3808
Year 3[d]	0.52	1.04	0.99	1.32	2.05	1.26	.0007	.4362

[a] SUP = Supplemented with 0.57 kg of range cake (14% CP)

[b] *P*-value for t-tests of mean.

[c] *P*-value for TRT x Time ($P < 0.01$).

[d] *P*-value for TRT x Time ($P < 0.05$).

Table 3. Effect of glycerin treatment on insulin and glucose concentrations by sampling time

	Hours post glycerin administration									
	0	1	2	3	4	5	7	10	Linear	Quadratic
Glucose, mg/dl										
Year 1[b]	144.04	304.74	292.49	291.63	277.65	246.14	154.04	118.48	<.0001	<.0001
Year 2	130.80	253.79	245.03	232.53	213.17	187.93	158.81	148.31	.0002	<.0001
Year 3	137.90	226.37	210.01	187.69	169.22	151.77	137.13	133.68	<.0001	<.0001
Insulin, ng/ml										
Year 1[b]	0.14	Non-Est	0.47	0.94	0.86	0.94	0.53	0.14		
Year 2	0.49	0.91	1.38	1.76	1.97	1.51	0.87	0.88	.4201	<.0001
Year 3[c]	0.28	0.71	2.09	1.89	1.69	1.22	0.86	0.75	.6221	<.0001

[a] *P*-value for t-tests of mean.

[b] *P*-value for TRT x Time ($P < 0.01$).

[c] *P*-value for TRT x Time ($P < 0.05$).

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HAY SUBSTITUTION USING A CONTROLLED RELEASE DISTILLERS DRIED GRAIN SUPPLEMENT

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ABSTRACT: Determining the forage replacement value of a 24.0% CP controlled release distillers dried grain supplement was the basis for this study. Mixed age (3-10 yr.) range beef cows (n=108) were used to evaluate the effect of supplementation that began either 56d before calving or at the onset of calving. The research objective was to determine the substitution effect on potential change in cow weight, body condition score (BCS), 12th rib fat depth, reproductive performance, and calf weaning weight. Control, pre- and post-calving treatment groups consisted of 4 pen replicates of 9 cows per replicate; a total of 36 cows per treatment. The data was analyzed using the generalized least squares procedure of PROC MIXED. Once supplementation was initiated, it was fed continuously until May 1 (89.5d; 56d pre-calving, 33.5d post-calving). During the supplementation periods, and due to the 56d longer pre-calving supplementation period, pre-calving cows consumed the least amount of hay (P = 0.0001) and a greater amount of supplement per cow (P = 0.061). The post-calving treatment group consumed 41.9% more supplement per head per day overall (P = 0.055; 0.2735 vs. 0.3881 kg/day). Cow starting, ending, and overall weight change did not differ (P = 0.213), however, weight decline was numerically less among supplemented groups, and ending BCS did not differ (P = 0.469). Although ending BCS was not different, ending 12th rib ultrasound fat depth tended to be greater for supplemented cows (P = .092). Rebreeding pregnancy performance following pre- and post-calving supplementation did not differ for 1st (P = 0.564), 2nd (P = 0.172) and 3rd (P = 0.765) breeding cycles. The overall pregnancy rate (P = 0.66), and the percent of cows that did not become pregnant (P = 0.62) during a 63d breeding period also did not differ. At weaning, cow BCS did not differ (P = 0.825), weaning weight did not differ (P = 0.971), and calf weight gain per day of age did not differ (P = 0.484). Using a 24.0% CP controlled release distillers grain supplement as a forage replacement strategy resulted in comparable cow wintering, rebreeding, and calf performance.

KEY WORDS: Distillers Grain, Beef Cows, Forage Replacement

Introduction

Drought in the northern Great Plains region is common and often impacts hay production. Short hay supplies are often addressed by selling cattle and replacing hay shortages with purchased hay, annual forages, and co-products. Considering the readily available supply of corn distiller's dried grain with solubles (**DDGS**), the co-product is an obvious choice for forage replacement in gestating and lactating cow diets. Drying method is a primary factor in determining the overall quality of distiller's grain (Rasco et al., 1989). The nutritional components most affected were protein and NDF. Distillers grains and distillers grains with solubles contain significant amounts of both rumen degradable and rumen undegradable protein and the post-ruminal digestibility of the rumen undegradable protein is generally high (Ingalls, 1994; Stern et al., 1995 and O'Mara et al., 1997). Values for rumen by-pass protein for corn and DDGS are 54 and 47% of the CP value (NRC 1996), however, large variations can be found in the literature (Nakamura et al., 1994a; Grings et al., 1992; Powers et al., 1995). Nakamura et al. (1994a) reported rumen undegradable protein values ranging from 16 to 80% for DDG. Harty et al. (1998) reported rumen degradable protein values for DDGS averaged 53% (range: 40 – 68%) of the crude protein value. Stern et al., (1995) has reported similar values for rumen undegradable protein. Harty et al., (1998) tested DDGS samples and reported in vitro estimates of intestinal digestibility for rumen degradable protein averaging 77%, and ranging from 71 to 94%. Using the mobile bag technique, Ingalls (1994) and O'Mara et al., (1997) documented that the disappearance of individual amino acids in by-pass protein is quite high, ranging from 76 to 84% for corn DDG.

The average nutrient composition of corn DDGS is 29.5% CP, 46.0% NDF, 88.0% TDN, 10.3% fat, 0.32% calcium, 0.83% phosphorus, 1.07% potassium, and 10.56 mg/kg copper, and 0.40% sulfur (NRC 1996). Distiller's gains are used extensively in the cattle feeding industry and are a desirable nutrient source in forage-based cow diets. The relatively high fat content found in DDGS is expected to provide energy for lactation and may also be beneficial with respect to reproductive performance that is independent of caloric content. While dried DGs are considered to be highly desirable nutritionally, the co-product is fine textured and difficult to feed without waste under range conditions. One supplement delivery method is the use of a controlled release lick-tub system (**CR**).

Our research group hypothesized that daily hay dry matter fed to gestating and lactating range beef cows could be replaced with an experimental DDGS-based CR supplement without adversely effecting cow body condition, cow and calf performance, and reproductive performance.

Materials and Methods

All procedures used in this project were approved by the North Dakota State University Institutional Animal Care and Use Committee. Mixed age (3-10 yr.) range beef cows (n=108) were randomly assigned to the following

three treatments: 1) all hay control diet, 2) reduced hay diet plus a CR DDGS-based supplement beginning 56d pre-calving, and 3) reduced hay diet plus a CR DDGS supplement beginning at the onset of calving. Each treatment group consisted of 4 pen replicates of 9 cows/replicate; a total of 36 cows/treatment. Supplementation in the post-calving treatment was fed for 33.5 days.

Medium-quality alfalfa-bromegrass mixed hay (*Medicago sativa* and *Bromus inermis*) was fed throughout the study and was delivered to the cows daily using a Haybuster forage processor equipped with electronic scale. The bales fed were core sampled, composited weekly, and analyzed for percent CP, TDN, NDF, ADF, calcium, and phosphorus (Table 1). The CR DDGS supplements were offered continuously from the initiation of each treatment until the cows and their calves were turned out on crested wheatgrass (*Agropyron desertorum*) pasture the first week of May. Prior to the initiation of feeding, the experimental CR supplements were core sampled and analyzed for percent CP, NDF, ADF, IVDMD, IVOMD, calcium, and phosphorus (Table 1).

Table 1. Hay and Supplement Nutrient Analysis

	Alfalfa-Brome Hay DM (%)	*24.0% Controlled Release Suppl. DM (%)*
CP, %	13.3	27.78
TDN, %	51.6	-
NDF, %	58.5	12.85
ADF, %	39.7	2.54
IVDMD, %	-	85.75
IVOMD, %	-	63.39
Calcium, %	0.95	9.62
Phos., %	0.28	1.52

Measurements: Cows were weighed, condition scored, and an ultrasound fat depth measurement that was taken between the 12th and 13th ribs was recorded at the start and end of the supplementation period. During calving, as each calf was weighed and processed, the dam was visually scored for body condition (BCS). Final cow and calf weight, and BCS were recorded at weaning. Cows in the study were bred naturally and breeding cycle pregnancy rate was determined using regression analysis of ultrasound fetal cranial eye socket width.

Statistics: The data was analyzed using the generalized least squares MIXED procedures of SAS (1996).

Results

By design, the supplemented cows that received the CR DDGS supplement consumed less hay than the unsupplemented control cows. Among supplemented cows (Table 2), pre-calving cows consumed the least amount of hay (P = 0.0001; 17.68 vs. 18.10 kg/cow/d) and a correspondingly larger amount of supplement (P = 0.0614; 24.48 vs. 12.97 kg/cow) during the entire supplementation period (56d pre-calving and 33.5d post-calving). The post-calving supplementation group received supplement beginning at the onset of calving and continued until the cows were turned out on crested wheatgrass pasture, a period of 33.5d. Cows in the post-calving treatment consumed less total supplement per cow (P = 0.0614; 24.48 vs. 12.97 kg/cow), but average daily consumption was 41.9% greater (P = 0.055; 0.2727 vs. 0.3872 kg/cow/d) than the pre-calving cows.

Cow starting (P = 0.217), ending (P = 0.433), and weight change (P = 0.217) did not differ between treatments (Table 3); however, cow body weight decline was numerically less among the supplemented groups.

Initial (P = 0.938), calving (P = 0.854), and ending (P = 0.0.469) cow body condition score (BCS) did not differ (Table 3). Although visual BCS evaluation was not sensitive enough to detect a difference between treatments, body condition evaluation based on ultrasound external fat thickness over the 12th and 13th ribs identified a significant ending fat depth difference between control and supplemented cows (Table 3). On average, fat depth decline among the supplemented cows was 23.4% less than the unsupplemented control cows.

Calf performance has been summarized in Table 4. Hay and CR supplement feeding was terminated the first week of May when the cows and their calves were moved to Crestedwheat grass pasture; followed by native range pastures the third week of June. Calf birth weight (P = 0.507), May turnout calf weight (P = 0.872), final weaning weight (P = 0.971) and calf age at weaning (P = 0.381) did not differ.

First (P = 0.564), second (P = 0.172), and third (P = 0.765) breeding cycle pregnancy rates did not differ (Table 5). While breeding cycle pregnancy rates did not differ, there was a numerical 15% fewer number of cows pregnant in the first breeding cycle among cows supplemented pre-calving. The number of open cows (P = 0.620) and the overall percent pregnant (P = 0.66) did not differ between the control and supplemented treatments.

Discussion

This project was conducted during one of the most severe winters in North Dakota history. Multiple blizzards during the March – April calving period resulted in statewide calf death losses of approximately 85,000 head as reported through the North Dakota Extension Service. Calf death loss across all treatments in this experiment was 5.5%.

Corn distiller's dried grains with solubles are very difficult to feed without waste, due to their fine texture, unless the ingredient is included in TMR rations or in other types of limit-fed manufactured feed supplements. The release rate from the experimental 24.0%CP CR supplement used in this evaluation was desirable considering the many nutritional and environmental factors that can impact lick supplement release rate. Ideally, when used as a substitute feed for hay, it would have been more desirable to have a greater release rate/cow/d.

The significantly high consumption/cow/d observed after calving (41.9%) was not expected

considering the more consistent consumption level observed among those cows supplemented pre-calving. The greater observed response among the post-calving supplementation treatment would suggest that an acclimation period prior to the onset of calving would be a beneficial management strategy.

Implications

Utilizing a controlled release lick supplement system as a replacement for hay following drought is a very practical and effective way to deliver DDGS to range beef cows with virtually no waste.

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Table 2. Hay Consumption and Controlled Release 24% CP DDGS Supplement Intake

	Control	*Ctrl-Rel DDG Pre-Calving*	*Ctrl-Rel DDG Post-Calving*	*SE*	*P-Value*
Hay Intake:					
Hay, kg/Cow[a]	1616.4[a]	1539.3[b]	1574.7[c]	34.6	<.0001
Hay, kg/Head/Day[a]	18.58[a]	17.68[b]	18.10[c]	0.40	0.0001
Controlled Release Supplement Intake:					
Days Supplement Fed	-	89.75	33.5		
kg/Cow[a]	-	24.48[a]	12.97[b]	4.52	0.0614
kg/Cow/Day[a]	-	0.2727[a]	0.3872[b]	0.072	0.055

[a] Means with unlike superscripts differ (P < 0.10).

Table 3. Cow Performance Following Hay Replacement with a Controlled Release 24% CP DDGS Supplement

	Control	*Ctrl-Rel DDG Pre-Calving*	*Ctrl-Rel DDG Post-Calving*	*SE*	*P-Value*
Trial Length, Days	89.25	89.75	89.5	0.263	0.244
Cow Body Weight Change:					
Cow Start Wt., kg	689.4	685.6	679.8	26.24	0.217
Cow End Wt., kg	630.7	640.0	640.9	25.46	0.433
Cow Wt. Gain (Loss), kg	(58.7)	(45.6)	(38.9)	7.44	0.217
Cow Wt. Gain (Loss)/Head/Day, kg	(0.658)	(0.508)	(0.435)	0.084	0.213
% Weight Decline	8.51	6.65	5.72		
Cow Body Condition Score Change:					
Start BCS	6.39	6.42	6.39	0.233	0.938
Calving BCS	6.39	6.47	6.47	0.223	0.854
End BCS	5.75	6.06	5.83	0.317	0.469
BCS Increase or (Loss)	(0.64)	(0.36)	(0.56)	0.133	0.358
% BCS Decline	10.0	5.61	8.76		
Cow Ultrasound Fat Depth Change:					
Start Rib Fat Depth, mm	5.86	5.91	6.03	0.702	0.955
End Rib Fat Depth, mm[a]	3.58[a]	5.09[b]	5.00[b]	0.867	0.092
Rib Fat Depth Inc. (Decline), mm	(2.28)	(0.82)	(1.03)	0.548	0.185
% Rib Fat Depth Decline	38.9	13.9	17.1		

[a] Means with unlike superscripts differ ($P < 0.10$).

Table 4. Calf Performance Following Pre- and Post-Calving Hay Replacement with a Controlled Release 24% CP DDGS Supplement

	Control	*Ctrl-Rel DDG Pre-Calving*	*Ctrl-Rel DDG Post-Calving*	*SE*	*P-Value*
Cow Weight Change:					
Cow Weight at Calving, kg	668.3	682.5	684.1	31.02	0.460
Cow Weight at Weaning, kg	702.2	664.7	677.4	17.94	0.185
Cow Weight Gain (Loss)	33.9	(17.8)	(6.67)		
Weaning Cow BCS	6.22	6.02	6.03	0.248	0.825
Calf Performance:					
Calf Birth Weight, kg	44.6	43.1	43.0	1.06	0.507
Calf May Turnout Weight, kg	77.3	79.4	79.4	3.17	0.872
Calf Age at Weaning, Days	187.8	190.6	193.2	2.44	0.381
Calf Weaning Weight, kg	292.6	292.2	290.6	6.21	0.971
Calf Wt Gain/Day of Age, kg	1.32	1.31	1.28	0.023	0.484

Table 5. Rebreeding Performance Following Pre- and Post-Calving Hay Replacement with a 24% CP DDGS Controlled Release Supplement

	Control	*Ctrl-Rel DDG Pre-Calving*	*Ctrl-Rel DDG Post-Calving*	*SE*	*P-Value*
Breeding Cycle Pregnancy Rate:					
1st Breeding Cycle, %	52.8	38.9	55.1	11.14	0.564
2nd Breeding Cycle, %	23.4	38.9	24.9	5.83	0.172
3rd Breeding Cycle, %	21.3	19.4	13.4	7.85	0.765
Open, %	2.8	2.8	6.7	3.19	0.620
Overall Pregnancy, %	97.2	97.2	93.6	3.13	0.660

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EFFECTS OF CALF WEANING METHOD ON CALF STRESS, HORMONE CONCENTRATION, GROWTH PERFORMANCE, AND CARCASS ULTRASOUND CHARACTERISTICS[1]

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ABSTRACT: The objective was to determine the effects of calf weaning method on calf stress associated, hormone concentration, growth performance, and carcass ultrasound characteristics. Crossbred steer and heifer calves (n = 71) were stratified by BW and allotted randomly to 1 of 2 weaning treatments (**TRT**): conventional weaning (**CON**) or two-step weaning (**2P**) in a completely randomized design. Blood samples were collected concurrently with rectal temperature assessment, on d -7, -6, -4, 0, 1, 3, 7, and 10 relative to weaning (d 0) for determination of plasma cortisol and haptoglobin concentrations. A subset of calves (n = 12; 6 calves/TRT) were fitted with human pedometers to measure steps taken before and after weaning. On d 0, calves were allotted by TRT and sex to 12 feedlot pens (6 pens/TRT) for a 65 d background period. Calves were fed a growing diet (11.5% CP, 4.08 Mcal of NE_g; DM basis) for a 1-kg/d predicted ADG. Calf age at weaning and initial BW averaged 160 ± 2 d and 239 ± 3.8 kg for both treatments, respectively. Calf BW and DMI during backgrounding were similar ($P \geq 0.10$) across TRT; however, treatment x sex interactions occurred for ADG and G:F ($P = 0.001$ and $P = 0.01$, ADG and G:F, respectively). Furthermore, CON G:F from d 0 to 65 was greater than 2P G:F (0.19 vs. 0.15; $P = 0.05$). Rectal temperatures, cortisol concentrations, and haptoglobin absorbance were similar ($P \geq 0.10$) for weaning method. Pedometer steps recorded were similar ($P \geq 0.10$) across TRT. Carcass ultrasound characteristics did not differ ($P \geq 0.15$) for weaning method. These results suggest that two-step weaning has positive benefits of getting freshly weaned calves on feed faster than conventional weaning; however it may not alleviate all forms of calf stress from the weaning event as compared with conventional weaning.

KEYWORDS: acute-phase protein, calf stress, weaning method

Introduction

Conventional weaning, defined as the sudden removal of a calf from its dam and mother's milk (Haley et. al, 2005), is the traditional weaning method currently used by most cattle producers. It can be one of the most stressful experiences for young calves. During weaning, calves experience various events including loss of maternal contact, new diets, novel social environments, as well as transportation to new housing facilities (Enriques et al., 2010). Weaning initiates behavioral and physiological responses indicative of distress that are unfavorable to beef production and animal welfare (Lefcourt and Elasser, 1995; Stookey et al., 1997; Krebs et al., 2010), causing higher incidences of morbidity and mortality at feedlot arrival (Loerch and Fluharty, 1999).

Two-step weaning, a weaning alternative using anti-suckling nose tags, has been reported to reduce stress during the weaning process (Carter et al., 2010). The process allows calves to remain with their mothers, adjusting to milk removal prior to physical separation (Loberg et al., 2007). While wearing the anti-suckling nose tag, the calf can still graze, drink, groom and socialize with its mother (Haley et al., 2005). However, there has been little research evaluating how weaning stress influences marbling deposition during the growing period. We hypothesized that the two-step weaning method would reduce calf stress and improve calf growth and carcass characteristics compared with conventional weaning.

Our study's objective was to determine the effects of calf weaning method on calf stress, hormone concentration, growth performance, and carcass ultrasound characteristics on growing crossbred calves.

Materials and Methods

The experimental protocols were approved by the North Dakota State University Institutional Animal Care and Use Committee.

Animals and Management. The experiment was conducted at the North Dakota State University Hettinger Research Extension Center's (HREC) Southwest Feeders feedlot and two-129 ha pasture locations, one pasture located 4 km south of Hettinger, ND (Clement; T129N R95W, T129N R96W) and the other 8 km west of Hettinger (Fitch; T130N R96W). The Clement and Fitch pastures housed 36 and 35 cow-calf pairs, respectively. Seventy-one crossbred steer (n = 36) and heifer (n = 35) calves were utilized in this study (d -7 to 65). Before weaning, cow-calf

[1]Partial support for this research was provided by the USDA-ARS Northern Great Plains Research Laboratory, Mandan, ND Specific Cooperative Agreement No. 58-5445-7-315. *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.* The authors would like to thank Don Stecher and Donald Drolc for their assistance in conducting this trial.

pairs grazed the respective pastures containing portable wind breaks. Cow-calf pairs had ad libitum access to water, vitamin-mineral premix, and plain salt blocks. Pastures contained similar vegetation as described by Sebesta (2010). Two creep feeders, with oat grain as creep feed, were placed on the pastures 65 d before weaning. All calves were gathered and vaccinated for respiratory diseases, clostridial and *H. Somnus* diseases, and Mannheimia disease, and weighed 33 d before weaning to obtain pre-weaning calf BW.

Design, Treatments, and Diet. On d -7, cow-calf pairs were gathered on respective pastures. Calves were stratified by pre-wean BW and within stratification allotted randomly to 1 of 2 weaning treatments (**TRT**): traditional weaning (**CON**) or two-step weaning (**2P**) in a completely randomized design. Anti-suckling nose tags (QuietWean nose tags, JDA Livestock Innovations, Ltd, Saskatoon, Canada), a flexible, one-piece plastic tag, were placed into the nostrils of the 2P calves. The tags were monitored to ensure retention; any calf that lost their nose tag had another one reinserted. The tags remained in the 2P calves nostrils for 7 d and all calves remained on pasture with their dams until conventional weaning (d 0).

On d 0, cow-calf pairs were gathered on the respective pastures; calves were separated from their dams, loaded into livestock trailers and transported to the NDSU HREC Southwest Feeders feedlot. Calves were weighed, bled, rectal temperatures taken, pedometer measures recorded, and ultrasound carcass characteristics measured. Calves were allotted by TRT and sex to 1 of 12 feedlot pens (6 pens/TRT; 5 or 6 calves/pen) for a 65 d background period. Calf age at weaning and initial BW averaged 160 ± 2 d and 239 ± 3.8 kg, respectively.

During the first four days in the feedlot, calves had free-choice access to grass hay, plain salt blocks, and water in automatic electrically heated fence-line water fountains. On d 2 and 3, oat grain (9.7 kg/pen; DM basis) was offered; on d 3, calves were fed a growing diet (11.5% CP, 4.08 Mcal of NE_g; DM basis) in the form of a totally mixed ration (TMR) at 2.2 kg/calf (DM basis). The diet was formulated for a 1-kg/d predicted ADG (NRC, 2000), consisting of ground mixed hay, corn, barley, oat silage, a custom calf pellet containing monensin sodium (440 mg/kg; Elanco Animal Health, Indianapolis, IN), decoquinate (1,252 mg/kg) medicated crumbles (CHS Nutrition, Sioux Falls, SD), and calcium carbonate at 53.9, 16.5, 11.5, 9.9, 5.9, 1.7, and 0.6%, respectively (DM basis). The TMR was fed once daily (0900 h) from d 4 through 65, with adjustments to intake made daily.

On d 14, all calves were revaccinated for respiratory diseases, clostridial and *H. Somnus* diseases, and Mannheimia disease and implanted with an estrogenic implant (36 mg zeranol; Schering-Plough Animal Health Corp., Union, NJ).

Data Collection. Data measures (calf BW, blood samples, rectal temperatures, pedometer, and ultrasound measures) were collected before calf feeding on data collection days. Calves were checked daily for signs of illness. Calf BW was measured on d -7, 0, 7, 14, 21, 28, and 65. Two ultrasound technicians, certified by Centralized Ultrasound Processing center (CUP, Ames, IA, 2010), performed all ultrasonic scanning on d 0 and 65. Fat thickness, longissimus muscle area, 12-13th rib fat thickness and intramuscular fat percentage were measured (described previously by Kemp et al., 2002), while the marbling scores were calculations based on the intramuscular fat percentages. All images were interpreted with CUP UICS chute side software program (CUP, Ames, IA).

All calves were bled via jugular venipuncture (two-10 ml samples) and collected concurrently with rectal temperature assessment, on d -7, -6, -4, 0, 1, 3, 7, and 10 relative to weaning for determination of plasma cortisol and haptoglobin concentrations. All blood samples were placed immediately on ice and later centrifuged at 2,500 x *g* for 30 min at 4ºC for plasma collection. Plasma was removed and frozen at -20ºC for later analysis.

A subset of calves (n = 12; 6 calves/TRT) were fitted with human pedometers (GOsmart Tri-Axis pocket pedometer, model HJ-303, Omron Healthcare, Inc., Bannockburn, IL) on d -7 to measure steps taken before (d -4, and 0) and after weaning (d 3, 7 and 10). Pedometers were placed in plastic Ziploc bags to protect them from moisture; bags were fastened securely to the interior aspect of the calf's left rear leg above the metacarpophalangeal joint with veterinary wrap, and checked regularly for signs of swelling, discomfort, or pain. Pedometer measures were recorded and reset to zero on d -4, 0, 1, 3, and 7, with pedometers removed from the 12 calves' rear legs on d 10.

Sample analysis. Diet samples from the background period were collected weekly from each pen at feed delivery, composited, dried at 55ºC in a forced-air oven, and ground to pass through a 1-mm Wiley mill screen for nutritional analysis by a commercial laboratory (Midwest Laboratories, Omaha, NE). Concentrations of cortisol were determined using a bovine-specific commercial ELISA kit (Endocrine Technologies, Inc., Newark, CA). Concentrations of haptoglobin were determined according to procedures described previously (Makimura and Suzuki, 1982). All samples were analyzed in duplicate. The intra- and interassay CV were, respectively, 7.82 and 8.73% for cortisol, and 2.44 and 8.12% for haptoglobin.

Statistical Analysis. All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) and the Kenward and Roger approximation to determine the denominator degrees of freedom for the test of fixed effects. Rectal temperatures, plasma cortisol concentrations, plasma haptoglobin absorbances, pedometer data, and carcass ultrasound characteristics were analyzed as repeated measures, with calf as the experimental unit. Calf performance data were also analyzed as repeated measures; however, pen was the experimental unit. The model included all possible combinations of treatment, sex, and time (day). The test of sex main effects and treatment x sex interaction were removed from the model statement if the *F*-values were found to be not significant ($P > 0.05$). The specified term for the repeated statement was time (day), and calf was included as subject. The covariance structure utilized was univariate, which provided the best fit for these analyses according to the Akaike information criterion. Results are reported as least squares means and were separated by preplanned pairwise comparisons (DIFF). For all analyses, significance was set at ($P \leq 0.05$).

Results and Discussion

Nose tag retention. When analyzed, a treatment x sex interaction occurred: heifer calves had a 3.5 times increased incidence of lost nose tags than the steer calves (26.3 vs. 7.6%; $P = 0.05$).

Feedlot performance. No interactions or sex effect ($P \geq 0.23$) were observed for calf BW. Calf BW were similar ($P \geq 0.10$; Table 1) across TRT. Average daily gain ($P = 0.14$) was similar across TRT and TRT x day ($P > 0.05$); however, a TRT x sex interaction ($P = 0.001$) was observed for ADG. Steer CON calves had the highest ADG, followed by 2P steers, 2P heifers, and CON heifers (1.31, 1.02, 0.89, and 0.88 kg, respectively; $P \leq 0.01$) during the study. No interactions or TRT effects ($P \geq 0.10$) were observed for DMI. Steer DMI tended to be 4.5% greater than heifer DMI (6.30 vs. 6.03 kg/d; $P = 0.06$); however, this was expected as steer calves generally have greater DMI than heifer calves.

While no TRT x day interaction occurred for G:F ($P = 0.89$), a TRT x sex interaction ($P = 0.01$) was observed. The CON steers had the highest G:F, followed by 2P heifers, 2P steers, and CON heifers (0.27, 0.24, 0.24, and 0.22, respectively; $P = 0.05$). Feed efficiency for CON was greater ($P = 0.05$) than 2P (Table 1).

Hormone concentrations and Physiological measures. Rectal temperatures, cortisol concentrations, and haptoglobin absorbance were not affected by TRT ($P \geq 0.10$). Calf rectal temperatures were affected by day ($P < 0.001$; Figure 1). According to Cooke and Bohnert (2010), rectal temperatures above 39 ºC are associated with signs of an acute phase reaction in young cattle. This increase in rectal temperature reported here may be an effect of possible heat stress exhibited in the calves, since the outdoor ambient temperature that day was 29.7ºC, which produced a temperature-humidity index (**THI**) adjusted for wind speed and solar radiation, of 72.8. This THI, according to Mader et al. (2006), has the potential for heat stress to occur in livestock. From d -4 to d 1, both TRT showed elevated rectal temperatures (40.1, 39.7, and 39.2ºC vs. 40.0, 39.6, and 39.1ºC on d -4, 0, and 1; 2P and CON, respectively). Except for receiving pre-weaning shots, the calves were not acclimated to much human handling before this study, which may well have contributed to the elevated temperatures reported here.

Haptoglobin absorbance did not show a sex ($P = 0.93$) or TRT x sex interaction ($P = 0.81$), but a day effect ($P < 0.002$) was observed (Figure 2). Both TRT groups had elevated plasma haptoglobin absorbance occurring on d 10. This response is likely the result of seven cases of tissue trauma (shoulder injuries) that were not treatment related.

Although interactions for TRT x sex ($P = 0.95$) and TRT x day ($P = 0.06$) did not occur for cortisol, cortisol concentrations were affected by day ($P < 0.001$) and sex ($P = 0.03$; Figure 3). Peak cortisol concentrations for 2P (52.7 ng/ml) occurred on d -4, 72 h post nose tag insertion, while peak cortisol concentrations for CON (43.6 ng/ml) occurred on d -6, 24 h after the calves were first handled. Generally, plasma cortisol concentrations spike immediately following a stress (Lefcourt and Elasser, 1995). As to why the plasma cortisol concentrations were numerically higher on d -4 than d -6 for 2P calves may be a result of increased human handling that occurred when some cow-calf pairs escaped the Clement pasture while the majority of the calves were corralled in the handling facility. The corralled calves remained in the facilities until the stray pairs were gathered, which may have increased cortisol levels in the calves. A smaller, second cortisol peak was observed on d 3. This may be a result of heat stress experienced by the calves, since the outdoor ambient temperature was 28.1 ºC (d 3), with an adjusted THI of 70 (Mader et al., 2006). Another contributing factor may be the late feeding because of data collections. Heifer calves had 5.5 ng/ml greater ($P = 0.03$) cortisol concentrations than steer calves.

Pedometer steps recorded were similar ($P \geq 0.10$) across TRT; additionally, no sex effect ($P = 0.82$) nor interactions ($P = 0.92$) were observed. Still, a day effect ($P = 0.03$) was observed for the number of steps taken daily (Figure 4). The 2P calves took more steps/d by d -4 because the 2P calves were unable to suckle and relied on pasture forages and creep feed for their daily nutrition. Conversely, CON calves took more steps/d by d 3 due to their dependency on milk and the social bonds with their dams.

Ultrasound measures. No interactions or TRT effects ($P \geq 0.15$) occurred for carcass ultrasound characteristics (Table 2). The 12-13th rib fat thickness (**URFAT**), longissimus muscle area (**ULMA**), and marbling score (**MARB**) were affected by sex ($P \leq 0.04$), while fat thickness (**UFAT**), intramuscular fat (**UIMF**), longissimus muscle area (**ULMA**), and marbling score (**MARB**) were affected by day ($P \leq 0.003$; Table 2). As expected, increases in UFAT, UIMF, ULMA and MARB would be observed because the calves had been on a higher plain of nutrition (1-kg/d ADG diet) for 65 d. Overall, heifer URFAT were 3.4 cm greater ($P < 0.001$) than steer URFAT; this increase in rib fat thickness may be a function of the heifers beginning to approach pubescence, as females tend to increase internal fat stores during this time (Hall et al., 1995). However, LMA for heifers were 6.7 cm smaller ($P = 0.002$) than steer LMA, with a 0.45 numeric increase ($P = 0.04$) in steer MARB as compared with heifer MARB at the end of the background period.

Implications

As the cattle industry has increased scrutiny by its consumers and outside entities to utilize more animal friendly production methods, cattle producers must consider less stressful, more humane alternative methods of weaning calves than conventional weaning. These results suggest that two-step weaning has positive benefits of getting fresh weaned calves onto feed faster than conventional weaning. Our results indicate that two-step weaning may not alleviate all forms of calf stress from the weaning event as compared with conventional weaning. Continued evaluation of two-step weaning is necessary.

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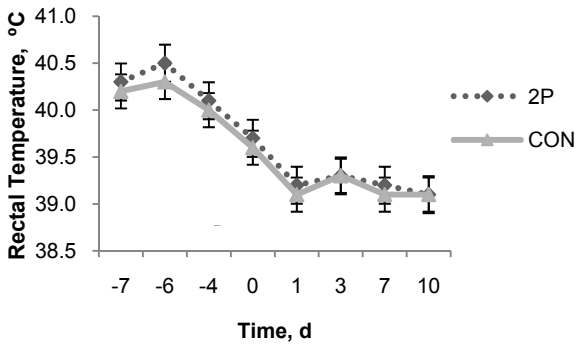

Figure 1. Effect of weaning method on calf rectal temperatures (ºC; ±SEM). Effects of treatment ($P = 0.10$), day ($P < 0.001$), and treatment x day ($P = 0.70$).

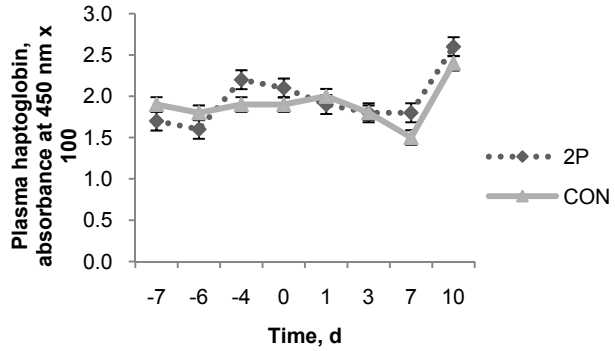

Figure 2. Effect of weaning method on plasma haptoglobin concentration (absorbance at 450 nm x 100; ±SEM). Effects of treatment ($P = 0.69$), day ($P = 0.002$) and treatment x day ($P = 0.43$).

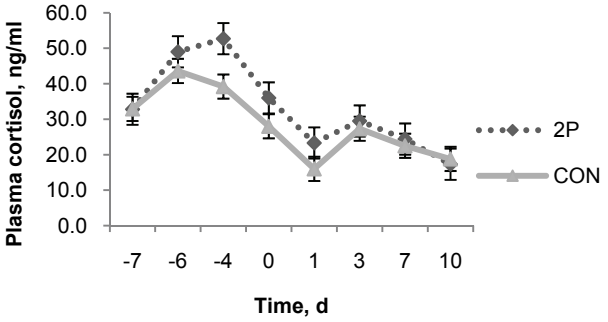

Figure 3. Effect of weaning method on plasma concentration of cortisol (ng/ml; ±SEM). Effects of treatment ($P = 0.13$), day ($P < 0.001$) and treatment x day ($P = 0.06$).

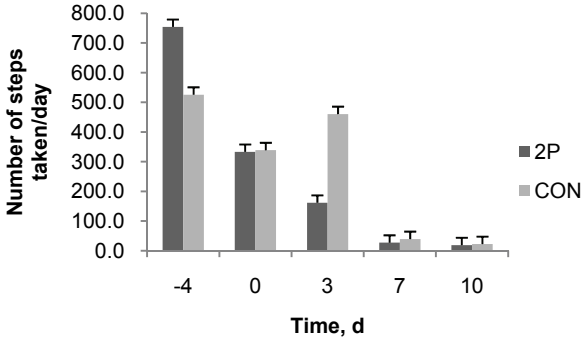

Figure 4. Effect of weaning method on number of steps taken/day (±SEM). Effects of treatment ($P = 0.91$), day ($P = 0.03$) and treatment x day ($P = 0.09$).

Table 1. Effect of weaning method on calf growth performance

	Treatment[1]			*P*-values[3]		
Item	CON	2P	SEM[2]	TRT	Day	TRT x Day
No. head	36	35	-	-	-	-
No. pens	6	6	-	-	-	-
Initial weight, kg	237	241	3.8	0.99	< 0.001	0.66
Day -7 to 0						
Weight, kg	234	231	4.4	0.99	< 0.001	0.66
ADG, kg	-0.44	-1.39	0.4	0.14	< 0.001	0.61
Day 1 to 7						
Weight, kg	233	234	5.1	0.99	< 0.001	0.66
DMI, kg/d	3.5	3.4	0.09	0.10	< 0.001	0.69
ADG, kg/d	-0.17	0.40	0.6	0.14	< 0.001	0.61
G:F	-0.06	0.10	0.2	0.85	< 0.001	0.89
Day 8 to 14						
Weight, kg	262	264	4.2	0.99	< 0.001	0.66
DMI, kg/d	4.70	5.25	0.31	0.10	< 0.001	0.69
ADG, kg/d	4.22	4.27	0.6	0.14	< 0.001	0.61
G:F	0.89	0.81	0.1	0.85	< 0.001	0.89
Day 15 to 21						
Weight, kg	263	261	4.8	0.99	< 0.001	0.66
DMI, kg/d	5.5	5.9	0.14	0.10	< 0.001	0.69
ADG, kg/d	0.01	-0.35	0.5	0.14	< 0.001	0.61
G:F	-0.001	-0.06	0.1	0.85	< 0.001	0.89
Day 22 to 28						
Weight, kg	274	271	5.4	0.99	< 0.001	0.66
DMI, kg/d	6.51	7.11	0.25	0.10	< 0.001	0.69
ADG, kg/d	1.65	1.38	0.3	0.14	< 0.001	0.61
G:F	0.25	0.19	0.04	0.85	< 0.001	0.89
Day 29 to 65						
DMI, kg/d	8.39	9.0	0.36	0.14	< 0.001	0.69
ADG, kg/d	1.28	1.41	0.10	0.14	< 0.001	0.61
G:F	0.16	0.16	0.02	0.85	< 0.001	0.89
Overall, d 0 to 65						
Final weight, kg	322	323	7.6	0.99	< 0.001	0.66
Weight gained, kg	88	83	5.1	0.53	-	-
DMI, kg/d	7.1	7.6	0.26	0.19	-	-
ADG, kg/d	1.4	1.2	0.1	0.10	-	-
G:F	0.19	0.15	0.01	0.05	-	-

[1]Treatments: CON = Conventionally weaned calves; 2P = Two-step weaned calves.
[2] Standard Error of Mean; n = 6.
[3]*P*-values for the tests of main effects of treatment (TRT), day, and treatment x day interaction (TRT x day).

Table 2. Effect of weaning method on real-time ultrasound carcass characteristics

	Treatment[1]			*P*-values[3]		
Item	CON	2P	SEM[2]	TRT	Sex	Day
RTU[4] measurements						
Age at first RTU, d	160	160	2	-	-	-
Initial weight at first RTU, kg	239	239	3.8	0.99	0.59	< 0.001
Initial ULMA, cm^2	43.1	40.0	1.6	0.29	0.002	< 0.001
Initial URFAT, cm	1.22	2.33	0.74	0.15	< 0.001	0.52
Initial UFAT, cm	0.37	0.35	0.02	0.98	0.18	0.02
Initial UIMF, %	2.71	2.64	0.18	0.85	0.74	< 0.001
Initial MARB[5]	3.64	3.51	0.20	0.53	0.04	0.003
RTU measurements						
Age at final RTU, d	225	225	-	-	-	-
Final weight at final RTU, kg	322	323	7.6	0.99	0.59	< 0.001
Final ULMA, cm2	54.3	53.7	1.9	0.29	0.002	< 0.001
Final URFAT, cm	1.61	3.11	1.04	0.15	< 0.001	0.52
Final UFAT, cm	0.39	0.41	0.02	0.98	0.18	0.02
Final UIMF, %	3.74	3.87	0.15	0.85	0.74	< 0.001
Final MARB	4.29	4.15	0.24	0.53	0.04	0.003

[1]Treatments: CON = Conventionally weaned calves; 2P = Two-step weaned calves.
[2]Standard error of Mean; n = 6.
[3]*P*-values for test of main effects of treatment (TRT), sex, and day.
[4]RTU = real-time ultrasound; ULMA = ultrasonically scanned longissimus muscle area, cm^2; URFAT = ultrasonically scanned 12-13th rib fat thickness, cm; UFAT = ultrasonically scanned fat thickness, cm; UIMF = ultrasonically scanned intramuscular fat percentage, %.
[5]MARB = Marbling score, correlated to IMF percentage.

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IMMUNOGLOBULIN TRANSFERENCE FROM MATERNAL COLOSTRUM AND COLOSTRUM SUBSTITUTE IN HOLSTEIN CALVES IN MEXICALI

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ABSTRACT. The objective of this study was to evaluate Holstein cows' colostrum quality and immunoglobulin transfer in calves (TIGS). A colostrum meter (hydrometer) was used to evaluate colostrum of the first four milkings after calving of 188 cows. Calves at birth were assigned to two groups: Group 1 (100 bull calves) received maternal colostrum (CM) consuming two liters during the first six and 12 hours after birth; and Group 2 (83 heifer calves) that received substitute colostrum (CS), 0.250 kg dissolved in two liters of warm water during the first 6 and 12 hours after birth. Blood samples were obtained (10 ml) at 12 and 24 h of age, in order to evaluate TIGS in both groups. Immunoglobulin analysis was carried out by ELISA technique. In both groups 2 liters of milk substitute were given every 12 hours until weaning and an initiation concentrate was provided (21 % CP) beginning the first week of life. Analyses were carried out by GLM procedure (General Linear Models) of the Statistical Analysis System program (SAS Institute Inc). IGS concentration of colostrum was higher (P<.0001) in the first milking (96.530 ± 1.580 mg/dl) as compared to the following three milkings, (63.673 ± 1.580; 40.498 ± 1.580 and 25.260 ± 1.580 mg/dl, respectively). IGS concentration in colostrum was higher (P<.0001) in cows that calved during summer and winter (60.932 ± 1.738 and 59.272 ± 2.014 mg/ml, respectively), than during autumn (49.266 ± 2.099mg/dl). According to the lactation number, cows with two to seven lactations had a higher (P<.0001) IGS concentration (52.003 ± 2.255, 57.591 ± 1.523, 56.706 ± 1.961, 58.990 ± 2.140, 62.922 ± 2.271 and 61.871 ± 3.475 mg/dl, respectively), than first calving heifers (45.349 ± 1.359 mg/dl). TIGS was higher (P<.0001) in calves that consumed CM (1143 ± 111.41 mg/dl) than calves that consumed CS (409. 59 ± 120.53 mg/dl). It is concluded that there is a noticeable effect on IGS concentration and colostrum quality by time after calving, season of calving, and lactation number. Maternal origin IGS were absorbed better than the ones from substitute colostrum.

Key Words: Calves, Colostrum substitute, Holstein cows

Introduction

Normally calves at birth have an agammaglobulinemia (Tizard, 1992), condition that makes them susceptible to diverse diseases (Bouda et al. 1994), causing high morbidity and mortality, (Pijoan,1997; Quiroz et al. 1998). Beseer et al. (1991) and Quiroz et al. (1998) state that colostrum is richer in total solids than milk (22 vs 12 %), due to its high protein content (17.6 vs. 3.3 %) of which IGS is almost half (47 %) of that amount. Medina (1994) indicates that colostrum quality has a relationship with IGS concentration that is to say the higher the IGS concentration the higher the colostrum quality. (Fleenor and Stott, 1980; Bouda et al. 1994). A relationship has also been established between the IGS concentration and density, that is to say it is an indication of colostrum quality. IGS present in colostrum are IG G, IG M and IG A, with the first one being more predominant, while the other two are present at a lower concentration (Tizard, 1992). First milking colostrum contains a higher concentration of IGS as compared with the following postpartum milkings (Stott et al. 1979). During several years several colostrum supplements have been given to provide passive immunity to calves (first 4 to 8 weeks after birth) in order to avoid health crisis and improve calf survival (Abel Francisco and Quigley 1993). Studies on immunity transfer have promoted the implementation of a field test that is known as colostrum metering, which allows the selection of colostrums with high antibody content to be given to calves (Fleenor and Stott, 1980). The objectives of this study were: a).-To determine the quality of colostrum during the first four postpartum milkings; b).- Evaluate TIGS in calves.

Materials and Methods

The study was carried out in the dairy farm Establo Volcan del Valle S.P.R. de R.L. located in the Mexicali valley. Colostrum quality was evaluated in Holstein cows during the first 36 hours after calving and immunoglobulin transference to calves (TIGS). Calving happened from the 20th of June 2004 up until the 20th of March 2005. Colostrum was evaluated by hydrometer during the first four postpartum milkings (0, 12, 24 and 36 h) in 188 cows. Blood samples were obtained from the calves at 12 and 24 h of age, in order to evaluate immunoglobulin transference (TIGS) and were assigned to two groups: Group 1 (100 bull calves) received maternal colostrum (CM) consuming two liters during the first six and 12 hours after birth; and the Experimental Group (83 heifer calves) that received substitute colostrum (CS). Immunoglobulin (IGS) analysis was carried out by ELISA technique. Calves were weighed at birth and at weaning, that happened at 49 to 88 days of age of the calves; males consumed two liters of colostrum from their mothers during the first six and twelve hours after birth; females consumed one envelope (250 g) of colostrum substitute dissolved in 2 liters of warm water during the first 6 and 12 hours after birth, then the offspring were given 2 liters of milk substitute every 12 hours until weaning, and an initiation concentrate (21 % CP) from the first week of life. Analyses were carried out by GLM procedure (General Linear Models) of the Statistical Analysis System program (SAS Institute Inc, 2002).

RESULTS AND DISCUSSION

Table 1 shows results of IGS concentration of the first postpartum milkings. IGS concentration was higher (P<.0001) during the first milking (96.530 ± 1.580) than during the following milkings (12, 24, 36 h) which had 63.673 ± 1.580; 40.498 ± 1.580 and 25.260 ± 1.580 mg/dl, respectively. The amount of the first two milkings is excellent (50 to 123 mg/dl), the third milking was acceptable (30 to <50 mg/dl) while the fourth milking was low (<30 mg/dl). Colostrum quality of the first four milkings reflects changes in composition as the cow is milked after calving (Fleenor and Stott, 1980; Medina, 1994). In this study, according to the Fleenor and Stott (1980) classification, the first milking had excellent quality (50 to 123 mg/ml).

Table 2 shows results of IGS concentration according to the season in which cow calving occurred. Cows that calved during summer and winter had a higher concentration (P<.0001) of IGS (60.932 ± 1.738 and 59.272 ± 2.014 mg/dl, respectively) than cows that calved during autumn (49.266 ± 2.099). Quality during summer and winter seasons was excellent (50 to 123 mg/dl), while during autumn quality was acceptable (30 to <50 mg/dl). There are few studies carried out in relation to the effect of seasons on TIGS and colostrum quality (Nardone et al. 1997 and Morin et al. 2001).

Table 3 shows IGS concentration results according to the lactation number of the cow. Cows with 2, 3, 4, 5, 6, and 7 lactations had a higher (P<.0001) concentration of IGS (52.003 ± 2.255; 57.591 ± 1.523, 56.706 ± 1.961, 58.990 ± 2.140, 62.922 ± 2.271, and 61.871 ± 3.475, mg/dl, respectively) of excellent quality (50 to 123 mg/dl), than the first partum heifers that had low IGS concentration (45.349 ± 1.359 mg/dl) and acceptable quality (30 to <50 mg/dl). First calving heifers produce less colostrum volume and very low IGS concentration as compared to cows on their second and third calving (Medina 1994).

In Table 4, TIGS results of calves according to the treatment group are shown. TIGS in calves were higher (P<.0001) in the group that received maternal colostrum both in blood samples taken at 12 as well as 24 h of age (1116.39 ± 110.52 and 1143 ± 111.41 mg/dl, respectively), than in calves that received substitute colostrum (409.77 ± 119.57 and 409.59 ± 120.53, mg/dl respectively). There is quite some controversy about the level of risk associated with low IGS levels in calf serum (Pijoan, 1997), since statistical differences have not been found in calf diarrhea indices in relation to low IGS levels (5.9 mg/ml) as compared to those of high levels. Levels found in this study are not in agreement with other studies reported (Besser et al. 1991) since they indicate that < 10 mg/ml levels at 48 hours of age are indicative of a failure in immunoglobulin transference, and in this study sanitary problems and deaths did not occur.

CONCLUSIONS

These results indicate that colostrum quality is affected as time goes by after calving, season of the year and lactation number of the cow. Maternal colostrum immunoglobulin absorption is more effective than other origin ones, and therefore it is recommended that in future research pasteurizing of maternal colostrum be programmed to be able to administer it to all newborn offspring (females and males) and not incur in sanitary risk by using substitute colostrums.

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Table 1. Mean and standard error of IGS concentration in 752 milkings from the first four milkings of 188 cows according to lactation number.

Postpartum Milking	Number of Animals	Immunoglobulin Concentration	Colostrum Quality*	VP <.0001
Postpartum Milking (hs)		Mean and S. E.		(P<.0001)
1 (12)	188	96.530 ± 1.580	1	(P<.0001)
2 (24)	188	63.673 ± 1.580	1	(P<.0001)
3 (36)	188	40.498 ± 1.580	2	(P<.0001)
4 (48)	188	25.260 ± 1.580	3	(P<.0001)

* Hydrometer Indicates IG concentration: Quality 1= Green color or Excellent and Plus (50 or more mg of IG/dl); Yellow color or Acceptable and Good (from 31 to 50 mg of IG/dl); and Red color or poor (≤30 mg of IG/dl).

Table 2. Mean and standard error of IGS concentration in 752 milkings of 188 cows according to the season of calving

Season* of calving	Number of milkings	Mean and S. E.	Colostrum Quality*	VP <.0001
Summer	232	60.932 ± 1.738	1	(P<.0001)
Autumn	315	49.266 ± 2.099	2	(P<.0001)
Winter	205	59.272 ± 2.014	1	(P<.0001)

* Seasons: Summer (22nd of June to 21st of September); Autumn (22nd of September to 21st of December); Winter (22nd of December to 20th of March).
** Hydrometer Indicates IG concentration: Quality 1= Green color or Excellent and Plus (50 or more mg of IG/dl); Yellow color or Acceptable and Good (from 31 to 50 mg of IG/dl); and Red color or poor (≤30 mg of IG/dl).

Table 3. Mean and standard error of IGS concentration in 752 milkings of 188 cows according to lactation number

Lactation Number	Number of Animals	Mean and S. E.	Colostrum Quality*	VP <.0001
1	50	45.349 ± 1.359	2	(P<.0001)
2	15	52.003 ± 2.255	1	(P<.0001)
3	44	57.591 ± 1.523	1	(P<.0001)
4	29	56.706 ± 1.961	1	(P<.0001)
5	22	58.990 ± 2.140	1	(P<.0001)
6	20	62.922 ± 2.271	1	(P<.0001)
7	8	61.871 ± 3.475	1	(P<.0001)

* Hydrometer Indicates IG concentration: Quality 1= Green color or Excellent and Plus (50 or more mg of IG/dl); Yellow color or Acceptable and Good (from 31 to 50 mg of IG/dl); and Red color or poor (≤30 mg of IG/dl).

Table 4. Mean and standard error of IGS Transference in 183 calves according to treatment with maternal or substitute colostrum

VARIABLES	TREATMENT		
	Substitute Colostrum	Maternal Colostrum	
Number of Animals	83	100	
IGS Transference (h)	Mean and S. E.	Mean and S. E.	VP <.0001
12 h	409.77 ± 119.57	1116.39 ± 110.52	(P<.0001)
24 h	409. 59 ± 120.53	1143 ± 111.41	(P<.0001)
Total	821.68 ± 205.47	2256.11 ± 189.92	(P<.0001)

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EFFECTS OF TEMPERAMENT ON PERFORMANCE AND CARCASS TRAITS OF RANGE-ORIGINATED FEEDER CALVES

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ABSTRACT: The objective was to evaluate the effects of temperament on performance and carcass traits of feeder calves originated from a range cow-calf operation. Ninety-seven Angus × Hereford calves (62 heifers and 35 steers) were evaluated for BW and temperament at weaning (d 0). Temperament was assessed by chute score (1–3 scale) and exit velocity (EV), which was subsequently converted into an EV score (1 = EV < 1 SD from the mean; 2 = EV within 1 SD from the mean, and 3 = EV > 1 SD from the mean). Calves were classified for temperament according to combined chute and EV scores [calm < 2 (n = 56), moderate = 2 (n = 25), and aggressive > 2 (n = 16)]. All calves were managed similarly in a single group during the preconditioning (60 d), growing (137 d), and finishing (110 d) phases. Calf BW was determined at the end of each phase. Trained personnel and a USDA grader evaluated carcass traits following a 24-h chill. Weaning age was similar (P = 0.59) across temperament classes. Weaning BW tended (P = 0.10) to be reduced for aggressive vs. moderate and calm calves (185.8, 192.0, and 197.8 kg, respectively). Average weaned calf value was $629.5, $656.5, and $656.7 for aggressive, calm, and moderate calves, respectively. No temperament effects were detected (P > 0.23) on performance during preconditioning, growing, or finishing phases. However, hot carcass weight tended (P = 0.15) to be reduced for aggressive vs. moderate and calm calves (352.5, 363.3, and 362.2 kg, respectively). Backfat thickness and KPH were reduced (P < 0.03) for aggressive vs. moderate and calm calves (1.20, 1.47, and 1.33 cm of backfat; 2.02, 2.44, and 2.46% for KPH, respectively). Carcass yield grade was improved (P = 0.04) whereas marbling score tended to be reduced (P = 0.09) for aggressive vs. moderate and calm calves (2.71, 3.15, and 2.99 for yield grade; 422, 460, and 445 for marbling score, respectively). Average carcass value was $1,102.5, $1,151.7, and $1,119.2 for aggressive, moderate, and calm calves, respectively. In summary, aggressive temperament is detrimental to performance and profitability of range-originated feeder calves at weaning and upon slaughter.

Key Words: performance, range calves, temperament

Introduction

For over a century, temperament has been defined as the behavioral responses of cattle when exposed to human handling (Fordyce et al., 1988). As cattle temperament worsens, their response to human contact or any other handling procedures becomes more aggressive and excitable. Cattle temperament has been shown to be detrimental not only to personnel safety, but also to productivity of beef operations. As an example, our research group demonstrated that aggressive beef females have reduced reproductive performance compared to cohorts with adequate temperament (Cooke et al., 2009; Cooke et al., 2010).

However, the deleterious effects of excitable temperament in cattle are not limited to reproduction. Previous research reported that feedlot calves with excitable temperament have decreased growth rates compared to calm cohorts (Voisinet et al., 1997a). These outcomes were mainly attributed to reduced feed intake because temperamental cattle spend more time inspecting their surroundings and reacting against "threats" instead of consuming their diets (Nkrumah et al., 2007). Also, excitable temperament has detrimental effects on carcass quality by decreasing final carcass weight, carcass yield grade, and meat tenderness, and increasing percentage of bruised and dark carcasses (Fordyce et al., 1988; Voisinet et al., 1997b). However, all the research studies associating temperament and feedlot performance evaluated calves originated from cowherds maintained in drylot and intensive systems, which differ in terms of overall temperament compared to the herds reared in Oregon's extensive rangeland scenarios (Fordyce et al., 1985). Also, the majority of research studies associating temperament and carcass quality evaluated *Bos indicus* cattle, and similar studies should be conducted with *B. taurus* cattle, which commonly exhibit excitable temperament and represent the majority of calves in the Oregon and U.S. beef industry. Therefore, the objective of this study was to evaluate the effects of temperament on performance and carcass traits of *B. taurus* feeder calves originated from a range cow-calf operation.

Materials and Methods

The experiment was conducted in accordance with an approved Oregon State University Animal Care and Use Protocol, and was divided into preconditioning (d 0 to 60), growing (d 61 to 197) and finishing phases (d 198 to 307). The preconditioning phase was conducted at the Eastern Oregon Agricultural Research Center, Burns. The growing (Top Cut; Echo, OR) and finishing (Beef Northwest; Boardman, OR) phases were conducted at commercial feedyards.

Ninety-seven Angus × Hereford calves (62 heifers and 35 steers) were evaluated for BW and temperament at

weaning (d 0). Temperament was assessed by chute score and chute exit velocity (EV). More specifically, chute score was assessed by a single technician when calves were restrained in the chute based on a 3-point scale, where 1 = no movement or occasional shifting, 2 = constant shifting with occasional shaking of the chute, and 3 = continuous and violent movement and shaking of the chute. Chute EV was achieved by determining the speed of the calf exiting the squeeze chute by measuring rate of travel over a 1.8-m distance with an infrared sensor (FarmTek Inc., North Wylie, TX). Chute EV was subsequently converted into an EV score (1 = EV < 1 SD from the mean; 2 = EV within 1 SD from the mean, and 3 = EV > 1 SD from the mean). Calves were classified for overall temperament class according to combined chute and EV scores [calm < 2 (n = 56), moderate = 2 (n = 25), and aggressive > 2 (n = 16)]. All calves were managed similarly in a single group during the preconditioning, growing, and finishing phases. Calf BW was determined at the end preconditioning and growing phases. Calves were slaughter at a commercial packing facility (Tyson Fresh Meats, Inc.; Pasco, WA) at the end of the finishing phase. Hot carcass weight was collected at slaughter. Finishing BW was calculated based on hot carcass weight adjusted to a 63% dressing percentage (Loza et al., 2010). Following a 24-h chill, trained personnel assessed carcass backfat thickness at the 12th-rib and LM area, whereas all other carcass measures were recorded from a USDA grader. Calf value at weaning or upon preconditioning were calculated based on local prices (available at: http://www.centraloregonlivestockauction.com/marketreports.htm; assessed on February 25, 2011). Carcass sale value was $143.70 per 45 kg of hot carcass weight.

Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for performance traits contained the effects of temperament class (calm, moderate, or aggressive), sex, and the resultant interaction. Data were analyzed using calf (temperament × sex) as the random variable. Results are reported as least square means and separated using a single-df orthogonal contrast (aggressive vs. calm and moderate). Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.15.

Results & Discussion

No temperament × sex interactions were detected for any of the variables analyzed (P > 0.24); therefore, all results reported herein include data from steers and heifers All performance results are described in Table 1. Weaning age was similar (P = 0.59) across temperament classes. However, aggressive calves tended (P = 0.10) to have reduced weaning BW compared to calm and moderate cohorts. No differences were detected for preconditioning ADG (P = 0.91), hence aggressive calves also tended (P = 0.14) to have reduced BW at the end of preconditioning compared to control and moderate cohorts. As a result, calf value at weaning or after preconditioning was the lowest for aggressive calves. No temperament effects were detected for BW and ADG during growing and finishing phases.

Table 1. Performance traits of calves according to temperament at weaning.

	Temperament[1]				
Item[2]	C	M	A	SEM	*P*[3]
Weaning age, d	152.3	151.6	148.5	2.4	0.34
Weaning BW, kg	197.8	192.0	185.8	3.9	0.10
Weaning value, $	656.5	656.7	629.5	-	-
Preconditioning ADG, kg/d	0.23	0.31	0.28	0.04	0.91
Preconditioning BW, kg	211.7	210.9	202.6	4.0	0.14
Preconditioning value, $	700.6	714.5	690.4	-	-
Growing phase ADG, kg/d	1.16	1.17	1.18	0.03	0.51
Growing phase BW, kg	370.8	370.8	365.6	5.4	0.51
Finishing phase ADG, kg/d	1.78	1.80	1.70	0.05	0.23
Finishing phase BW, kg	572.4	576.7	559.6	8.7	0.23

[1] Temperament classification based on chute score and exit velocity; C = calm temperament, M = moderate temperament, and A = aggressive temperament.
[2] All calves were managed similarly in a single group during the preconditioning (60 d), growing (137 d), and finishing (110 d) phases. Calf BW was determined at the end of preconditioning and growing phases. Finishing BW was calculated based on hot carcass weight (assuming 63% dressing; Loza et al., 2010).
[3] P-value relative to single-df orthogonal contrast (aggressive vs. calm and moderate)

All carcass results are described in Table 2. Hot carcass weight tended (P = 0.15) to be reduced for aggressive calves compared to calm and moderate cohorts. Backfat thickness and KPH were also reduced (P < 0.03) in aggressive calves compared to calm and moderate cohorts. Carcass yield grade was improved (P = 0.04) whereas marbling score tended to be reduced (P = 0.09) for aggressive vs. moderate and calm calves. As a result, mean carcass sale value was the lowest for aggressive calves.

Table 2. Carcass traits of calves according to temperament at weaning.

	Temperament[1]				
Item[2]	C	M	A	SEM	*P*[3]
Hot carcass weight, kg	362.2	363.3	352.5	5.4	0.15
Fat,[4] cm	1.33	1.47	1.20	0.06	0.03
LM area, cm^2	87.9	87.5	87.6	1.6	0.96
KPH, %	2.46	2.44	2.02	0.11	0.01
Yield grade [5]	2.99	3.15	2.71	0.12	0.04
Marbling [6]	444.7	459.9	422.7	12.1	0.09
Retail product,[7] %	49.8	49.4	50.4	0.3	0.03
Carcass sale value, $	1,119.2	1,151.7	1,102.5	-	-

[1] Temperament classification based on chute score and exit velocity; C = calm temperament, M = moderate temperament, and A = aggressive temperament.
[2] All calves were managed similarly in a single group during the preconditioning (60 d), growing (137 d), and finishing (110 d) phases. Calf BW was determined at the end of each phase for ADG calculation.
[3] P-value relative to single-df contrast (aggressive vs. calm and moderate)
[4] Backfat thickness measured at the 12th rib.
[5] Calculated as reported by Lawrence et al. (2010).
[6] Marbling score: 400 = Small^{00}, 500 = Modest^{00}.
[7] USDA Retail Yield Equation = 51.34 – (5.78 × backfat) – (0.0093 × hot carcass weight) – (0.462 × KPH) + (0.74 × LM area)

These results indicate that calves with aggressive temperament were lighter at weaning compared to cohorts with adequate temperament (calm and moderate temperament), and this BW difference persisted until slaughter based on results detected for hot carcass weight. Further, aggressive calves had reduced carcass backfat and marbling compared to cohorts with adequate temperament, which suggests that carcass development and fat deposition was delayed in aggressive calves mainly due to reduced weaning BW. Differently than previous research efforts (Nkrumah et al., 2007; Cafe et al., 2010), temperament did not influence feedlot ADG in the present study. However, to our knowledge, the effects of temperament on weaning BW are novel, influence calf overall productivity, and potentially impact profitability of beef producers that either market calves at weaning or retain ownership until slaughter. The reasons to why aggressive calves were lighter at weaning is unknown and deserve further investigation. Potential theories include reduced milk production and maternal ability of aggressive brood cows rearing aggressive calves given that temperament is a moderately heritable trait (Shrode and Hammack, 1971; Stricklin et al., 1980), reduced milk and feed intake of aggressive sucking calves, detrimental effects of temperament on calf health and physiologic parameters (Cooke et al., 2009; Burdick et al., 2010), or even a direct genetic interactions among temperament and performance traits. Therefore, additional research is warranted to assess the relationship between temperament and weaning weights in beef calves

Implications

Range-originated feeder calves with aggressive temperament have impaired BW at weaning compared to cohorts with adequate temperament, and such BW difference persists until slaughter and results in impaired carcass quality. Therefore, temperament directly impacts profitability of range beef operations that market calves at weaning, or retain ownership until slaughter.

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EFFECTS OF ISOFLAVONES ON PUBERTY AND PREGNANCY RATES IN EWE LAMBS

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ABSTRACT. Soy isoflavones in humans have shown to increase circulating estrogen levels. Female infants consuming formula high in soy proteins tends to cause early onset of puberty and the development of secondary sex characteristics from high levels of estrogen in their blood. This study was designed to determine if ewe lambs consuming diets high in soy proteins would have increased levels of circulating estrogen and have an earlier onset of puberty and ultimately higher conception rates. Sixty-six Rambouillet and 15 Suffolk ewe lambs (98 d of age) were blocked by breed and randomly assigned to one of three treatments. Treatment differences were only the source of protein ingredient in the diet, either cottonseed meal (CSM) or soybean meal (SBM). Treatment 1 was the control diet without any SBM, only CSM as the protein, treatment 2 diet had half SMB and half CSM as the protein and treatment 3 had only SMB as the protein. Diets were formulated to be isonitrogenous and isocaloric. At weaning ewe lambs were weighed and a serum sample was collected to measure estrogen. Ewe lambs had ad libitum access to their diets for 90 d. At which time they were weighed and another serum sample was collected to measure change in circulating estrogen levels. Additionally, twice per week serum samples were collected to measure serum progesterone levels as an indicator of attainment of an estrous cycle. On d90 fertile males were placed with the ewe lambs for 45 d while remaining on their treatment diets. Following ram removal ewes were joined into one group until lambing. On d175 pregnancy rates and number of multiple fetuses were determined using ultrasound. No differences *($P > 0.05$)* in weight gain or conception rates were observed among treatments. However, ewe lambs receiving soy protein in their diets, regardless of level, had 7 times higher *($P < 0.05$)* levels of serum estrogen levels than those not receiving soy proteins in their diet. Therefore, results indicate that consumption of diets containing soy proteins, post-weaning, will result in elevated serum estrogen levels.

Keywords: *Isoflavones, Puberty, Sheep*

Introduction

In recent years the advances in technology have been a major factor for the current progressive turn in the livestock industry, and more importantly, the field of reproductive physiology. Not only have advances in technology increased the quality of livestock, but also have enabled producers to consequently increase the amount of revenue changing hand, and in turn, aid an already recessive economy. The particular area in which major improvement could be made, particularly in the sheep and goat industry, is allowing the producer to have the ability to maximize the potential of ewe lambs being able to reach puberty, and ultimately conceive and give birth to offspring at an earlier age. This would not only allow the industry to produce more fall born lambs, but also allow the numbers to increase, which to an industry whose numbers are declining, higher volume, higher quality livestock is a must.

Unfortunately, there have been few studies on the effect soy protein isoflavones have on animals. There have been limited studies on the effects they have in humans, but general observations have been made and an attempt to correlate them to female puberty has been made. In a study by Fallon and Enig (2009), only 14.7 percent of Caucasian girls and almost 50 percent of African-American girls showed signs of early puberty. This has been linked to soy based infant formula in which the African-American girls had consumed at a higher rate. The formula in question has boosted plasma estradiol concentrations anywhere from 13,000 to 22,000 times higher than estradiol concentrations in infants on cow's milk formula. It is estimated that an infant exclusively fed soy formula receives the estrogenic equivalent (based on body weight) of at least five birth control pills per day (Fallon and Enig, 2009). Therefore the objectives of this study were to determine the effects isoflavones in the diets of developing pre-pubertal Rambouillet and Suffold ewe lambs have on the attainment of puberty and early reproductive activity.

Materials and Methods

Sixty-six freshly weaned Rambouillet ewe lambs and 15 Suffolk ewe lambs were randomly assigned to one of three treatments. Treatments consisted of all ewes receiving a mixed diet that meets the NRC (NRC, 2007) requirements for weaned, young, growing ewes with varying levels of isoflavones; 1) containing no isoflavones, 2) containing ½ the level as reported to impact developing young girls (Table 1), 3) level reported to impact growing young girls (Table 1). Actual level was reviewed by the human nutrition lab at Iowa State University. Soybean meal replaced cottonseed meal in the control diet to meet the required isoflavone levels needed while maintaining similar crude protein levels. All diets were isonitrogenous and isocaloric to eliminate nitrogen and energy as the factor influencing development (Table 1). Isoflavone analysis was conducted by the

human nutrition laboratory at Iowa State University. The three main isoflavones relevant to this study were daidzein, genistein, and glycitein, and to be activated must have met levels of 477, 666, and 150 mg/g respectively. Animals were housed in small groups with ad libitum access to shade, clean fresh water, and their respective diets. Following 120 days of feeding they were placed, in treatment groups, with intact rams for mating. Rams were fitted with marking harnesses designed to place a colored mark on the female's back when they are mated. Mating activity was monitored daily. Body weights were taken at d0 and d90 to monitor growth and development.

Statistical Analysis. Data was analyzed using the GLM procedure of SAS (SAS Institute, Cary, NC). Individual animal served as the experimental unit, and differences were considered significant when $P \leq 0.05$. Variables measured were initial weights, ending weights, total and periodic gain. Reproductive activity was analyzed using the Categorical models of SAS since data was collected as yes or no data.

Results and Discussion

The goal of this research and the following results of this research are to have the ability to relay benefits or non-beneficial messages correlating to the feeding of diets high in soy isoflavones. There are both positive and negative connotations dealing with high soy protein related diets. With females being our primary concern, growth and reproductive performance was of importance

During the experiment, several ewe lambs experienced rectal prolapses and were removed from the data. Fortunately, the numbers of affected by prolapses were equal across all treatments (two/treatment). Additionally, approximately mid-way through the trial, two dogs attacked the ewes in treatment three, resulting in three being removed from the trial and the remaining ewe lambs in this treatment temporarily reduced feed intake. Therefore, results involving treatment three should be interpreted with caution.

Performance

No differences ($P > 0.05$) were identified in growth performance (Table 2). All treatments had similar initial weights, final weights, gain and average daily gain. Therefore, diet composition had little or nothing to do with animal growth and any differences in reproductive performance are related to ingredients rather than nutrient levels.

Pregnancy Rate

No statistical differences ($P > 0.05$) in pregnancy rate were detected; however, a trend was evident (Table 2). Unfortunately, in this project, total number of ewes was relatively small when using a Qui-square analysis (Categorical Model in SAS) and becomes a relatively weak statistical test. There was a 17% difference in conception rates between treatments 1 and 3 for the Rambouillets while the Suffolk lambs were identical across all treatments. Realizing that within our study, this was not statistically significant, it could be economically important to a larger producer. For example, an operation with 1000 head of ewes had a 17% increase in conception rate, this could easily mean that there would be 170 more lambs to be born. Inevitably, a larger sample size would dictate that a 17% increase in conception rate would suggest a significant difference and therefore should be investigated a larger scale than the current experiment.

Reproductive Activity

Reproductive activity as measured by circulating progesterone showed no difference ($P > 0.05$) among treatments. In fact, only 8% of all ewe lambs, regardless of treatment, actually showed progesterone levels adequate for a reproductive cycle (Salisbury et. al., 2000). These results are quite interesting considering the fact that over 70% of all ewes bred and conceived within 30 days of taking the final blood sample. However, this discrepancy is similar to that observed by Salisbury et. al (2000) where they had similar results in a study comparing reproductive rates in ewe lambs on different protein levels when measuring progesterone levels in the serum. As in ours, their ewe lambs actually conceived and gave birth to lambs. It can be speculated that the lack of exposure to a ram may have played a role in the lack of hormonal evidence of cyclicity when in fact they bred once they had a male placed with them. This phenomenon is described in detail by Bearden et. al. (2004) where he described the impact of the male on reproductive activity in all species. They explain that the impact is even greater on prepubertal females in their first breeding season.

Additionally, blood sampling ceased at d 47 which was the day prior to the attack by dogs in order to reduce stress on the ewes. Therefore, if blood sampling was to have continued there may have been a difference in progesterone levels because the ewes would have been closer to their normal breeding season. It is obvious by the conception rates that ewes experienced reproductive activity, but it was just not detected in the serum progesterone levels.

Isoflavone Concentrations

Isoflavones are found in any plant product and are defined as a substance that has a hormonal effect on mammalian beings (Ursin et. al., 2006; Higdon, 2009). It is also stated upon ration analysis at the Iowa State University Food Science and Human Nutrition Laboratory that the three main isoflavones that concerns our study were daidzin, genistin, and glycitin. Daidzin, genistin, and glycitin had an activation rate of 477, 666, and 150 mg/g, respectively. Treatment 1 was the control and contained no isoflavones. Treatment 2 was the low soy diet and the sheep in treatment 2 ingested 48,785 mg/d of daidzein, 67,932 mg/d of genistein, and 15,300 mg/d of glycitein (Table 3). While the ewe lambs in treatment 3 ingested 92,061 mg/d of daidzein, 128,538 mg/d of genistein, and 28,950 mg/d of glycitein (Table 3).

Circulating Estrogen

Estrogen level is a major factor in female reproductive tract growth Bearden et. al. (2004). Therefore, serum estrogen levels were measured in the ewes prior to the feeding treatments and again at the conclusion of the feeding treatments as an indicator of potential reproductive tract growth and the ability to conceive. Differences ($P < 0.05$; Table 4) were indentified in the initial samples (d0) between the control ewe lambs and the low soy treatment. There were also differences ($P < 0.05$) on d47 between the control and both soy treatments. Since there were differences at the initiation of the experiment, the difference between the initial and final concentrations was calculated to determine the actual change in concentration level following the feeding of diets with differing level of soy isoflavones. Change in concentration followed the same pattern as final concentration with the control ewes being lower than both soy treatments ($P < 0.05$; Table 4). These findings are consistent with that reported by Fallon and Enig (2009) when they found that young females consuming even moderate amounts of the soy isoflavones had a considerable increase in circulating estrogen levels. Therefore, females should see an increase in reproductive tract development consistent with early puberty (Bearden et. al. 2004).

Implications

The results of this study suggest that ingestion of an increased level of soy isoflavones will have an effect on estrogen levels. In addition, it seems that conception rate was dictated at a lower degree, however, the use of soy isoflavones could still be used in a practical sense. Producers that run large operations would obtain a greater benefit than those raising farm flocks, primarily due to volume of sheep. Instead of feeding out ewe lambs on a traditional West Texas diet with a cottonseed meal base, producers could feed them out using a soy bean meal based diet. This should allow the ewe lambs to ingest a relative amount of soy isoflavones, consequently increasing their estrogen levels. Ultimately, this should allow the ewe lambs to reach puberty in an earlier time frame, thus allowing the producer to likely have a higher conception rate in his ewe lambs. Consequently, if this happened that likelihood of more lambs being on the ground would in turn suggest that more revenue would be generated for the producer. One more lamb per ewe per season of service would allow the female to produce more lamb throughout her productive life, ultimately maximizing her profit potential.

Table 1. Ingredient composition and nutrient composition of experimental diets on an as fed basis

Ingredient	Ration 1	Ration 2	Ration 3
Corn%	45.00	45.00	45.00
Cottonseed Hulls%	22.00	29.00	30.00
Soy Bean Meal %	0.00	9.00	17.00
Cottonseed Meal %	15.00	9.00	0.00
Alfalfa pellets %	12.50	2.50	2.50
Molasses %	3.00	3.00	3.00
Sheep Mineral Premix %	2.50	2.50	2.50
TDN	62.31	63.46	64.43
CP	14.04	14.07	14.00
Neg	0.91	0.94	0.97

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Table 2. Growth, performance and pregnancy differences in Rambouillet and Suffolk ewe lambs consuming diets with differing levels of soy isoflavones, no statistical differences among treatments ($P \leq 0.05$) .

	TREATMENT 1		TREATMENT 2		TREATMENT 3	
	Rambouillet	Suffolk	Rambouillet	Suffolk	Ramboullet	Suffolk
Initial Weight, kg	36.95	35.00	38.95	36.14	38.23	35.45
Final Weight, kg	50.77	51.59	54.64	51.05	52.23	47.95
Total Gain, kg	13.82	16.59	15.68	14.89	14.00	12.50
Daily Gain, kg/d	0.15	0.18	0.17	0.17	0.16	0.14
Conception Rate, %	60	75	70	75	77	75

Table 3. Soy isoflavone intake by Rambouillet and Suffolk ewe lamb consuming diets containing three different levels of soy proteins.

	Daidzein	Genistein	Glycitein	Totals
Isoflavone Levels, mg/g of SBM	477.0	666.0	150.0	N/A
Ration 1 (control), mg/d	0.0	0.0	0.0	0.0
Ration 2 (low soy- 102 g/d), mg/d	48.9	67.9	15.3	132.0
Ration 3 (high soy-193 g/d), mg/d	92.1	128.5	28.9	249.6

Table 4. Serum estrogen concentration in Suffolk and Rambouillet ewe lambs consuming diets with different levels of soy isoflavones.

	Treatments			
	Control	Low Soy	High Soy	SE[a]
Day 0, ng/ml	2.0[b]	8.1[c]	4.6[bc]	2.15
Day 47, ng/ml	2.6[b]	19.9[c]	17.4[c]	6.02
Change, ng/ml[d]	0.6[b]	11.7[c]	12.9[c]	5.11

[a]Most conservative standard error of the least squares mean.
[b,c]Mean in the same row with differing super scripts are different ($P < 0.05$).
[d]Change in estrogen concentrations from d0 to d47.

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A PROCEDURE TO REDUCE COLLECTED SAMPLE SIZE FOR NUTRIENT ANALYSIS OF HAY CORES

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ABSTRACT: When sampling large lots of hay for nutrient analyses, the number and quantity of cores required to obtain a representative sample often results in producers arbitrarily subsampling in order to reduce the volume of sample sent to a testing lab. This can bias results due to improper subsampling technique; consequently, we compared 2 methods of sampling 4 different baled hays from eastern Oregon (alfalfa, alfalfa/grass, grass, and grass seed straw) using a Penn State Sampler. We obtained 2 cores (A & B) from each bale, 13 cm apart, from 4 lots of 20 bales of each forage type. The A & B cores were grouped by forage type within lot. The first method used 100% of the A cores from each lot (CON) and the second method involved subsampling the B cores from each lot via a quadrant method (SUB) in which the cores were mixed well, spread out on a plywood sheet labeled with 9 quadrants (13 × 13 cm), and approximately 33% of the overall sample (the middle, vertical column of a tic-tac-toe arrangement) was obtained for analyses. Samples were dried (55°C; 96 h), ground (1-mm screen), and analyzed for CP, NDF, and ADF. In addition, TDN was estimated for all forages [82.38-(0.7515*ADF)]. Results were analyzed with the MIXED procedure of SAS and LSMEANS were separated using LSD protected by a significant F-test ($P \leq 0.05$). In tests of fixed effects, no differences were noted between CON and SUB (sampling method; $P > 0.30$) or the interaction of sampling method and forage type ($P \geq 0.09$) for NDF, ADF, TDN, and CP; differences were noted due to forage type ($P < 0.001$) for each nutrient. The take home message from this data is that CON and SUB LSMEANS for NDF (61.4 vs 61.2%), ADF (32.1 vs 31.9%), TDN (58.2 vs 58.4%), and CP (12.0 vs 12.1%) were not affected by sampling procedure. We do not recommend routine subsampling of cored hay samples; however, these data indicate that subsampling can be used to reduce sample size if proper attention to procedures is followed.

Key Words: forage testing, hay, sampling

Introduction

Hay sampling and nutritional analyses are important components of most nutritional programs for ruminant livestock. This information is critical for ration formulation, determining hay value, and allocating hays within an operation's inventory to the appropriate classes of livestock.

A common question when sampling hay is how many bales must be sampled to get a representative sample of the lot of hay. The National Forage Testing Association (**NFTA**; Putnam, 2011; Putnam and Orloff, 2011) recommends a minimum of 20 bales (one core sample per bale) with up to 35 bales for large lots (100 to 200 ton) or if hay nutritional quality is expected to be very variable. In addition, NFTA strongly recommends that core samples for each lot of hay are combined into a single sample, not subsampled, and sent to a laboratory for testing. Depending on the coring device, this can result in a large volume of sample collected. Nevertheless, NFTA also suggests that the sample of cores from each lot of hay weigh approximately 225 g (Putnam, 2011; Putnam and Orloff, 2011) which may not be possible when using some probes and/or with large lots of hay. Furthermore, most forage testing laboratories request that from 8 to 20 bales be sampled for each lot of hay and/or suggest that each group of cores from a lot of hay fit within a "gallon" bag. This is to minimize the volume of sample the laboratories must process prior to analysis. Consequently, with large lots of hay or hay that is assumed to be highly variable in nutrient content, individuals or laboratories often manually subsample when the number of cores collected yields greater than 225 g. This can result in improper subsampling and nutrient analyses that are not representative of the lot of hay.

Consequently, we designed a study to evaluate a subsampling procedure for cored hay samples. If successful, this procedure will allow for reduction of sample size while not affecting nutrient analyses compared with hay cores that are not subsampled.

Materials and Methods

We obtained core samples using a Penn State Sampler (Nasco, Fort Atkinson, WI) from 4 baled hays common to eastern Oregon. The hays were alfalfa (0.9 × 1.2 × 2.4 m bales), grass/alfalfa (2-tie small bales), Chewings fescue grass seed straw (0.9 × 1.2 × 2.4 m bales), and meadow foxtail (1.5 m diameter round bales). We obtained 2 cores (A & B) from each bale, 13 cm apart, from 4 lots of 20 bales of each hay type. Coring technique followed the procedure recommended by NFTA (Putnam, 2011). The A & B cores were grouped by hay type within lot.

The first sampling method used 100% of the A cores from each lot (CON) and the second method involved subsampling the B cores from each lot via a quadrant method (SUB) in which the cores were mixed well, piled in the middle of a plywood sheet labeled with 9 quadrants (13 × 13 cm) and spread to cover all quadrants, and approximately 33% of the overall sample (the middle, vertical column of a tic-tac-toe arrangement) was obtained for analyses. Samples were dried (55°C; 96 h), ground (1-

mm screen), and analyzed for CP (Leco CN-2000; Leco Corp., St. Joseph, MI) and NDF (Robertson and Van Soest, 1981) and ADF (Goering and Van Soest, 1970) using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Co., Fairport, NY). In addition, TDN was estimated for all forages [82.38-(0.7515*ADF)].

Data were analyzed with the MIXED procedure of SAS (SAS Inst., Inc., Cary NC). The model included sampling method, hay type, and the resultant interaction with degrees of freedom calculated by the Satterthwaite procedure. In addition, replication within hay type was used to specify variation using the RANDOM statement. The LSMEANS were separated using LSD protected by a significant F-test ($P \leq 0.05$).

Results & Discussion

Differences in hay type were observed for CP, NDF, ADF, and TDN ($P < 0.001$; data not shown); however, no differences were noted for the interaction of method × hay type (Table 1; $P \geq 0.09$) or sampling method ($P > 0.30$). Consequently, overall CON and SUB LSMEANS for CP, NDF, ADF, and TDN were, on a DM basis, 12.0 vs 12.1% (SEM = 0.22), 61.4 vs. 61.2% (SEM = 0.28), 32.1 vs. 31.9% (SEM = 0.31), and 58.2 vs. 58.4% (SEM = 0.23), respectively. These data indicate subsampling using the procedure described herein is an acceptable method to reduce sample size without biasing results compared with cores that were not subsampled.

Nutrient analyses can only be as good as the sample collected. Therefore, it is critical to obtain a representative sample from each lot of hay. Unfortunately, there is no definitive recommendation for the number of bales to sample for nutrient analysis with respect to varying lot size and hay type. A study from Kansas State University provides sampling recommendations for 99%, 95%, and 80% confidence intervals for the CP content of alfalfa, prairie hay, and sorghum-sudan hay determined to within 1% or 0.5% CP of the actual mean (Blasi, 2011). The recommendations are specific to each forage type; however, the general recommendation is to sample 20% of the bales in a lot of hay to obtain a representative sample for CP analysis. However, the most commonly accepted recommendation by the forage industry is to use a minimum of 20 bales (one core per bale) and to sample more bales for larger lots of hay or if the hay is assumed to be very variable in nutrient composition (NFTA; Putnam, 2011; Putnam and Orloff, 2011).

The National Forage Testing Association recommends that the amount of sample obtained from each lot of hay be approximately 225 g to assure that the amount of sample is an easily managed and processed size (Putnam, 2011; Putnam and Orloff, 2011). This may not be possible for large lots of hay or hay that is highly variable in nutrient composition. Consequently, many hay growers, livestock owners, nutritionists, and forage testing laboratories subsample when samples from a lot of hay exceed 225 g. Even though this is not a recommended practice by NFTA (Putnam, 2011; Putnam and Orloff, 2011), our data suggests that the subsampling method described herein can be an acceptable practice with cored hay samples greater than 225 g.

Implications

Subsampling cored hay samples that are greater than 225 g by spreading the sample over 9 quadrants, arranged in a tic-tac-toe layout, and collecting 33% of the original sample volume (3 quadrants) does not bias nutritional results and is an effective way to reduce sample size for laboratory analysis.

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Table 1. Influence of sampling method[a] and hay type on nutrient concentration (DM basis)

	Hay					
Nutrient, %	Alfalfa	Alfalfa/Grass	Grass	Grass Seed Straw	SEM[b]	P-Value[c]
CP					0.22	0.70
Control	21.8	15.4	5.2	5.6		
Subsample	21.2	15.6	5.2	6.4		
NDF					0.28	0.31
Control	43.9	58.9	64.7	78.1		
Subsample	43.5	57.7	65.0	78.4		
ADF					0.31	0.42
Control	25.8	27.9	32.7	42.1		
Subsample	25.8	27.1	32.8	42.1		
TDN					0.23	0.42
Control	63.0	61.4	57.8	50.8		
Subsample	63.0	62.0	57.7	50.8		

[a] 2 cores (A & B) were obtained from each bale, 13 cm apart, from 4 lots of 20 bales of each hay type. The A & B cores were grouped by hay type within lot. The first method used 100% of the A cores from each lot (Control) and the second method involved subsampling the B cores from each lot in order to obtain approximately 33% of the original sample volume (Subsample).

[b] n = 4; Method Effect SEM

[c] Method Effect; no Method × Hay Interaction ($P \geq 0.09$).

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THE VIABILITY AND ECONOMICS OF COMPOSTING ON-FARM FEEDSTUFFS AND ANIMAL WASTE IN NORTHERN MONTANA

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ABSTRACT: The objective of this demonstration project was to examine the technical and economic viability of composting in northern Montana during winter months. Composting occurred at Northern Agricultural Research Center in November and December of 2008 and 2009. The compost consisted of cattle manure/bedding material, wheat straw, and spoiled corn silage. Windrows were constructed in 2008 (W1 and W2) and 2009 (W3, W4 and W5) on a flat clay loam soil. Moisture content of the blend was analyzed at the beginning and windrows were irrigated until 50% was achieved to optimize aerobic composting. The compost was turned, with an elevating face Vermeer compost turner, twice weekly. A 90 cm probe with data logger was utilized to record internal temperatures and oxygen levels of the windrows. Ambient temperature readings were recorded daily. Maximum and minimum mean daily ambient temperatures in 2008 were 6.9 and -31.1°C, respectively. In 2008, W1 and W2 reached a high temperature of 60.6±3.97°C 7 d after irrigation and 63.6±4.55°C 9 d after irrigation, respectively. Core temperatures in W1 and W2 exceeded 40°C 120 and 24 h after irrigation, respectively. Core temperatures remained above 40°C for day 26 and 30 d. In 2009, maximum and minimum mean daily ambient temperatures during the study were 10.0 and -33.3°C. High temperatures in W3, W4, and W5 were reached on d 10, 4, and 5 after irrigation (52.9±6.16, 65.7±3.36, 67.6±7.95°C), respectively. Temperatures exceeded 40°C immediately after irrigation in W4 and W5 and 5d after irrigation in W3. Core temperatures remained above 40°C for 14, 25, and 25 d. Even though the minimum ambient temperature reached -31.1°C, there were no lasting negative effects on the composting process. During the coldest periods the core temperatures remained above 35°C. Costs were tracked for cleaning the pens ($2.10/yard), composting ($4.74/yard) and spreading manure or finished compost ($1.14/yard). It was determined that composting is a technically viable option in northern Montana.

Key words: Compost, Animal Waste, Beef

Introduction

The current model of animal feeding relies heavily on imported nutrients in the form of feed, replacement stock, and commercial fertilizer. Nutrient exports are primarily represented by animals, food, fiber or crops sold, and sometimes exported manure. However, animal feeding operations (AFOs) often have a positive nutrient balance; i.e.: more nutrients than they can use. Additionally, long term land application of manure based on nitrogen rates can result in high soil phosphorus, thereby restricting manure use until phosphorus levels can be reduced through crop harvest and export. It may take many years to draw down soil phosphorus levels in this manner.

The nutrient management planning process can identify an operation's nutrient balance. Many AFOs may be in need of manure management options that not only focus on agronomic use of manure on-site, but also manure export to other enterprises. In 2010 MSU Extension, the Montana Agricultural Experiment Station, and a private custom manure services company identified and tested markets for exporting compost and raw manure within the region. Transportation distances varied; a separate aspect of these case studies will examine economics. The initial work simply identified and worked with introducing regional manure resources to users external to AFOs. The objective of this demonstration project was to examine the technical and economic viability of composting in northern Montana during winter months.

Materials & Methods

Composting occurred in November and December of 2008 and 2009. In 2008, the compost consisted of 163.3 t of dried cattle manure and bedding material from a permitted feedlot, 38.1 t of wheat straw, and 38.1 t of year-old spoiled corn silage to bring the carbon:nitrogen (C:N) ratio to a calculated value of approximately 30:1. The C:N ratio was within the recommended optimum range for US composting guidelines. Wheat straw and corn silage were produced at Northern Agricultural Research Center (NARC). Two windrows (W1, 66.8 m long by 3.7 m wide and W2, 76.8 m long by 3.7 m wide) of the composting blend were constructed on a flat surface of clay loam soil. Moisture content was analyzed to be 12.5% at the beginning of the project after the compost materials were blended and the windrows were constructed.

In 2009, compost consisted of 180.5 t of dried cattle manure and bedding material from a permitted feedlot and 20.2 tons of wheat straw and corn stalks from NARC. Three windrows (W3, W4, and W5) 57.3 m long by 3.0 m wide, 84.7 m long by 3.0 m wide and 218 m long by 3.0 m wide, respectively were constructed on a flat surface of clay loam soil. Moisture content of W3, W4, and W5 was analyzed to be 58.0%, 70.4%, and 56.7%, respectively.

The compost was turned with an elevating face compost turner (CT-670, Vermeer, Pella, IA) twice weekly, weather permitting. A 90 cm data logger (Windrow Manager, Green Mountain Technologies, Bainbridge Island, WA) was placed into the windrows 3 times weekly

to record internal temperature and oxygen levels of each windrow at a depth of 45 and 89 cm. After initial readings, water was added to W1, W2, and W5 as it was being turned bringing the moisture content for each windrow to approximately 50% (5 was already over 50%). Water addition took place on December 3 and 5, 2008 and November 20, 2009. Ambient temperature readings were recorded daily at 0800 h (National Oceanic and Atmospheric Administration, National Weather Service Cooperative Observer Site: Fort Assiniboine; Site ID: ASNM8; Site Number: 24-3110-03: Lat/Lon: 48.29.54, 109.47.50: Elevation: 2613 ft.). Grab samples of each windrow were taken and samples were tested at the beginning and end of the composting period to test for maturity of the compost.

Results and Discussion

Maximum and minimum mean daily ambient temperatures for the 47 d trial in 2008 were 6.9°C and -31.1°C, respectively. From the beginning of the trial to when the windrows were irrigated (d 16 and 14, respectively) no composting or aerobic activity was occurring evidenced by the low temperatures (mean temp W1 and W2; Figure 1) and high oxygen levels (Figure 2). Oxygen levels in the windrows were >16% and >6% before irrigation in W1 and W2, respectively. After irrigation, aerobic bacteria immediately began digesting the feedstocks within the windrows, evidenced by the rapid increase in temperature and decrease in oxygen (Figures 1 and 2). Maximum and minimum mean daily ambient temperatures for the 34 day trial in 2009 were 10.0°C and -33.3°C respectively. In 2009 aerobic composting immediately commenced upon blending and the initiation of regular turnings as evidenced by high temperatures (Figure 3 and 4) and decreasing oxygen level (Figure 5). Mean core temperatures in W1 and W2 were >40°C 120 h and 24 h after irrigation, respectively. Composting occurs most rapidly when temperature are >40°C (Trautmann et al, 1996). Even though all the windrows in 2009 tested >50% moisture, W3 did not recover after turning as seen by temperature and oxygen levels, therefore W3 was irrigated to increase the composting process. In both 2008 and 2009 oxygen levels consistently returned to 20% immediately after aerating the windrows, however within 24 h of turning, oxygen levels < 2% were observed. The consistent drop in oxygen levels indicates a very rapid aerobic digestion of feedstocks and available oxygen in all windrows across both 2008 and 2009. Windrow 1 and W2 reached a high temperature of 60.6°C 7 d and 63.6°C 9 d after irrigation, respectively. High temperatures in W3, W4 (65.7°C), and W5 (67.6°C) were reached on d 10, 4, and 5 after irrigation, respectively. Temperatures exceeded 40°C immediately in W4 and W5 and 5 d after irrigation in W3. Core temperatures remained above 40°C for 14 d for W3 and 25d for both W4 and W5. Oxygen levels below 5% result in anaerobic conditions within the windrows, and oxygen becomes the limiting factor (Trautmann et al, 1996), hence the requirement for such frequent turning of the windrows in this trial for both 2008 and 2009. The wheat straw and year old corn silage in this composting system broke down rapidly and added little to the overall bulk density. To combat the low oxygen levels in the center of the windrows, our procedure resulted in turning quite frequently, even in 2009 when corn stalks were added to the blend. In future compost runs, adding different carbon sources including wood chips for bulking agents may promote increase aeration and infiltration and oxygen availability within the windrows.

Composting systems can achieve a significant reduction of pathogens when the compost is maintained at minimum operating conditions of 40°C for 5 d, with temperatures exceeding 55°C for at least four hours of this period (Trautmann et al., 1996). Core temperatures within this trial remained above 40°C for 26 d in W1 and 30 d in W2 (Figure 1) and 14 d in W3, 20 d in W4 and 20 d in W5. Even though the minimum ambient temperature reached -31.1°C and -15.4°C in 2008 and 2009 respectively, there appeared to be no lasting negative effect as the compost was able to maintain and generate appropriate optimum temperatures after each turning. Microbial activity was sufficient for composting as evidenced by the independent temperature trends of windrow temperature and ambient temperature. For windrow composting methods, maintaining 55°C for 15d is sufficient to destroy weed seeds (Trautmann et al, 1996; Wilen, 1997). Once the temperature of the windrows maintain 55°C, the environment in compost windrows have sufficient moisture and temperature to break dormancy of hard seeds followed by thermal kill of seedlings (Egley, G.H., 1990).

Ambient temperature and precipitation events appeared to have little effect on the compost. During the coldest periods of the experiment, ambient temperature was <-17°C for 6 d in 2008 and <-12°C for 4 d in 2009; core temperatures remained above 35, 47, 36, 47, and 50°C in W1, W2, W3, W4, and W5 respectively. In 2008, 4.22 cm measurable precipitation in the form of snow fell on W1 and W2, with 1.42 cm falling on d 2. In 2009 there was 1.75 cm precipitation in 8 precipitation events throughout December Compost was deemed to be mature when it conformed to US Composting Council guidelines including: C:N ratio, pH, organic matter, and moisture content (Alexander, 2003).

Labor and equipment usage were recorded for each step of the composting project. Costs were based on $12/hour labor and estimates of actual equipment costs, fuel usage and economic depreciation for machinery. Results indicate that cleaning pens cost $2.10/yard (of finished compost produced), composting cost $4.74/yard and applying the finished compost to agricultural land cost $1.14/yard. The value of the compost is determined to be highest value of these possible uses. First, the compost value determined by the price at which it could be sold to an off farm customer (landscaping, land applications, gardeners). Second, the value determined by the reduced amount of commercial fertilizer applied due to the nutrients supplied by the compost. Third, the value determined by the increased productivity of land that would not have otherwise received a fertilizer application. This project estimated the cost of compost production but did not

estimate the value of the compost produced due to the wide variation in compost value.

Implications

Composting is a viable option at any time in the semi-arid region of Northern Montana. In this trial the most important factor was to reach appropriate moisture content (>35%) after blending the carbon and nitrogen sources to initiate the composting process and further research is planned.

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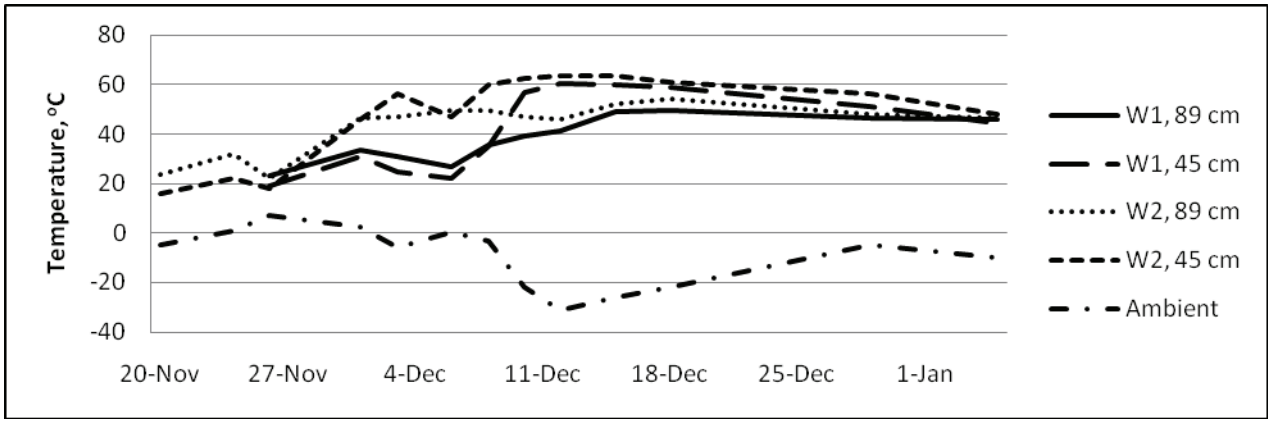

Figure 1. Mean core temperatures of two compost windrows (W1 and W2) in November and December 2008 composed of 163.3 t of dried cattle manure and bedding material from a permitted feedlot, 38.1 t of wheat straw, and 38.1 t of year-old spoiled corn silage to bring the carbon:nitrogen (C:N) ratio to a calculated value of approximately 30:1; and associated mean ambient temperatures near Havre, Montana. After initial readings, water was added to the windrows as it was being aerated (turned) bringing the moisture content for each windrow to approximately 50%. Water addition took place on December 3 and 5, 2008.

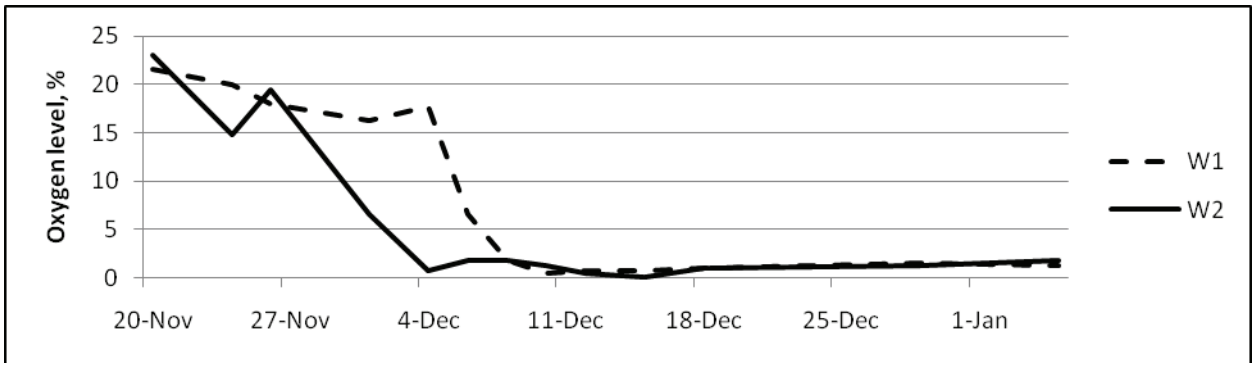

Figure 2. Oxygen levels of two compost windrows (W1 and W2) in November and December 2008 composed of 163.3 t of dried cattle manure and bedding material from a permitted feedlot, 38.1 t of wheat straw, and 38.1 t of year-old spoiled corn silage to bring the carbon:nitrogen (C:N) ratio to a calculated value of approximately 30:1. After initial readings, water was added to the windrows as it was being aerated (turned) bringing the moisture content for each windrow to approximately 50%. Water addition took place on December 3 and 5, 2008.

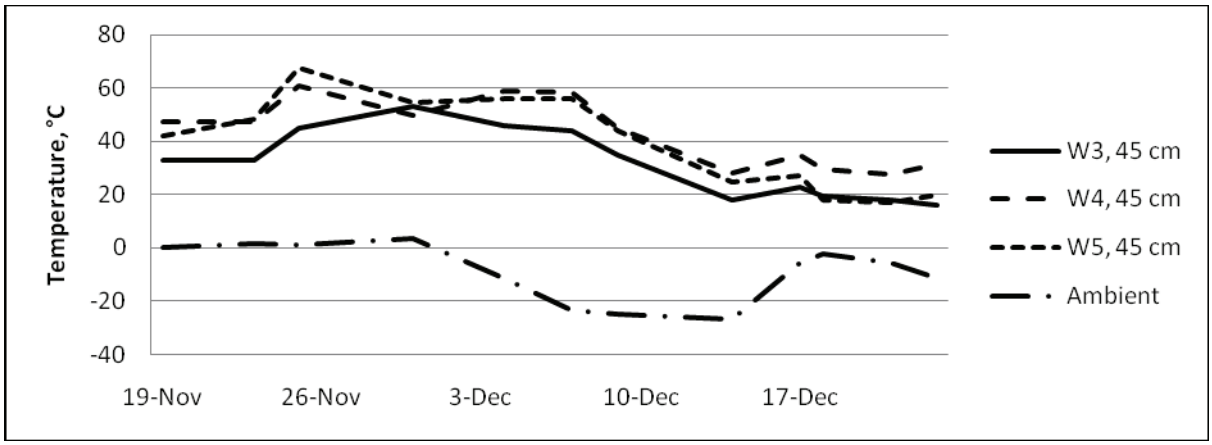

Figure 3. Mean core temperatures of three compost windrows (W3. W4, and W2) in November and December 2009 composed of 180.5 t of dried cattle manure and bedding material from a permitted feedlot and 20.2 t wheat straw and corn stalks to bring the carbon:nitrogen ratio to a calculated value of approximately 30:1.

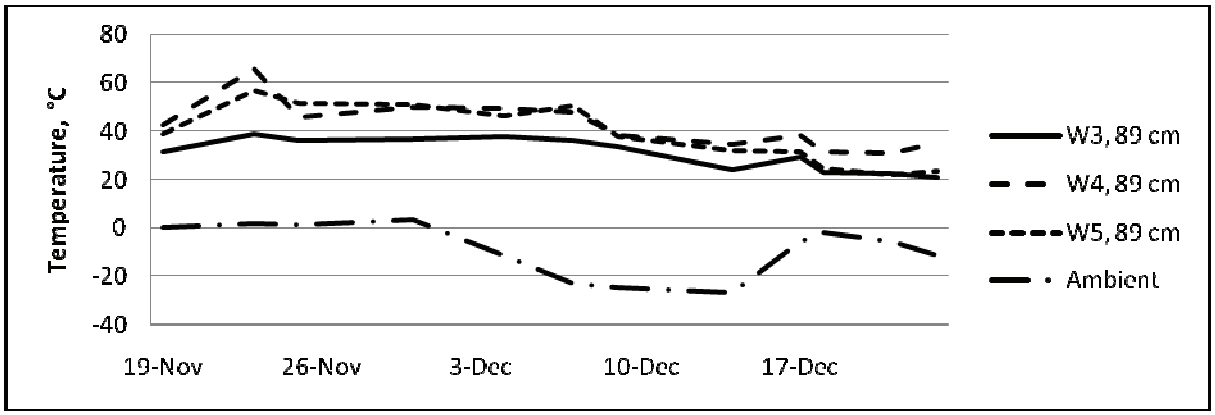

Figure 4. Mean core temperatures of three compost windrows (W3. W4, and W2) in November and December 2009 composed of 180.5 t of dried cattle manure and bedding material from a permitted feedlot and 20.2 t wheat straw and corn stalks to bring the carbon:nitrogen ration to a calculated value of approximately 30:1.

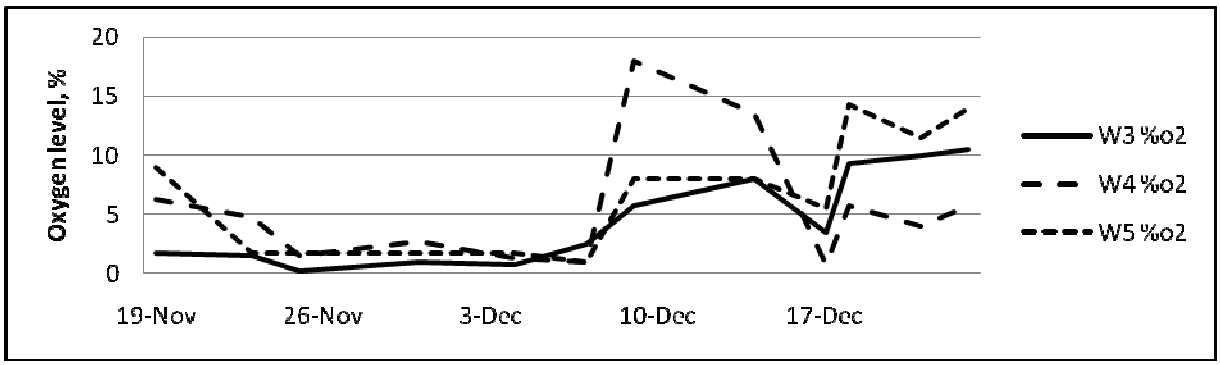

Figure 5. Oxygen levels of three compost windrows (W3. W4, and W2) in November and December 2009 composed of 180.5 t of dried cattle manure and bedding material from a permitted feedlot and 20.2 t wheat straw and corn stalks to bring the carbon:nitrogen ratio to a calculated value of approximately 30:1.

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DETERMINING THE VIABILITY OF BEEF CATTLE MORTALITY COMPOSTING IN NORTHERN MONTANA

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ABSTRACT: The object of this demonstration project was to determine the viability of composting mortalities in northern Montana, which is characterized as a cold semi-arid environment. Mature cows (n=3) were composted in individual bins (B1, B2 and B3) and calves (n=11) were composted in two bins (B4 and B5) containing multiple calves. The animals that were composted included calving losses from the 2010 calving season and animals euthanized according to best management practices for animals that were unsalvageable. The bins for the mature cows were constructed with 4 large straw square bales (0.91 x 0.91 x 2.44 m). Bins were constructed in February, 2010 at Northern Agricultural Research Center on flat clay loam soil surface and composting continued through March 2011. Sawdust was used as a base material (approximately 45 cm) for moisture retention and to prevent runoff, the animal was then placed on the base material, covered with year old spoiled corn silage and capped with a layer of sawdust. The bins were recapped with sawdust as needed. The bins for the calves were constructed using small square straw bales (45 x 45 x 91 cm) on three sides and a large square straw bale on one side, base and fill material were the same as used in the cow bins. Calves were placed in the bins as mortalities occurred and then covered with year old spoiled corn silage. Once the bin was full it was capped with sawdust and another bin was constructed. No predator, nuisance or domestic animal disturbance was observed. Very little insect and fly activity was observed. The maximum temperature for B1, B2, B3, B4 and B5 was 65.7±1.72, 63.5±2.84, 64.8±4.00, 70.4±1.10, 68.1±2.33°C occurring 22, 7, 32, 22, and 32 d after mortality was placed in bin, respectively. All bins containing mature cows reached 40°C temp 24 hours after construction. Bins containing calves reached 40°C 9 d after construction. Bins were excavated at 130 d and no soft tissue remained only skeletal structures and hair. Additional work is planned; however it appeared that large domestic animal composting can occur in northern Montana.

Keywords: Mortality Compost, Beef

Introduction

Disposal of agricultural mortalities is becoming of great concern to both the industry and the general public. Appropriate disposal methods may include incineration, burying the carcass (at least six feet deep, yet above seasonal high ground water, and sufficiently far away from water sources to prevent contamination), and transporting the animal to a certified rendering facility or landfill. All these options are expensive and very time consuming in much of Montana and the Northern Great Plains. For permitted confined animal feeding operations (CAFO), management plans must be in place to appropriately dispose of the animals. These plans include appropriate distances from water sources, and plans if a pandemic crisis reaches the feeding operation. Abandonment in a desolate area on the farm or ranch is no longer an appropriate manner of disposing of domestic animals. The objective of the demonstration trial was to investigate the potential to dispose of beef cattle mortalities of various ages and sizes in Northern Montana, and to provide educational opportunity regarding the practice. If Montana's semi-arid, cold environment can support mortality composting, best management plans for producers in the Northern States can be developed and prepared for mortalities or pandemic events as a viable management option. Although it is not recommended to sell or export compost derived from the mortality composting process this material can be reused in composting future mortalities. Some targeted land application on the site of origin could be considered.

Material and Methods

Bins were constructed in the winter of 2010 at Northern Agricultural Research Center (NARC), near Havre, Montana on flat clay loam soil surface protected from storm water run-on. The animals that were composted included calving losses from the 2010 calving season and animals euthanized according to best management practices for animals that were unsalvageable (no animals were euthanized solely for the purposes of the demonstrated research). As death losses occurred, mature cows (n = 3, average wt = 904 kg) were composted in individual bins (B1, B2 and B3) and new born calves (n = 11) were composted in two bins (B4 and B5) containing multiple calves. Mature cows consisted of multiple breeds, including one Hereford, one Angus and one Simmental cross bred cow but were from the same herd. The bins for the mature cows were constructed with four large straw square bales (0.91 x 0.91 x 2.44 m). Bins were constructed in February, 2010 and composting continued in the bins relatively undisturbed through March 2011. Sawdust was used as a base material (approximately 45 cm) for moisture retention and to prevent runoff, the animals were then placed on the base material, covered with year old spoiled corn silage and capped with a layer of sawdust. The spoiled silage is used to provide a carbon based substrate and

biologically active material. The bins were recapped with sawdust as needed throughout the 12 month period. The bins for the calves were constructed using small square straw bales (45 x 45 x 91 cm) on three sides and a large square straw bale on one side, base and fill material were the same as used in the cow bins. In this project bins were constructed; however, mortality composting has occurred in non-contained windrows. It is believed predator/scavenger disturbance may be more likely in non-contained windrows. Calves were placed in the bins as mortalities occurred and then covered with year old spoiled corn silage. Once the bin was full it was capped with sawdust and another bin was constructed in preparation for future mortalities that could occur. A 90 cm data logger (Windrow Manager, Green Mountain Technologies, Bainbridge Island, WA) was placed into the bins monthly to record internal temperature and oxygen levels of each windrow at a depth of 45 and 89 cm. Mortality composting is static, meaning the bins or windrows are not turned at regular intervals as they are in conventional composting. Aeration occurs passively, and some pockets of anaerobic decomposition occur; over time, the system shifts between aerobic and anaerobic composting. Turning the material after a 3-6 month static period will re-oxygenate the mass and aid in finishing the process.

Results and Discussion

No predator, nuisance or domestic animal disturbance was observed. Very little insect and fly activity was observed. No complaints or personal observations of offensive smell were cited after capping the bins. Daily ambient maximum and minimum temperatures in February, 2010 were 5±5.3°C and -31±6.8°C while March, 2010 was 19.4±6.6°C and 18.9±5.9°C, respectively. All bins containing mature cows reached 40°C 24 hours after bin construction. Bins containing calves reached 40°C 9 d after bin construction and final capping. Indicating the conditions and substrate provided an adequate environment for initiating the composting process. Monthly internal temperatures for each bin are illustrated in Figure 1 to 5. Oxygen was never observed in appreciable levels in any of the bins indicating anaerobic composting was taking place or limited amounts oxygen was passively infiltrating the bins so oxygen measures are not included. The maximum temperature for B1, B2, B3, B4 and B5 was 65.7, 63.5, 64.8, 70.4, 68.1°C occurring 22, 7, 32, 22, and 32 d after the mortality was placed in bin, respectively. Bins were excavated 130 d after beginning the mortality composting process (June 28, 2010) and no soft tissue remained, only skeletal structures and some hair. Looper et al. (2002) reported similar results at 4 m when composting manure and dairy cattle. Bins were re-closed and sealed with sawdust and left undisturbed until March 2011. Upon re-excavating in March 2011, one year from initiating the project all mortality bins contained skeletal bones. All appeared to have reached temperatures to produce a black charred appearance in direct proximity to the skeleton. Calf bones were more brittle and appeared to be more broken down by the process than the mature cow bones. The long bones of the mature cows could not be physically broken by the force of a normal sized human; however some larger long bones of the calves could easily be broken. These results are similar to other mature cow and calf compost projects (Looper et al., 2002; Xu et al, 2010, Stanford et al., 2009). The one year-old mortality compost bin materials were completely excavated and were mixed together. The blend of compost and remaining bones will be the substrate for future compost in 2011. Additional work is planned; however it appeared that large domestic animal composting can occur in northern Montana and similar climates.

Implications

Access to appropriate co-composting materials could be a barrier to implementing the practice. However, if an operation has access to free or affordable carbon material the practice is feasible and can be done with less time and equipment relative to burial on-site. The demonstrated research at NARC in Northern Montana in a semi arid environment provides educational opportunities to regional stakeholders. Additionally, winter weather conditions associated with the region did not appear to hamper the process. With proper site selection, the same area can be used season after season for mortality management in an efficient and safe manner.

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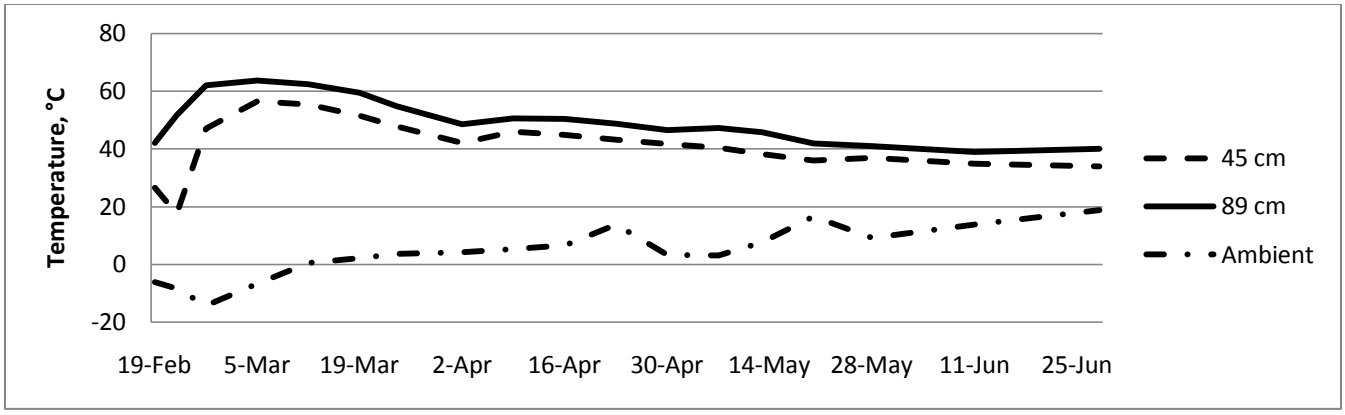

Figure 1. Ambient and mean internal core temperatures of Bin 1 for a mature cow placed in a mortality bin constructed with 4 large straw square bales (0.91 x 0.91 x 2.44 m). The cow (wt = 476 kg) was placed on a bed of sawdust (approximately 45 cm) then a layer of spoiled corn silage was placed around the cow and the bin was capped with a final layer of sawdust. The project was initiated on February 11, 2010 at Northern Agricultural Research Center, near Havre, MT.

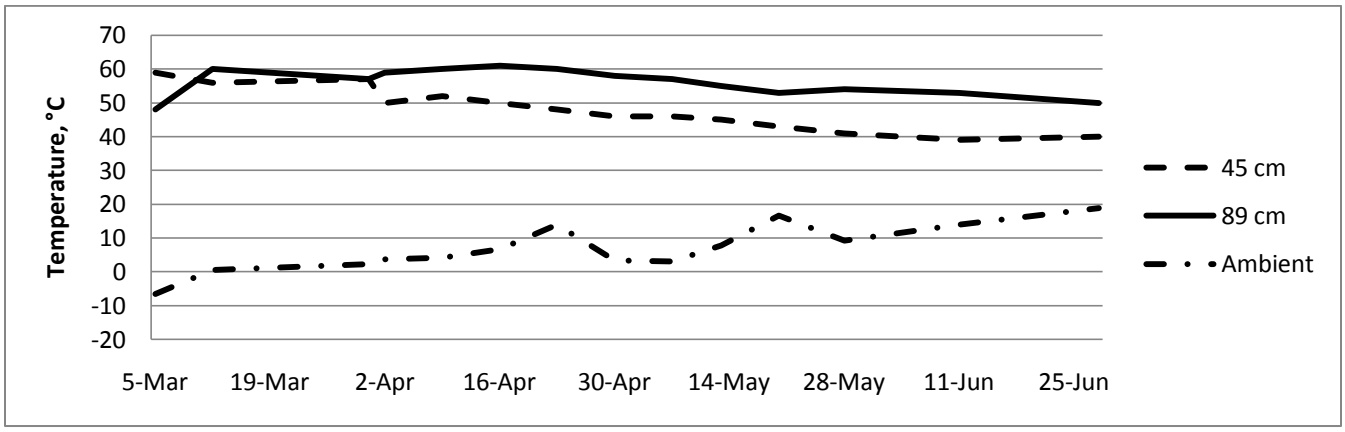

Figure 2. Ambient and mean internal core temperatures of Bin 2 for a mature cow placed in a mortality bin constructed with 4 large straw square bales (0.91 x 0.91 x 2.44 m). The cow (wt = 549 kg) was placed on a bed of sawdust (approximately 45 cm) then a layer of spoiled corn silage was placed around the cow and the bin was capped with a final layer of sawdust. The project was initiated on February 26, 2010 at Northern Agricultural Research Center, near Havre, MT.

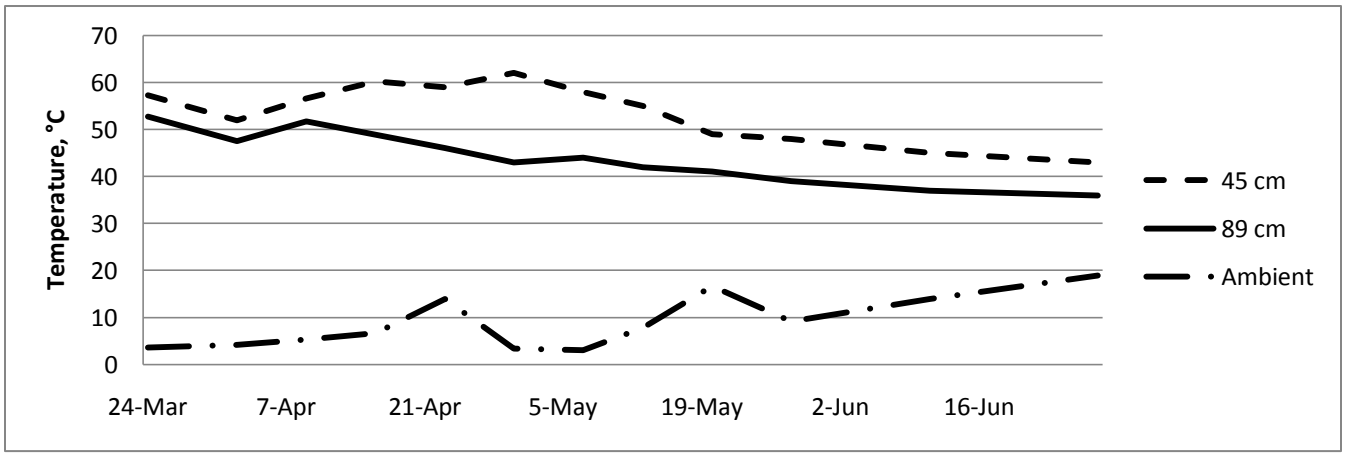

Figure 3. Ambient and mean internal core temperatures of Bin 3 for a mature cow placed in a mortality bin constructed with 4 large straw square bales (0.91 x 0.91 x 2.44 m). The cow (wt = 599 kg) was placed on a bed of sawdust (approximately 45 cm) then a layer of spoiled corn silage was placed around the cow and the bin was capped with a final layer of sawdust. The project was initiated on March 15, 2010 at Northern Agricultural Research Center, near Havre, MT.

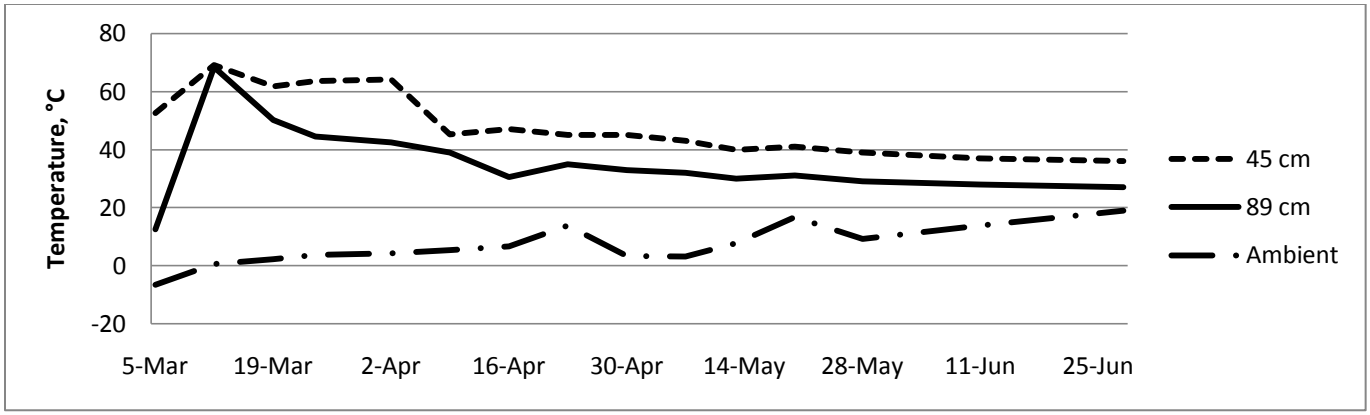

Figure 4. Ambient and mean internal core temperatures of Bin 4 for newborn calves placed in a mortality bin constructed with small square straw bales (45 x 45 x 91 cm) on three sides and a large square straw bale (0.91 x 0.91 x 2.44 m) on one side. The calves (n=5, total wt = 147 kg) was placed on a bed of sawdust (approximately 45 cm) then a layer of spoiled corn silage was placed around the calves as mortality occurred and the bin was capped with a final layer of sawdust when the bin was appropriately full of calf mortalities. The bin was capped March 10, 2010 at Northern Agricultural Research Center, near Havre, Montana.

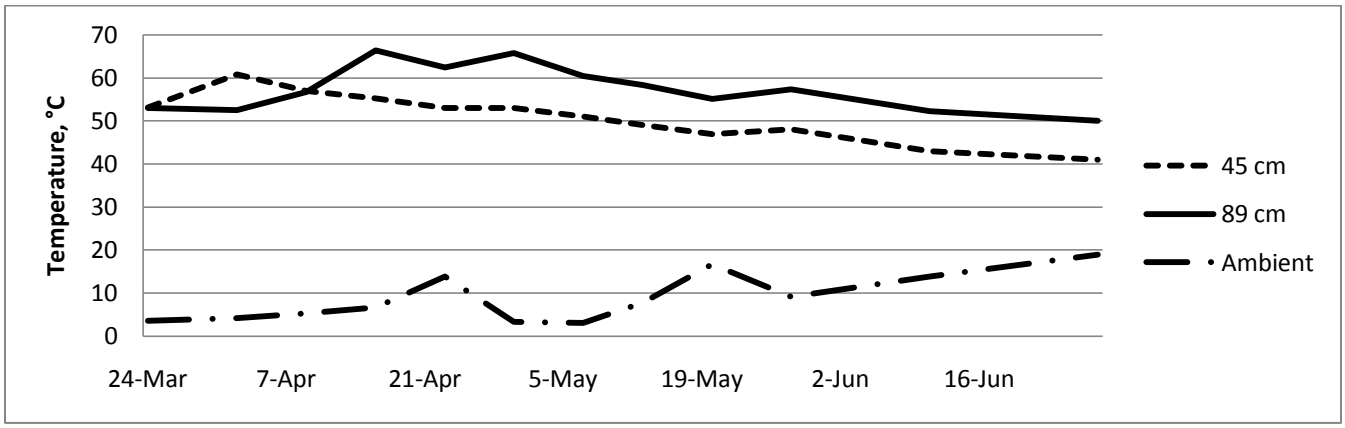

Figure 5. Ambient and mean internal core temperatures of Bin 5 for newborn calves placed in a mortality bin constructed with small square straw bales (45 x 45 x 91 cm) on three sides and a large square straw bale (0.91 x 0.91 x 2.44 m) on one side. The calves (n=6, total wt = 186 kg) was placed on a bed of sawdust (approximately 45 cm) then a layer of spoiled corn silage was placed around the calves as mortality occurred and the bin was capped with a final layer of sawdust when the bin was appropriately full of calf mortalities. The bin was capped April 9, 2010 at Northern Agricultural Research Center, near Havre, Montana.

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EXTENSION PROGRAMMING RESULTS IN NATURAL RESOURCE IMPROVEMENT AND COLLABORATION

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ABSTRACT: In 1993, the Oregon Legislature charged the Oregon Department of Agriculture with enforcement of agricultural sources water pollution. This legislation caused an evolution of agricultural water quality planning and enforcement that culminated in 2009. As a result, Oregon State University Extension Service (OSUES) worked cooperatively with Soil and Water Conservation Districts (SWCD) to provide educational workshops (Cows and Creeks (CC)) over a seven year period (540 participants) to improve collaboration among stakeholders (governmental agencies, non-profits and landowners) and reinforce scientific-based decision making to improve management and regulatory oversight of natural resources and water quality. Evaluation of the program was conducted via printed, multiple-choice survey, mailed to 151 participants (33% return rate). The respondents (n=50) were livestock/land owner/manager (84%), governmental agency (30%) and non-profit (20%). Respondents (affiliation not a variable) stated relationships improved with government agencies (49%), land/livestock owners (45%), and non-profit organizations (38%). Forty-two percent of respondents indicated they utilized financial and/or technical aid from various agencies. The respondents secured more than $1,000,000 in grant funding and personal contributions to enhance riparian function. Of 21 people that received funding, 70% believed the projects resulted in a return on investment as indicated by livestock performance, recreational opportunities, habitat for wildlife and fisheries, water quality standards and/or farm/hay production. Forty-eight percent of respondents implemented at least one restoration project or changed management to improve riparian function. As a result of CC, the respondents (n=50) observed improvement with the following; cow/calf performance (24%), riparian vegetation (42%), fish habitat (20%), bank stabilization (36%), and stream flow (18%).

Key Words: Extension Service, collaboration, natural resource improvement

Introduction

In 1993, the Oregon Legislature charged the Oregon Department of Agriculture (ODA) with enforcement responsibilities of nonpoint source pollution originating from agricultural sources. This legislation caused an evolution of agricultural water quality planning and enforcement that as of 2009 is in full force and effect. Water quality regulations have sparked interest among resource mangers as they relate to livestock production among producers, government and non-profit funding agencies and public land agencies. As a result, Oregon State University Extension Service (OSUES) worked cooperatively with Soil and Water Conservation Districts (SWCD) to provide educational workshops entitled "Cows and Creeks" (CC) over a seven year period to improve collaboration among stakeholders (governmental agencies, non-profits and landowners) and reinforce scientific-based decision making to improve management and regulatory oversight of natural resources, and water quality.

Thirteen workshops were held at six different locations in Central and Eastern Oregon. Experts with backgrounds in University Extension and research, public land management, and private consulting were asked to share knowledge on various topics such as livestock grazing strategies, water quality regulation and monitoring, fish habitat, riparian function, confined animal feeding operations, cost share programs and livestock performance. Local county government, OSUES, private business, SWCD and State and Federal agencies provided funding for these workshops. During the 7 years, a total of 540 people participated in the workshops, many attending all or more than one. Evaluation of economic, social, and environmental impacts of Extension programs is important in understanding if program content is meeting clientele needs and addressing concerns.

Materials and Methods

Research approval from Oregon State University Internal Review Board (IRB) for human subjects was attained prior to survey distribution. The survey focused on five evaluation areas; description of the audience, demographics, economic impacts, social impacts, and environmental impacts.

Questions in the audience section were designed to identify the affiliation of the participant, motivation for attending CC, and specific programs attended by the participant. All questions in this section were multiple choices allowing the respondent to "mark all that apply".

Demographics evaluated if the respondent owned or managed property on a stream, river or creek. If so, respondents were asked questions about the land use, miles of stream, river, or creek owned/managed, and if the waterways had anadromous or listed fish populations, and the 303(d) Status (water quality concern). All questions were multiple choice. The land use questions were "mark all that apply" multiple choice.

Economic impact section asked if the respondent utilized cost-share programs, value of grant and personal

cash and/or in-kind projects in which cost-share was utilized, and agency from which technical and/or financial assistance was used. The section also asked if the projects resulted in a return on investment. All questions were multiple choice.

Environmental impact was evaluated by asking if the respondent adopted particular management techniques or natural resource improvements. This section was a multiple choice question which allowed respondents to mark all that apply.

In the social impact section, respondents were asked if relationships with land owners/managers, public agencies, and non-profit organization had improved, worsened or not changed. Respondents were also asked how CC influenced their opinion of livestock and wildlife grazing, recreation, and farming/haying in riparian areas. A list of 15 organizations was listed and respondents were asked to rank the top three organizations in which they would seek assistance in dealing with riparian management, water quality and other natural resource issues. Further questions asked multiple choice questions about the program timing, location, and pricing and if they would attend future sessions.

The survey was mailed to 151 past participants as documented by program signup sheets. Self addressed, postmarked envelopes were provided for surveys to be returned. Summary statistics were used to analyze the data.

Results and Discussion

Surveys were returned at a 33% return rate (50 surveys). Of those responding, many participants attended more than one program over the seven years (2003, 2004, 2005, 2007, 2009), totaling 76 participants in multiple programs.

Description of Audience

The respondents (n=50) include livestock (44%) and land owners/managers (40%), Agency personal from Forrest Service, Bureau of Land Management, Oregon Department of Fish and Wildlife, and Natural Resource Conservation Service (30%) as well as nonprofit organizations such as Watershed Council, Soil and Water Conservation Districts, and Weed Advisory Boards (20%), and Other (20%). The reason that percentages sum up to be greater than 100 % is some individuals described themselves with more than one affiliation, most commonly, livestock owner/manager was marked in conjunction with landowner/manager.

Of the 50 respondents, 28 (56%) managed or owned property along a stream, river or creek, representing up to one mile of water-frontage (18%), 15 (30%) of respondents managed 1-10 miles of water frontage and 4 (8%) of the respondents managed water frontage greater than 10 miles. Twenty-eight percent (28%) of the respondents owned or managed waterways that were listed as 303 (d) water quality limited and 56% of the respondents owned/managed waterways that had listed and/or anadromous fish populations.

Property owner or managers indicated that the primary use for the land was most livestock grazing (60%), followed by farming/haying (12%) and timber (8%). Secondary use for the property included farming/haying (30%), livestock grazing (20%), timber (16%), and recreation (14%). Some respondents indicated more than one primary and/or secondary use.

Economic Impact

A total of 21 (42%) respondents indicated that as a result of Cows and Creeks, they had utilized cost-share programs with the financial and/or technical aid from various agencies. Of which, 67% of these individuals indicated NRCS as a source of funding, followed by Oregon Watershed Enhancement Board (OWEB) (52%), SWCD (43%), Oregon Department of Fish and Wildlife (ODDFW) (33%), and Watershed Council (19%). Although OSUES does not provide funding, (38%) of these respondents indicated they received technical aid from OSUES.

Grant funding from all funding sources was estimated to be at least $420,000. Landowners reported they contributed additional cash and/or in-kind investments similar to that obtained through grants. Additional in-kind contributions were made by technical assistance providers including SWCD's, OSUES and NRCS. These estimates likely underestimate the value of both grant funds and in-kind/personal cash. The survey required marking one of five categories (< $5,000; $5,000-$10,000; $10,000-$20,000; $20,000-$50,000; >$50,000). The value of the grant and personal contribution was estimated by totaling the least value indicted, for example, if one marked $10,000-$20,000, the researchers valued the contribution at $10,000. A total of nine respondents indicated grant or personal contribution was greater than $50,000, with the researchers only accounting for $50,000 for each of these respondents.

Seventy percent (70%) of the respondents that received funding believed the projects they completed resulted in a return on investment as indicated by improved livestock performance, recreational opportunities, habitat for wildlife and fisheries, water quality standards and/or farm/hay production. Additionally, three individuals thought there was no return on investment even though they indicated livestock performance was improved. Likewise, three other grant recipients were not sure if there was a return on investment.

Social Impact

As a result of Cows and Creeks, relationships between landowners, agencies and non-profit organizations were built and strengthened. Respondents (affiliation not a variable) indicated that relations improved with government agencies (49%), landowners/livestock owners (45%), and non-profit organizations (38%). Respondents also indicated a seven percent increase in willingness to partner and/or cooperate with other agencies, organizations, and/or landowners to deal with natural resource concerns.

Respondents have many resources available to them for assistance with riparian management, water quality and natural resource issues. Of 15 different organizations, respondents were asked to rank their top three organizations (Table 1). If ranking was not

considered, OSUES, SWCD, NRCS, and Watershed Councils were most likely to be utilized for information. If the participants ranked their choices, OSUES was most likely to be asked for information first. Likewise, the top four organizations as listed above remained ranked as one of the first three choices for information. These results indicate the CC was successful at identifying important resources for stakeholder education, technical and financial support from local, state and federal organizations.

As a result of CC, 72% of respondents said they formed an opinion that livestock grazing can occur in riparian areas without environmental damage, 6% disagreed, 12% did not comment and 10% were neutral (Table 2). Most respondents believed farming/haying could also be accomplished without environmental damage (66%). Fewer respondents believed that recreation (48%) and wildlife grazing (54%) could occur without environmental damage although workshop topics did not address these areas.

Environmental Impact

A total of 24 respondents (48%) reported they implemented at least one restoration project or changed management to improve riparian function (Table 3). Livestock grazing management was changed by creating riparian pastures (6%) and fencing riparian areas (18%). Implementation of rotational grazing was implemented by 26% of the respondents. Respondents also reported constructing hardened crossings/water gaps (14%) and off-site watering structures (22%). Bio-engineered projects included in-stream structures (jetties, water weirs, drop-step structures, etc.) (14%), stream bank stabilization structures (16%) and riparian plantings (14%). Eight percent (8%) reported they began to use fish screens.

Cows and Creeks was successful at influencing management changes within riparian zones. Improvements observed (frequency) by the respondents included cow/calf performance (24%), riparian vegetation (42%), fish habitat (20%), bank stabilization (36%), and stream flow (18%). Although only 20% of the respondents indicated improvement in water quality, we speculate water quality has likely improved on all of the operations that reported improved stream flow, riparian vegetation and more stabile banks.

Conclusion

The Cows and Creeks Program was successful at improving relationships among governmental agencies, landowners/livestock owners, and non-profit organizations. This program secured funding of more than $1,000,000 of grant and personal and other contributions to make improvements or management changes to improve riparian function and water quality. Collectively, these funds and relationships changes resulted in new management strategies that improved both livestock production and natural resources and are likely contributing to improved water quality. If more Cows and Creeks workshops were held in Oregon, 64% of the respondents would return (34% unsure, many of who indicated it depended on content).

Table 1. Survey Question: If you need assistance in dealing with riparian management, water quality and other natural resource issues, you would contact the following organizations: (Please rank **only the top 3 organizations.** 1= will contact first, 2= will contact second, 3 = will contact third).

Organization	If ranking was not considered, only frequency.[2] (n=121)	Removed Respondents that did not Rank Choices[1] (n=31)		
		Ranked as 1st Choice.[3]	Ranked as 2nd Choice.[3]	Ranked as 3rd Choice.[3]
OSUES	21 %	29%	26%	16%
SWCD	20%	23%	13%	26%
NRCS	18%	19%	19%	19%
Watershed Council	10%	10%	16%	13%
Livestock Owner/Manager	6%	6%	0%	0%
Land Owner/Manager	5%	3%	6%	0%
ODFW	5%	3%	3%	13%
Forrest Service	2%	6%	3%	0%
ODA	2%	0%	10%	0%
Oregon Div. of State Lands	2%	0%	0%	3%
Oregon Native Desert Assoc.	1%	0%	3%	0%
BLM	0%	0%	0%	0%
Sierra Club	0%	0%	0%	0%
Nature Conservancy	0%	0%	0%	0%
Other	1%	0%	0%	3%
No Answer	7%			

[1]Some respondents marked three organizations, but did not rank them.

Table 2. Survey Question: As a result of OSU Cows and Creeks, I have formed an opinion that livestock grazing, wildlife grazing, recreation and farming/haying in riparian areas can be accomplished without environmental damage.

Activity	(n=50)					
	Strongly Agree (%)	Agree (%)	Neutral (%)	Disagree (%)	Strongly Disagree (%)	No Answer (%)
Livestock Grazing	34	38	10	6	0	12
Wildlife Grazing	20	34	18	14	0	14
Recreation	6	42	28	4	4	16
Farming/Haying	18	48	14	4	2	14

Table 3. Survey Question: As a result of attending OSU Cows and Creeks I have implemented the following:

	Frequency	(n=50)
Fenced Riparian Areas	9	18%
Changed Season of Use	7	14%
Riparian Pastures	3	6%
Rotational Grazing	13	26%
Off-Site Watering	11	22%
Water Gap/Hardened Crossing	7	14%
Cost-Share Project for Rehabilitation of Riparian Area	6	12%
Routinely Monitor Vegetation and Water Quality	8	16%
In-Stream Structures	7	14%
Bank Stabilization	8	16%
Riparian Plantings	7	14%
Fish Screens	4	8%
Other	3	6%
No Answer	26	52%
Total Respondents that Answered Question	24	48%

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VACCINE STORAGE AND BEEF QUALITY ASSURANCE PRACTICES AMONG IDAHO BEEF PRODUCERS

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ABSTRACT: To be fully effective and provide consumers with safe, high quality beef, animal health products must be stored and administered properly. It is recommended that many animal health products be stored at temperatures ranging from 2 to 7°C. Vaccine efficacy may be compromised if vaccines are stored in faulty refrigerators or when thermostats are improperly set. Data recorders were used to log refrigerator temperatures at 10-min intervals for 48 h on 129 Idaho beef operations. On-site surveys were used to gauge the use and adoption of animal health product management and basic beef quality assurance (BQA) practices. Operations in the study included cow-calf (91%), feedlot (4%), and a combination of cow-calf and feedlot (5%). Size of operation was categorized as follows: 1 to 25 cows (7%), 26 to 50 cows (5%), 51 to 100 cows (15%), 101 to 200 cows (12%), and more than 200 cows (61%). Refrigerator locations included: kitchens (19%), mud rooms (15%), barns (13%), garages (10%), and on porches (11%). Refrigerator ages were ≤ 5 yr (16%), 6 to 10 yr (27%), 11 to 15 yr (23%) and > 15 yr (34%). One-third of refrigerators maintained temperatures in the recommended range for more than 95% of the recording time and 32% of refrigerators maintained temperatures in the recommended range for less than 5% of the recording time. Forty-seven of the surveyed producers were BQA certified and 47 were not certified and had not attended a training session. Equal proportions (34%) of certified and non-certified producers' refrigerators maintained temperatures in the recommended range 95% of the time. Thirty percent of certified producers' refrigerators and 36% of non-certified producers' refrigerators maintained recommended temperatures less than 5% of the time. On-site surveys show that Idaho beef producers have implemented basic BQA practices. Almost all (95%) producers cited the use of the neck region of beef cattle for injections. Producers have implemented acceptable chute-side practices for vaccines, such as mixing modified live vaccines on an as-needed basis (113 operations), protecting vaccines from sunlight (113 operations), and keeping vaccines in coolers (121 operations).

Key Words: Vaccine, Storage, Beef Quality Assurance

Introduction

To maintain consumer demand for beef, the beef industry has found it necessary to address and eliminate quality shortfalls. A component of this effort is the periodic audits the industry conducts. In the most recent National Beef Quality Audit, some carcass quality defects have been identified as the result of improper use of animal health products (NCBA, 2005). Animal health products must be stored and administered properly. The Code of Federal Regulations (USDA, 2006) states that biological products shall be protected at all times against improper storage and handling with products being kept under refrigeration at 2 to 7°C. The code also states that biological products be deemed worthless after the expiration date has passed. Results from an Arkansas study (Troxel and Barham, 2009) show that beef producers were generally careful in storing animal health products under refrigerated conditions, but a great deal of variation existed in refrigerator settings, maintenance, and function. They concluded that more than 76% of refrigerators tested were unacceptable for storing animal health products. A similar study in Nevada (Torell, 2006) found that approximately 25% of refrigerators tested were unacceptable for animal health product storage.

An additional component of the beef industry's effort to provide consumers with safe, high quality beef and beef products is the beef quality assurance (**BQA**) program. The goal of BQA is to create awareness among producers and focus their attention on the day-to-day management practices that influence beef carcass quality. Beef quality assurance practices help to enhance carcass quality by providing a means to prevent residues, pathogen contamination and various carcass defects. According to Idaho BQA Program statistics, approximately 1,400 producers have participated in BQA training and information sessions and approximately 800 producers have completed the requirements for BQA certification. A survey of the U.S. beef industry indicates that BQA trained producers have improved their animal health product management and basic BQA practices (USDA, 2008). As an example, the survey showed that more than 20% of producers changed their vaccine storage and handling, more than 40% of producers changed injection site selection, and more than 20% of producers changed their record keeping methods as a result of a BQA training session.

The objectives of this study were to conduct on-site surveys to: 1) determine the temperature range, location, age and effectiveness of refrigerators where animal health products are stored, 2) determine the status (expired, open, etc.) of products and identify product handling practices, and 3) gauge the level of implementation of various BQA practices among Idaho beef producers.

Materials and Methods

From December 1, 2009 to June 30, 2009, University of Idaho Extension faculty (Extension educators and

specialists) conducted on-site visits of beef cattle operations across the state of Idaho. During the visits, LogTag data loggers (Model Trix-8; LogTag, Auckland, NZ) were programmed to record temperatures at 10-min intervals and were placed in refrigerators where animal health products were stored. The data loggers remained in refrigerators for at least 48 h. Once the data loggers were removed from the refrigerators, the temperature data was downloaded using a LogTag Interface Cradle and Analyzer software (LogTag, Auckland, NZ) and made available for summary and analysis. Prior to the data loggers being placed in the refrigerators, Extension faculty assessed the contents of the refrigerators. In addition, the status (opened, expired, etc.) of the animal health products in the refrigerators was assessed. On-site surveys were also administered to producers during the farm/ranch visits. The surveys were used to gather demographic information, BQA certification status, animal health product purchasing habits, animal health product care and storage practices, chute-side practices and record keeping practices. The survey protocol used was approved by the University of Idaho Human Assurances Committee (Project #09-065). Data from the temperature loggers and surveys were entered into spreadsheets for summarization and the data was analyzed using the PROCSURVEYFREQ routine in SAS Version 9.1 (SAS Inst., Inc., Cary, NC).

Results and Discussion

One hundred, twenty-nine Idaho beef producers participated in the study and completed on-site surveys. To gain an understanding of the types of participating operations, producers were asked to categorize their operations as cow-calf, feedlot, or a combination of cow-calf and feedlot. Results are shown in Table 1. A large percentage of producers categorized their operations as cow-calf (91%), followed by a combination of cow-calf and feedlot (5%), and feedlot (4%).

Idaho's beef industry is made up of operations that are quite varied in size. One method of determining operation size is to use cow numbers. The percent of study participants owning 1 to 25, 26 to 50, 51 to 100, 101 to 200 and more than 200 cows was 7%, 5%, 15%, 12%, and 61%, respectively. While representation is spread fairly evenly across most of the cow number categories, the data from Table 2 suggest the majority of study participants are large (greater than 200 cows) producers.

The types of refrigerators (Table 3) found on Idaho farms and ranches and evaluated in the study included freezer-on-top (70%), side-by-side (11%), mini-refrigerators (10%), all-in-one units (6%), and freezer-on-bottom (3%). The location of refrigerators (Table 4) varied greatly, but most were found in kitchens (19%), mud rooms (15%), barns (13%), garages (10%), and on porches (11%). The age of refrigerators (Table 5) tested were ≤5 yr (16%), 6 to 10 yr (27%), 11 to 15 yr (23%), and > 15 yr (34%). The results related to refrigerator type, location, and age, are similar to the Arkansas results reported by Troxel and Barham (2009). In that study, freezer-on-top was the most frequent type of refrigerator and freezer-on-bottom was the least frequent. The location of refrigerators is difficult to compare due to the variety of locations noted in the studies. However, kitchens, barns, and garages ranked toward the top in both studies. The age of refrigerators tended to be older in the current study versus those in the Arkansas study. Approximately 85% of refrigerators in the current study were placed in the three older age categories compared to approximately 80% of the refrigerators in the Arkansas study being placed in the three newer age categories.

A total of 2,257 bottles of animal health products were found in the refrigerators of Idaho producers. Of those animal health products, 463 (20%) were expired and 614 (27%) were opened or previously used. Troxel and Barham (2009) found approximately 12% of the animal health products in Arkansas refrigerators expired and approximately 30% opened. Forty-seven percent of the refrigerators in Idaho contained food and 69% of refrigerators contained drinks for human consumption. Approximately 42% of the refrigerators contained animal health products meant for use in animals other than beef cattle.

As stated previously, it is recommended that many animal health products be stored under refrigeration at 2° to 7°C. The overall average temperature of the refrigerators tested was 2°C. While it may seem that most refrigerators performed adequately to store animal health products, a closer examination of the refrigerator temperatures is needed. Table 6 shows the importance of closely monitoring temperatures of refrigerators used for animal health product storage. Only 33% of refrigerators maintained temperatures in the recommended range 95% of the recording time. At the other end of the scale, 32% of refrigerators maintained temperatures in the recommended range less than 5% of the time. Twenty-seven percent of the refrigerators evaluated by Troxel and Barham (2009) maintained temperatures in the recommended range 95% of the time and 24% of the refrigerators they tested maintained temperatures less than 5% of the time. These results show that many refrigerators are unable or set improperly to maintain temperatures in the recommended range.

According to the results of the on-site surveys, 47 producers were BQA certified and 47 producers were not. The remaining 35 producers cited attendance at BQA programs but had not completed the requirements for BQA certification. Equal proportions (34%) of BQA certified and non-certified producers had refrigerators that maintained temperatures in the recommended range 95% of the time. Thirty percent of certified producers' refrigerators and 36% of non-certified producers' refrigerators maintained recommended temperatures less than 5% of the time.

On-site surveys were also used to determine the level of implementation, or adoption, of some basic BQA practices by Idaho beef producers. Since the beginning of BQA, it has been recommended that producers move injection sites to the injection-site triangle in the neck region of beef cattle. The on-site survey results show that 95% of producers use the neck region only for injections versus locations that are handier or more accessible. Another indication of how producers have accepted recommendations related to proper injections is how they

choose the route of administration. Eighty-nine percent of producers indicated they determine the proper route of administration by reading and following animal health product label information.

According to survey results, Idaho producers have implemented acceptable chute-side vaccine handling and use practices. To be fully effective, modified-live vaccines need to be mixed near the time of use, kept cool and protected from ultraviolet light. One hundred, thirteen producers (88%) mix their vaccines as needed versus mixing them at the beginning of the day and using them for extended periods. The same number of producers (113) indicated they protect their vaccines from sunlight during storage and use. One hundred, twenty-one producers (94%) keep their vaccines in coolers during use.

Record keeping is a key element of BQA. Feed, feed additive, drug, chemical, and animal processing and treatment records are some of the types of records that producers should consider collecting. It is recommended that producers keep the records for three years. Results of the on-site surveys show that a majority of producers (52%) keep their records for at least three years. Fourteen percent of producers indicated they keep their records for two years and 6% said they retain their records for a year. Twenty-eight percent of the respondents did not specify a length of time for record retention.

Implications

The small number of refrigerators that were able to maintain the recommended temperatures for at least 95% of the time underscores the need to regularly monitor the temperatures of refrigerators where animal health products are stored. Vaccines and other animal health products may be compromised if they are stored in faulty refrigerators or in refrigerators whose thermostats are improperly set. Producers should purchase a thermometer, place it in the refrigerator with their animal health products, and check and record the temperatures regularly. Most animal health products have expiration dates included on their labels or package inserts. The product's effectiveness may be compromised if used after the expiration date. All animal health products that have reached their expiration date should be discarded according to package directions. With less than half of the producers being BQA certified, opportunities exist to showcase the value and benefits of BQA and provide avenues for producers to become certified. Even though a majority of the producers were not BQA certified, it is encouraging that in most cases, large percentages of producers were using basic BQA practices.

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Table 1. Types of operations included in the study

Operation type	Operations, #	Operations, %
Cow-Calf	117	91
Feedlot	5	4
Combination (Cow-Calf and Feedlot)	7	5

Table 2. Number of cattle owned by producers

Number of cattle	Producers, #	Producers, %
1 – 25	9	7
26 – 50	7	5
51 – 100	19	15
101 – 200	15	12
201 +	77	61

Table 3. Types of refrigerators tested

Type of refrigerator	Refrigerators, #	Refrigerators, %
Freezer-on-top	90	70
Side-by-side	14	11
Mini-refrigerator	13	10
All-in-one unit	8	6
Freezer-on-bottom	4	3

Table 4. Location of refrigerators tested

Location	Refrigerators, #	Refrigerators, %
Kitchen	24	19
Mud room	19	15
Barn	17	13
Porch	14	11
Garage	13	10
Shop	9	7
Vet room	9	7
Storage room	8	6
Tack room	8	6
Basement	6	4
Bedroom	2	2

Table 5. Age of refrigerators tested

Age	Refrigerators, #	Refrigerators, %
< 5 yr	20	16
6 to 10 yr	36	28
11 to 15 yr	29	22
> 15 yr	44	34

Table 6. Refrigerators maintaining temperatures in recommended range (2° to 7°C)

Temperatures in range	Refrigerators, #	Refrigerators, %
> 95%	43	33
66 to 95%	17	13
36 to 65%	10	8
5 to 35%	17	13
< 5%	42	32

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Case Study: Low-Input Bunker Storage of Wet Distiller's Grain

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ABSTRACT: Traditionally the use of wet distiller′s grains (WDG) as a feedstuff has been limited to livestock producers with the capability of using truckload quantities of WDG within 7 to 21 d due to the short shelf life of WDG. Wet distiller′s grains can be stored in bags or mixed with forages and packed into bunkers. These storage methods require additional inputs (purchase and processing of forages, mixing and bagging equipment, fuel, labor) that increase the cost of storing WDG. Therefore, storage methods that require fewer inputs and less labor must be explored. The objective of this case study was to examine the feasibility of storing WDG in concrete bunkers without the addition of forage as a bulking agent. Approximately 68 metric tons (3 truckloads) of corn-based WDG was unloaded directly into 2 concrete bunkers and covered with 6 mil black plastic and tires. Samples of WDG were obtained from each truckload upon arrival and composited (d 0). Samples were then obtained from 3 locations within each respective bunker using a grain probe and combined to form a composite sample every 14 ± 5 d thereafter for the duration of the study (208 d). Samples were submitted to a commercial laboratory and analyzed for DM, CP, ADF, NDF, ADIN, Ca, P, S, and pH. Bunker served as the replicate and data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC) as a repeated measures design. Days in storage had no effect on DM, ADF, ADIN, Ca, S, or pH of WDG ($P > 0.05$), but did impact CP, NDF and P concentration ($P < 0.05$). Crude protein concentration of WDG tended to be greater from d 0 (30.92%) at d 42 (31.86%; $P = 0.09$), and was greater on d 167 (33.88%; $P < 0.05$). Concentrations of NDF tended to be greater than d 0 on d 69, 94, 181 ($P \leq 0.07$) and were greater on d 55, 137, 161, 167, 194, and 208 ($P < 0.05$). Phosphorous content tended to be less than d 0 on d 151, 161, 167 ($P \leq 0.12$) and tended to be greater on d 194 and 208 ($P \leq 0.18$). The results of this case study imply that low-input bunker storage of WDG, without the use of forage as a bulking agent, may be a feasible storage option for livestock producers interesting in using WDG in their operations.

Key Words: Distillers grains, bunker, storage

Introduction

The use of wet distiller's grains **(WDG)** as a feedstuff in livestock operations has increased substantially, but has been limited primarily to livestock producers with the capability of utilizing truckload quantities (approximately, 18 metric tons) of WDG within 7 to 21 days due to the short shelf life of WDG. The high moisture content of WDG (35% dry matter) has restricted the use of conventional methods of storing high moisture feedstuffs, as WDG alone cannot be mechanically compacted to exclude oxygen. However WDG has been successful ensiled with a variety of other feedstuffs including soyhulls and corn silage in silage bags (Kalsheur et al., 2002; 2003). More recently Adams et al., (2008) demonstrated that WDG may be successfully stored alone in silage bags or by combining WDG with forages in silage bags or concrete bunker silos. These storage methods utilize additional feedstuffs as bulking agents to facilitate compaction of WDG. Although these storage methods provide a means of effectively storing WDG they also require additional inputs (purchase and grinding/processing of forages, mixing and bagging equipment, fuel, and labor). These inputs add expense to the process of storing WDG and require several steps, making it difficult to process large quantities of WDG in a short period of time. Therefore, storage methods that require fewer inputs and less labor must be explored.

The objective of this case study was to examine the feasibility of storing WDG in concrete bunkers without the addition of forage as a bulking agent and to evaluate the effects of storage on subsequent nutrient composition of WDG.

Materials and Methods

Approximately 68 metric tons (3 truckloads) of corn-based WDG was unloaded directly into, 2 identical concrete bunkers (4.1 m (width) × 12.0 m (length) × 1.2 m (height) in September, 2009. Each bunker was filled approximately one-half full and the WDG was allowed to gradually slope toward the open of the bunker. Each pile of WDG was covered with 6 mil black plastic and tires. Grab samples of WDG were obtained from each truckload of WDG upon arrival and combined to form a composite sample (d 0). At the initiation of the project samples were scheduled to be taken every 14 d. However, the sampling protocol was interrupted due to cold temperatures during the fall and winter months of 2009/2010; the grain probe could not be inserted into the WDG without excessive force. Therefore, samples were obtained ever 14 ± 5 d for the duration of the study (208 d). Samples were collected from 3 locations within each respective bunker using a commercially available grain probe, inserted to a common depth of approximately 50 cm. Samples from the 3 locations were combined to form a composite sample within each respective sampling date. Samples were immediately frozen and were submitted at the conclusion of

the 208 d storage period to a commercial laboratory (SDK Laboratories, Hutchinson, KS) and analyzed for DM, CP, ADF, NDF, ADIN, Ca, P, S, and pH content.

Statistics. All data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The effects of storage day (time) on DM, CP, ADF, NDF, ADIN, Ca, P, S, and pH were evaluated as repeated measures design (covariance structure = autoregressive order one). Bunker served as the replicate. Data are presented as least squares means and differences were considered significant at $P < 0.05$.

Results and Discussion

Physical Observations. After 208 d in storage a thin layer of mold (approximately 0 to 5 cm thick) had developed across the surface of the WDG. There was very minimal mold on the surface of the WDG, where the plastic was sealed down tightly to the WDG by the tires used to hold the plastic sheeting in place. The WDG beneath the mold displayed no discernable off characteristics other than a slight acetic-acid type odor that dissipated within 48 h of opening the bunker. The absence of mold in the locations under the tires emphasizes the importance of establishing an oxygen-limiting environment. This would also tend to indicate that the amount of mold observed in this case study may have been further reduced if a tighter seal was created between the plastic sheeting and WDG.

Table 1. Effect of storage on chemical composition of wet distiller's grain stored in concrete bunkers

Item	0 day	208 day	SEM	*P*-Value[1]
DM, %	36.29	36.71	0.49	0.64
CP, %	30.92	30.78	0.37	< 0.05
ADF,%	11.47	13.67	0.60	0.24
NDF, %	25.0	26.94	0.41	< 0.05
ADIN, %	3.32	4.08	0.24	0.25
Ca, %	0.08	0.08	0.02	0.62
P, %	0.91	1.04	0.07	< 0.05
S, %	0.67	0.63	0.02	0.69
pH	4.0	4.0	0.10	0.43

[1]Effect of storage

Nutrient Analyses. Days in storage had no effect on DM, ADF, ADIN, Ca, S, or pH of WDG ($P > 0.05$; Table 1), but did impact CP, NDF and P concentration ($P < 0.05$). Crude protein concentration of WDG (Figure 1) tended to be greater from d 0 (30.92%) at d 42 (31.86%; $P = 0.09$), and was greater on d 167 (33.88%; $P < 0.05$). Concentrations of NDF (Figure 2) tended to be greater than d 0 on d 69, 94, 181 ($P \leq 0.07$) and were greater on d 55, 137, 161, 167, 194, and 208 ($P < 0.05$). Phosphorous content (Figure 3) tended to be less than d 0 on d 151, 161, 167 ($P \leq 0.12$) and tended to be greater on d 194 and 208 ($P \leq 0.18$). The final nutrient composition of WDG on a DM basis after 208 d in storage was 30.78% CP, 26.94% NDF, 13.67% ADF, 4.08% ADIN, 0.08% Ca, 1.04% P, and 0.63% S.

Wet distiller's grain appears to be a suitable feedstuff for low-input bunker storage systems. The relative acidity of WDG (initial pH = 4.0) likely restricts fermentation and degradation, if an oxygen-limiting environment can be created. Therefore, additional measures should be taken to ensure a tight seal at the surface of the WDG and plastic sheeting.

Implications

The results of this case study imply that low-input bunker storage of WDG, without the use of forage as a bulking agent, may be a feasible storage option for livestock producers interesting in using WDG in their operations.

Acknowledgements

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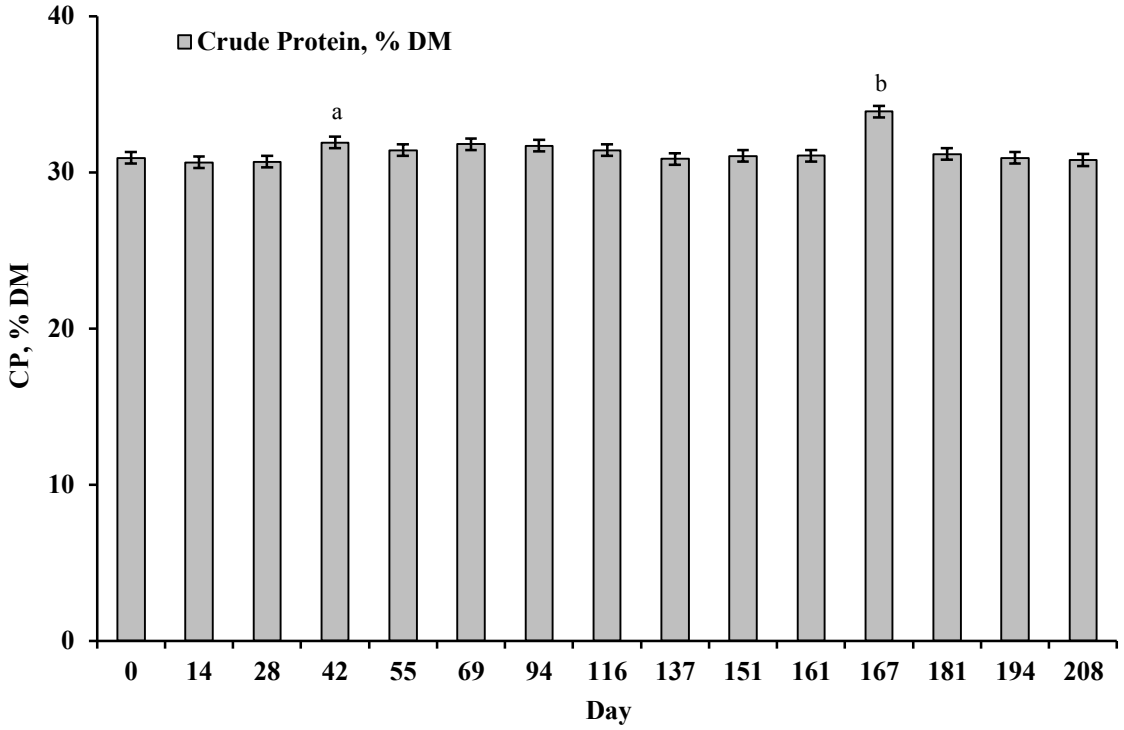

Figure 1. Effect of storage on CP content (DM basis) of wet distiller's grain stored in concrete bunkers. [a] tended to be different from d 0 ($P < 0.10$). [b] different from d 0 ($P < 0.05$).

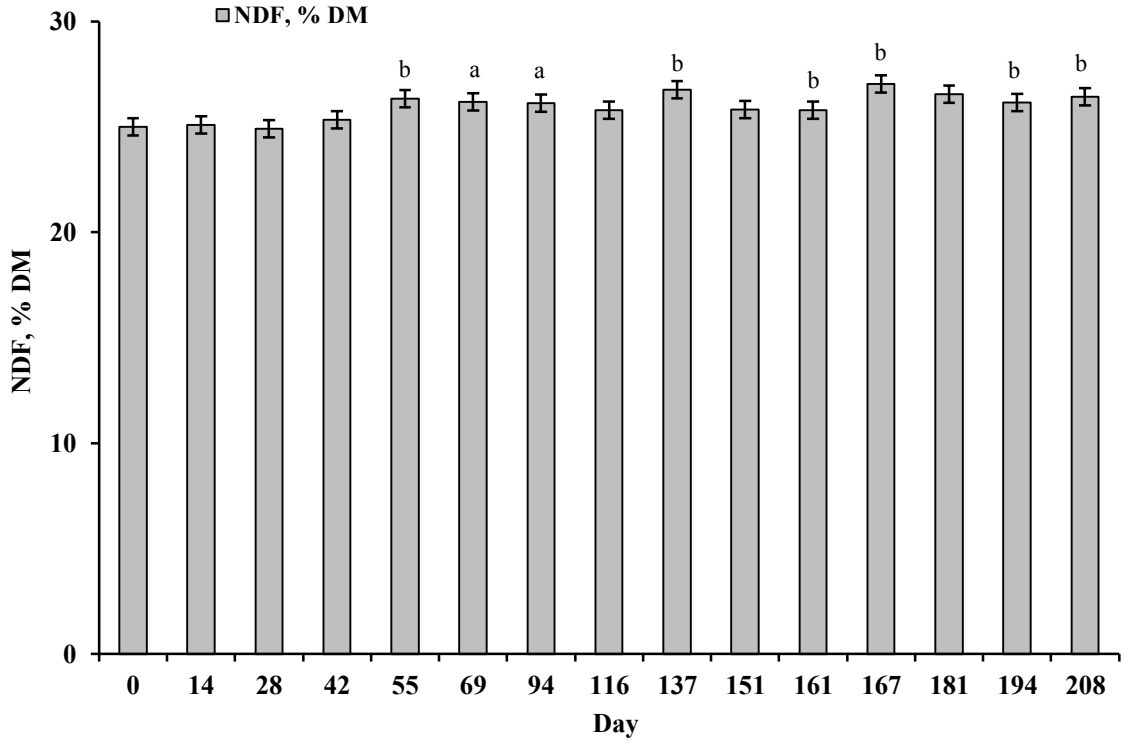

Figure 2. Effect of storage on NDF content (DM basis) of wet distiller's grain stored in concrete bunkers. [a] tended to be different from d 0 ($P < 0.10$). [b] different from d 0 ($P < 0.05$).

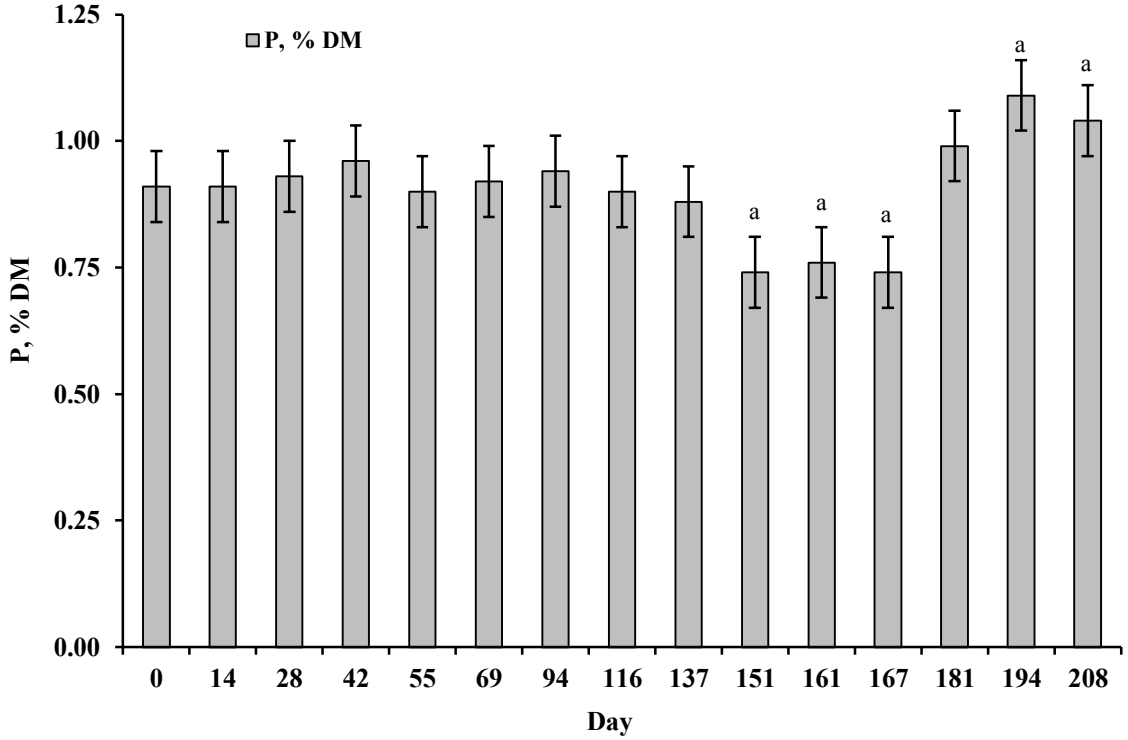

Figure 3. Effect of storage on P content (DM basis) of wet distiller's grain stored in concrete bunkers.
[a] tended to be different from d 0 ($P \leq 0.18$)

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EFFECTS OF FOUR LEVELS OF ZERANOL IMPLANTS ON LAMB GROWTH, CARCASS CHARACTERISTICS, NITROGEN BALANCE, AND BLOOD HORMONES[1]

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ABSTRACT: The objective of this research was to compare the growth performance, carcass characteristics, blood hormones, and nitrogen balance of lambs implanted with increased dosages of zeranol. One-hundred forty-four crossbred lambs (29.6 ± 0.3 kg BW) were utilized in a completely randomized design and placed into sixteen feedlot pens (4 pens/treatment) for a 116 d finishing study. Lambs were fed a 84.7% corn and 15.3% market lamb pellet (DM basis) ration ad libitum. Treatments were 0, 12, 24, and 36 mg zeranol (Ralgro, Schering-Plough), and lambs were implanted according to treatment on d 0. Lambs were weighed and feed refusals collected d 28, 56, 82, and 116. Blood samples were collected on d 0, 28, 56, 70, 82, 99, and 116 from 64 lambs (29.6 ± 2.1 kg BW) (subsample of 4 lambs per pen) and analyzed for thyroxine, triiodothyronine, and IGF-1. Thirty lambs (67.6 ± 3.4 kg BW) and 96 lambs (65.8 ± 5.1 kg BW) were harvested on d 84 and d 118, respectively. Carcass data were collected 24 h post-chill. A nitrogen balance study was also conducted to compare the effects of 0, 12, 24, or 36 mg zeranol on nitrogen balance in 16 crossbred lambs (34.8 ± 2.1 kg BW). There were no differences among treatments for BW, ADG, DMI, and G:F ($P \geq 0.33$) in the feedlot study. However, there was a linear increase for percent prolapse ($P = 0.006$); subsequently, there was also a linear increase in percent mortality ($P = 0.005$). Carcass characteristics, hormone concentration, nitrogen balance, and serum urea-nitrogen were not affected by treatment ($P \geq 0.07$). These results indicate zeranol increases percent prolapse and mortality, without increasing growth performance; therefore, its use in feedlot lambs is not recommended.

Key Words: hormones, lamb, nitrogen, zeranol

Introduction

Zeranol has been shown to improve growth performance in lambs implanted with 12 mg once (Field et al., 1993; Stultz et al., 2001; Salisbury et al., 2007), twice (Nold et al., 1992), or 3 and 5 times (Hufstedler et al., 1996) during the feeding period. Most research indicates zeranol does not alter carcass characteristics (Olivares and Hallford, 1990; Hutcheson et al., 1992; Salisbury et al., 2007). Zeranol has also been implicated in an increased incidence of prolapse (Arnsperger et al., 1976; Salisbury et al., 2007), resulting in decreased use of zeranol in the United States (Lupton, 2008). However, as many as half of market lambs fed in Mexico are implanted with zeranol (Amaya, G., personal communication). Currently, the effects of zeranol on growth performance, carcass characteristics, N balance, and the hormones triiodothyronine (**T3**), thyroxin (**T4**), and IGF-1 are unclear. Our objective for this study was to determine the effects of increasing dosages of zeranol on lamb growth performance, incidence of prolapse and mortality, carcass characteristics, blood hormones, and nitrogen balance. Three studies were performed to achieve this objective. The hypothesis tested was fourfold: lambs implanted with increased dosages of zeranol would have: 1) improved growth performance without altering carcass quality; 2) increased incidence of prolapse and mortality; 3) increased concentrations of T3, T4, and IGF-1; and 4) improved nitrogen balance.

Materials and Methods

All experimental protocols were approved by the North Dakota State University Animal Care and Use Committee prior to the initiation of the studies.

Study 1

The objective of this study was to determine the influence of increasing dosage of zeranol on lamb growth performance, incidence of prolapse and mortality, and carcass characteristics. Lambs were adapted to a diet which consisted of 84.7% corn and 15.3% commercial market lamb pellet (DM basis, Table 1) from a 100% creep pellet diet following weaning. Lambs were vaccinated for *clostridium perfringens* types C and D, as well as for tetanus (CD-T; Bar Vac CD-T, Boehringer Ingelheim,

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Ridgefield, CT). One hundred forty four spring-born crossbred lambs (wethers and ewes; initial BW 29.6 ± 0.3 kg; approximate age = 100 d) were stratified by weight and sex and assigned randomly to 1 of 16 pens (9 lambs/pen). Pens were assigned randomly to 1 of 4 treatments, with pen serving as experimental unit (n = 4 per treatment). Treatments were 0, 12, 24, or 36 mg/hd zeranol implant. Treatments were applied in a completely randomized design to evaluate the outlined objectives.

Lambs were implanted with zeranol (Ralgro, Schering-Plough Animal Health Corp., Union, NJ) according to treatment on d 0. Lambs were offered feed ad-libitum via bulk feeders and had continuous access to clean, fresh water and shade. Lambs which rectally or vaginally prolapsed were treated using techniques best-suited for each incidence, including the use of sutures, oxytetracycline, and general antibiotics.

The study was divided into four periods: d 0-28, d 29-56, d 57-82, and d 83-116. Lambs were weighed two consecutive days at initiation (d -1 and 0) of the trial and on d 82 and 116; single day weights were taken 28 and 56. Bulk feeders were emptied at the end of each period, and orts were weighed, sampled (approximately 2.0 kg), and dried to calculate period DMI. Ration and feed ingredient samples (approximately 2.0 kg) were collected every 28 d and analyzed for DM, OM, ADF, NDF, and N. Thirty (67.6 ± 3.4 kg BW) and 96 lambs (65.8 ± 5.1 kg BW) were harvested on d 84 and d 118, respectively, at Iowa Lamb Corporation (Hawarden, IA). Carcass data were collected 24 h post chill by trained university personnel. Data for lambs too light for shipment were included in growth performance analyses, but not carcass analyses.

Study 2

The objective of this study was to determine the influence of increasing dosage of zeranol on lamb T3, T4, and IGF-1 concentrations and T4:T3 ratio. Sixty four lambs (29.6 ± 2.1 kg BW; 2 wethers and 2 ewes per pen) from Study 1 were selected based on weight and sex to conduct a blood hormone study. Blood samples were collected via jugular venipuncture on d 0, 28, 56, 70, 82, 99, and 116 via serum separator Vacutainers (VWR, catalogue no. 14219-242). Vacutainers were placed on ice until transport to lab, refrigerated for 2 hours (2°C), centrifuged (3640 x g, 20 min) at room temperature, and serum was harvested and stored (-20°C). Serum samples were analyzed for T3 and T4 by chemiluminescence immunoassay using the Immulite 1000 system (Siemens Healthcare Diagnostics Inc., Los Angeles, CA). The intraassay CV were 4.33 and 4.07% for T3 and T4, respectively, and the interassay CV were 6.89 and 6.63% for T3 and T4, respectively. In addition, the T4:T3 ratio was calculated. Serum IGF-1 was quantified using the double antibody RIA (Berrie et al., 1995). The intraassay and interassay CV for IGF-1 were 12.8% and 13.8%, respectively.

Study 3

The objective of this study was to determine the influence of increasing dosage of zeranol on lamb N balance and serum urea-N concentration. Sixteen crossbred wethers (34.9 ± 2.1 kg BW) were utilized in a completely randomized design. Wethers were weighed on d 0 and 1, stratified by weight, and allotted randomly to treatments (n = 4 wethers/treatment) of 0, 12, 24 or 36 mg zeranol implants. Lambs were implanted with zeranol according to treatment and assigned randomly to individual metabolism crates on d 0. Wethers were housed in an enclosed room with lighting from approximately 0730 to 2000. Lambs were adapted to diets and processed as outlined in Study 1, but were also dewormed with ivermectin (Ivomec; Merial Limited, Duluth, GA). Rations were the same as Study 1 (Table 1), and were provided daily at 0830 at 130% of the average daily intake for the previous 5 d. Feed refusals from the previous day were determined prior to feeding.

The experimental period was 21 d. Dry matter intake was determined on d 14-20. Additionally, samples of corn, pellets, and the complete ration were collected on d 14-20 and dried at 55°C for 48 hours to determine DM. Orts were collected on d 15-21 and dried at 55°C for 48 hours. Total fecal and urine output were collected on d 16-21. A subsample of each daily fecal sample (7.5% of total, wet basis) was dried at 55°C for 96 h for calculation of fecal DM. Urine was composited daily by wether (10% of total; wet basis) and stored at 4°C. Sufficient 6 *N* HCL (100mL) was added daily to urinals to maintain urine pH < 3. Approximately 288 g of urine were collected from each urine subsample and stored at 4°C. On d 15-21, 10 mL of blood were collected via jugular venipuncture 4 h after feeding using Vacutainers (VWR, catalogue no. VT6430). Blood was cooled at 4°C for 2 h, centrifuged (3640 x g, 20 min), and serum harvested and stored (-20°C).

Dried fecal samples were ground through a Wiley mill (2-mm screen) and composited by lamb. Daily samples of corn, pellets, and the complete ration were composited for the collection period, and orts were composited by lamb on an equal weight basis (20%; as fed basis). Feed, orts, and fecal samples were analyzed for DM, OM, NDF, and ADF. Feed, orts, fecal, and urine samples were analyzed for N. Serum samples were analyzed for urea-N.

Statistical Analyses

Lamb performance, carcass, and blood hormone data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with pen serving as experimental unit, with missing data points from underweight lambs not included in the carcass data set. Repeated measures was used to analyze day effects for T3, T4, T4:T3, and IGF-1. The model included treatment, day, and day x treatment interaction. The covariance structure used was Compound Symmetry. Lamb N balance data were analyzed as a completely randomized design using the MIXED procedure of SAS with animal serving as experimental unit. Repeated measures was used to analyze day effect for serum urea nitrogen. The model included treatment, day, and day x treatment interaction. The covariance structure used was Autoregressive (1). When a significant *F*-test was observed ($P \leq 0.05$), linear, quadratic, and cubic contrasts were utilized to partition treatment effects with a critical *P*-value of ≤ 0.05.

Results and Discussion

Study 1

No differences were observed among treatments for final BW, ADG, DMI, or G:F (Table 2; $P \geq 0.33$). This agrees with some of the previous research that found no effect of zeranol implant on ADG (Wiggins et al., 1976; Nold et al., 1992; Field et al., 1993) or gain efficiency (Wiggins et al., 1976). However, the majority of the research indicates implanting feedlot lambs with 12 mg zeranol increases ADG (Hutcheson et al., 1992; Stultz et al., 2001; Salisbury et al., 2007) and G:F (Olivares and Hallford, 1990; Field et al., 1993; Stultz et al., 2001). Domínguez et al. (2010) reported lambs implanted with 24 mg zeranol had increased ADG and G:F compared with lambs implanted with 12 mg zeranol or lambs which were not implanted. In the present study, prolapsed lambs went off feed and this may have resulted in no differences among growth performances of lamb implanted with increased dosages of zeranol.

As zeranol dose increased, percent prolapse increased linearly ($P = 0.006$). Similarly, percent mortality also increased linearly ($P = 0.005$). Few studies (Arnsperger et al., 1976; Sluiter et al., 2007; Salisbury et al., 2007) have reported complications of prolapse resulting from zeranol implants, and no studies have reported problems with mortality. Studies examining the use of 2, 3, or more zeranol implants did not report any incidences of prolapse (Nold et al., 1992; Hufstedler et al., 1996). The finishing diets utilized by Sluiter et al. (2007), Salisbury et al. (2007), and the present study were high in concentrate (>70%). Anecdotal information suggests as much as 50% of Mexican feeder lambs are implanted with zeranol and raised on high forage diets, and do not experience the rate of prolapse that was observed in the present study (Amaya, G., personal communication). Arnsperger et al. (1976) noted that zeranol-implanted lambs raised on pasture did not experience the rate of prolapse observed in implanted lambs fed in drylot conditions. Salisbury et al. (2007) implicated high concentrate rations in contributing to increased incidence of prolapse. However, the increased incidence of prolapse reported in previous studies did not decrease growth performance (Arnsperger et al., 1976; Salisbury et al., 2007), as we believe to be the case in the present study.

There were also no differences ($P \geq 0.07$) in carcass characteristics in the present study. Zeranol has been shown to increase carcass weight, (Wilson et al., 1972; Stultz et al., 2001), decrease dressing percentage (Wiggins et al., 1979), and increase fat depth (Field et al., 1993). However, the majority of research indicates no effect of zeranol on carcass characteristics (Wiggins et al. 1976; Olivares and Hallford, 1990; and Sluiter et al., 2007).

Study 2

No differences were observed among treatments for T3, T4, and IGF-1 concentrations or T4:T3 ratio (Table 3; $P \geq 0.34$). Zeranol did not affect T4 in previous research with cattle (Price et al., 1983; Gopinath and Kitts, 1984; Doornenbal et al., 1987), but a time x implant interaction has been reported in implanted steers and bulls (Gray et al., 1986). Zeranol decreased T3 concentrations in implanted steers compared with non-implanted (Williams et al., 1991). Zeranol decreased T4 in lambs 42 d after implantation (Wiggins et al., 1979).

Rate of gain in steers is positively correlated with plasma concentrations of IGF-I (Ellenberger et al., 1989; Hersom et al., 2004). Zeranol increases IGF-1 and GH in lambs (Hufstedler et al., 1996; Thomas et al., 2000). Hammond et al. (1990) suggested IGF-1 concentration is related to nutritional status. As such, the increased incidence of prolapsed lambs going off feed may have affected the IGF-1 levels in lambs implanted with 24 and 36 mg zeranol.

Study 3

There were no differences among treatments for N intake, N excretion, N balance, or serum urea-N (Table 4; $P \geq 0.09$). Hufstedler and Green (1995) observed decreased urinary N excretion in lambs implanted with 12 mg zeranol, but N retention and absorption were not affected. Griffiths (1982) observed increased N retention in steers implanted with 36 mg resorcylic acid lactone. Sharp and Dyer (1971) also observed increased N retention in lambs implanted with 12 mg zeranol compared to non-implanted lambs. VanderWal et al. (1975) performed several studies analyzing N retention and found 36 mg zeranol increases N retention in beef calves (95-100 kg), while 72 mg zeranol did not improve N retention. These results could indicate response to zeranol is not dose dependent.

Implications

No differences were noted in growth performance, carcass characteristics, blood hormone concentrations, or N balance in lambs implanted with increasing dosages of zeranol. However, as zeranol dosage increased, incidence of prolapse and mortality increased linearly. Based on our research, we do not recommend increased dosage of zeranol for use in feedlot lambs fed high concentrate rations.

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Table 1. Ingredient and nutritional composition of diet fed to lambs implanted with increasing dosage of zeranol

Item	DM basis
Ingredient, %	
Whole Corn	84.7
Market Lamb Pellet[1]	15.3
Nutrient composition (analyzed)	
OM, %	95.41
CP, %	13.12
NDF, %	13.47
ADF, %	3.41

[1]Market Lamb Pellet contained: 0.22 g/kg chlortetracycline, 38% CP, 4.25% Ca, 0.6% P, 3.5% salt, 1.2 mg/kg Se, 52,920 IU/kg Vitamin A, 5,292 IU/kg Vitamin D, and 209 IU/kg Vitamin E.

Table 2. Effects of increasing dosage of zeranol on lamb growth performance, carcass characteristics, and health

	Treatment[1]						*P*-value[2]		
Item	0	12	24	36	SEM[3]	*P*-value	Linear	Quadratic	Cubic
Initial BW, kg	29.5	29.4	29.7	29.9	0.3	0.53	0.20	0.59	0.67
Final BW, kg	65.5	65.5	65.4	66.0	1.3	0.98	0.77	0.81	0.88
ADG, kg	0.34	0.33	0.32	0.30	0.02	0.64	0.23	0.75	0.90
Intake, kg DM·hd^{-1}·d^{-1}	1.70	1.68	1.74	1.74	0.05	0.77	0.42	0.85	0.53
G:F	0.22	0.22	0.21	0.20	0.01	0.33	0.10	0.53	0.84
Prolapse, %	2.78	5.55	24.98	27.75	6.31	0.03	0.006	1.00	0.26
Mortality, %	0.00	5.55	11.10	13.88	3.10	0.04	0.005	0.66	0.84
HCW, kg	34.0	33.4	33.4	33.2	0.58	0.81	0.35	0.89	1.00
Leg Score[4]	11.8	12.0	12.0	12.3	0.18	0.31	0.08	1.00	0.54
Conformation Score[4]	11.5	12.0	12.0	12.0	0.14	0.07	0.04	0.11	0.45
Fat Depth, cm[5]	2.49	2.49	2.74	2.62	0.08	0.14	0.11	0.45	0.11
Body Wall Thickness, cm	0.74	0.76	0.84	0.76	0.05	0.62	0.49	0.40	0.46
Ribeye Area, cm^2	17.7	17.3	16.3	16.5	0.50	0.17	0.05	0.52	0.47
Flank Streaking[6]	378.75	355.75	376.25	352.00	10.94	0.25	0.25	0.96	0.10
Quality Grade[4]	12.3	12.0	12.0	12.0	0.1	0.43	0.20	0.34	0.66
Yield Grade[7]	3.25	3.38	3.70	3.40	0.24	0.62	0.49	0.40	0.46
BCTRC, %[8]	45.58	45.43	44.70	45.05	0.36	0.35	0.18	0.50	0.33
Dressing, %	50.73	50.73	50.83	50.25	0.40	0.74	0.47	0.48	0.67

[1]Treatments: 0 (0 mg zeranol (Ralgro, Schering-Plough Animal Health Corp., Union, NJ) implant), 12 (12 mg zeranol implant), 24 (24 mg zeranol implant), 36 (36 mg zeranol implant).

[2]*P* – value for linear, quadratic, and cubic effects of increasing dosage of zeranol.

[3]Standard Error of Mean; n = 4.

[4]Leg score, conformation score, and quality grade: 1 = cull to 15 = high prime.

[5]Adjusted fat depth and yield grades.

[6]Flank streaking: 100-199 = practically devoid; 200-299 = traces; 300-399 = slight; 400-499 = small; 500-599 = modest.

[7]Yield Grade = 0.4 + (10 x adjusted fat depth, in).

[8]Boneless closely trimmed retail cuts, % = (49.936 – (0.0848 x 2.205 x HCW, kg) - (4.376 x 0.3937 x fat depth, cm) – (3.53 x 0.3937 x body wall thickness, cm) + (2.456 x 0.155 x ribeye area, cm^2)).

Table 3. Effects of increasing dosage of zeranol on triiodothyronine, thyroxin, and IGF-1 concentration and T4:T3 in lambs

	Treatment[1]						*P*-value[2]		
Item	0	12	24	36	SEM[3]	*P*-value	Linear	Quadratic	Cubic
Triiodothyronine, ng/mL[4]	1.97	1.85	2.03	1.91	0.08	0.38	0.95	0.98	0.07
Thyroxin, ng/mL[5]	94.6	94.4	97.0	89.9	4.8	0.77	0.60	0.49	0.57
Thyroxin : Triiodothyronine[6]	48.7	51.9	48.0	48.1	2.5	0.65	0.62	0.55	0.33
IGF-1, ng/mL[7]	327	301	276	314	20	0.34	0.48	0.13	0.49

[1]Treatments: 0 (0 mg zeranol (Ralgro, Schering-Plough Animal Health Corp., Union, NJ) implant), 12 (12 mg zeranol implant), 24 (24 mg zeranol implant), 36 (36 mg zeranol implant).
[2]*P* – value for linear, quadratic, and cubic effects of increasing dosage of zeranol.
[3]Standard Error of Mean; n = 4.
[4]*P*-values for triiodothyronine Treatment ($P = 0.31$), Day ($P < 0.001$) Treatment x Day ($P = 0.08$).
[5]*P*-values for thyroxine Treatment ($P = 0.77$), Day ($P < 0.001$) Treatment x Day ($P = 0.29$).
[6]*P*-values for thyroxine:triiodothyronine Treatment ($P = 0.65$), Day ($P < 0.001$) Treatment x Day ($P = 0.46$).
[7]*P*-values for IGF-1 Treatment ($P = 0.34$), Day ($P < 0.001$) Treatment x Day ($P = 0.13$).

Table 4. Effects of increasing dosage of zeranol on N intake, excretion, balance, and serum urea N concentration in lambs

	Treatment[1]						*P*-value[2]		
Item	0	12	24	36	SEM[3]	*P*-value	Linear	Quadratic	Cubic
Daily DMI, g/kg BW	20.8	19.9	18.2	19.1	2.0	0.30	0.47	0.66	0.70
Daily N intake, g/kg BW	0.425	0.381	0.380	0.412	0.053	0.90	0.88	0.48	0.96
Daily N Excretion, g/kg BW									
Fecal	0.117	0.109	0.114	0.106	0.014	0.94	0.65	0.99	0.69
Urinary	0.080	0.062	0.073	0.064	0.006	0.16	0.17	0.44	0.09
Daily N Balance, g/kg BW	0.227	0.210	0.192	0.242	0.041	0.84	0.88	0.42	0.72
Serum urea-N, m*M*[4]	4.14	2.89	3.44	3.07	0.38	0.09	0.09	0.20	0.09

[1]Treatments: 0 (0 mg zeranol (Ralgro, Schering-Plough Animal Health Corp., Union, NJ) implant), 12 (12 mg zeranol implant), 24 (24 mg zeranol implant), 36 (36 mg zeranol implant).
[2]*P* – value for linear, quadratic, and cubic effects of increasing dosage of zeranol.
[3]Standard Error of Mean; n = 4.
[4]*P*-values for serum urea-N Treatment ($P = 0.09$), Day ($P = 0.57$) Treatment x Day ($P = 0.96$).

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FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF CALVES FROM DAMS WITH DIFFERENT LEVELS OF WINTER SUPPLEMENTATION DEVELOPED WITH OR WITHOUT FEED RESTRICTION DURING THE POSTWEANING PERIOD[1]

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ABSTRACT: The objective of this research was to evaluate the impacts of 2 levels of supplemental feed provided to cows during late gestation and 2 levels of feed provided to their sons during postweaning development on subsequent feedlot performance and carcass characteristics. Bull calves (n = 56) were born from dams receiving adequate (1.8 kg/d; **ADEQ**) or marginal (1.2 kg/d; **MARG**) winter supplementation. After weaning, bulls were developed on ad-libitum (**Control**) or 27% less feed (**Restricted**) for ~140 d. Bulls were then band-castrated and placed on an 80% corn finishing diet ad libitum. Individual intakes were measured with a GrowSafe system for the final ~100 days of the finishing period. Cattle were harvested at commercial packing plant and carcass data were collected. The analysis of variance model included dam winter supplementation, bull postweaning treatment, and their interaction. Restricted calves gained less ($P < 0.01$) during the postweaning phase than Control calves (0.63 vs 1.16 ± 0.03 kg/d, respectively). Postweaning treatment did not impact feed intake during the finishing phase ($P = 0.30$; 13.0 vs 12.6 ± 0.34 kg/d for Restricted and Control, respectively; as-fed basis). However, ADG during the finishing phase exhibited a postweaning × dam treatment interaction ($P = 0.03$), where Restricted steers from MARG dams gained the most (1.55 ± 0.05 kg/d) and Control steers from MARG dams gained the least (1.26 ± 0.05 kg/d). Steers from ADEQ dams were intermediate (1.45 and 1.36 ± 0.05 kg/d for Restricted and Control). Restricted and Control steers had similar ($P \geq 0.63$) final BW (601 vs 622 ± 7 kg), HCW (357 vs 374 ± 5 kg), back fat thickness (1.12 vs 1.19 ± 0.05 cm), ribeye area (86.5 vs 88.4 ± 1.35 cm^2), intramuscular fat percentage (5.86 vs 5.69 ± 0.21%),and yield grade (2.69 vs 2.81 ± 0.08). Calves restricted during postweaning development gained more efficiently, and had similar carcass characteristics to their ad libitum-fed counterparts.

Key words: Postweaning development, uterine programming, finishing

[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 authors and does not imply its approval to the exclusion of other products that may be also suitable.

Introduction

Harvested feedstuffs are a major input cost for range-based cow-calf producers. A long term study at Fort Keogh has evaluated the influence of 2 levels of nutritional input during heifer development and winter supplementation on lifetime productivity in beef females (Roberts et al., 2009). Dietary treatments imposed on cows in this experiment resulted in a uterine programming effect in their heifers (Roberts et al., 2010). Bull calves in this experiment also received 2 levels of nutritional input during the postweaning period, but little work has been done to assess feedlot performance and carcass characteristics of these calves. Therefore, the objective of this research was to evaluate the impacts of 2 levels of supplemental feed provided to cows during late gestation and 2 levels of feed provided to their sons during postweaning development on subsequent feedlot performance and carcass characteristics.

Materials and Methods

Research protocols were approved by the Fort Keogh Livestock and Range Research Laboratory Animal Care and Use Committee. Cattle used in this study were a stable composite population (**CGC**; ½ Red Angus, ¼ Charolais, ¼ Tarentaise). Beginning in 2001, cows in this herd were randomly assigned to be fed levels of harvested feed from December to March of each year that were expected to result in either marginal (**MARG**) or adequate (**ADEQ**) nutrition while grazing dormant winter forage through this period, based on average quality and availability of winter forage (Roberts et al., 2009). Each group of cows was managed on separate pastures during the winter to allow differential feeding. Cows were supplemented with alfalfa hay every other day for 72 d (Dec 6 to Feb 17). Amount fed was equivalent to 1.84 or 1.13 kg hay/day for each ADEQ or MARG cow, respectively. Cows were then transferred to small calving pastures and fed 10.25 (ADEQ) or 8.06 (MARG) kg alfalfa hay/cow each day.

Bull calves (n = 203) born from ADEQ or MARG dams during spring 2009 (avg birth date = Apr 9) were allotted by weight at weaning to 6 pens (n = 33 or 34 per pen). Bulls were fed ad-libitum (65% silage, 10% barley, 10% corn, 10% ground alfalfa hay, and 5% supplement, as fed) for the first 56 d postweaning, at which time 3 pens were assigned to continue being fed ad libitum (**Control**) and 3 pens to be fed 80% (as-fed basis) of that consumed

while fed ad-libitum (**Restricted**). Bulls were weighed every 28 d and amount of feed provided to Restricted bulls were adjusted to be 80% of that consumed by Control bulls on a common BW basis. Feeding treatments were imposed for 140 d. The diet during this period consisted of 65% silage, 20% corn, 10% ground alfalfa hay, and 5% supplement, as fed. At the end of the 140-d postweaning treatment, ultrasound measurements of LM area, back fat, and percent intramuscular fat were taken as described by Roberts et al. (2007), using an Aloka SSD-500 ultrasound equipped with a 17.2 cm, 3.5 MHz, linear array transducer (Aloka Co. Ltd., Wallingford, CT) and the Beef Image Analysis software (Designer Genes Technologies LLC, Gustine, TX).

For the present study, 56 of the 203 bulls were band-castrated after the 140-d trial and placed on a corn finishing diet ad libitum (78.9% corn, 12.7% alfalfa hay, 4.2% silage, and 4.3% supplement, as fed). These bulls were selected from sires with offspring represented in each dam treatment and individual treatment classifications. Individual intakes were measured with a GrowSafe system for the final ~100 days of the finishing period, and steers were weighed every ~28 d. Cattle were harvested on Nov 18, 2010 at a commercial packing plant (Cargill, Fort Morgan, CO) and carcass data were collected by an independent company (Diamond T Livestock Services, Inc.).

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model for postweaning-phase measurements included dam winter supplementation treatment, postweaning treatment, their interaction, and dam age (2, 3, and 4+ years of age). Calf age was used as a covariate, and sire was a random effect. The model for feedlot phase measurements included dam winter supplementation treatment, postweaning treatment, their interaction, and dam age. Calf age, average feed intake and the interaction of postweaning treatment and feed intake were used as covariates. Sire was a random effect. Means were separated using PDIFF.

Results and Discussion

Restricted calves gained less ($P < 0.01$; Table 1) during the postweaning phase than Control calves and had lower BW, fat thickness, and intramuscular fat percentage at the end of the postweaning phase ($P \leq 0.03$). These results are similar to those observed in heifers with the same postweaning feed treatments at this location (Roberts et al., 2007), and similar to those observed by Hersom et al. (2004) with steers targeted for low and high rates of gain on wheat pasture.

Area of the LM (**LMA**) at the end of the postweaning phase exhibited a postweaning treatment × dam winter supplementation interaction ($P = 0.02$; Table 2), where Control bulls had the largest LMA regardless of dam treatment, Restricted bulls from MARG dams had the smallest LMA, and Restricted bulls from ADEQ dams were intermediate. Likewise, heifers from this herd developed with a similar postweaning restriction had smaller LMA than heifers developed on an ad libitum diet (Roberts et al, 2007). Bulls born to MARG dams weighed less (P = 0.04) at the end of the postweaning period than bulls born to ADEQ dams (366 vs 383 ± 7.3 kg for MARG and ADEQ, respectively). Larson et al. (2009) also found that different dam nutritional treatments during late gestation impacted weights of steer progeny at weaning and feedlot entry. Dam winter supplementation treatment did not impact any other measurements ($P \geq 0.19$).

Postweaning treatment did not impact feed intake during the finishing phase ($P = 0.30$; Table 1). However, ADG during the finishing phase exhibited a postweaning × dam treatment interaction ($P = 0.03$; Table 2), where Restricted steers from MARG dams gained the most and Control steers from MARG dams gained the least, while steers from ADEQ dams were intermediate. Interestingly, ranking of the postweaning × dam treatment means for ADG during the finishing phase were inverse from those observed for LMA taken at the end of postweaning treatment (Table 2). Furthermore, differences observed in ADG during the finishing phase resulted in similar ($P = 0.73$; Table 1) final BW for both postweaning treatments. Restricted and Control steers had similar ($P \geq 0.63$) final BW, HCW, back fat thickness, LMA, intramuscular fat percentage, and yield grade. These results are much like those observed by Hersom et al. (2004) where steers were targeted for high or low gain on wheat pasture, with the exception of ADG during the finishing phase and final BW. Hersom et al. (2004) found similar weight gains during finishing regardless of rate of gain on wheat pasture, which resulted in lower final BW for low-gain steers.

Bulls from 2-yr-old dams tended (P = 0.08; Table 3) to have higher ADG during the postweaning phase. Age of dam impacted BW, LMA, and back fat thickness at the end of the postweaning phase ($P \leq 0.05$). Progeny of 2- and 3-yr-old cows weighed less at the end of the postweaning phase than those from mature cows. Likewise, LMA for calves from 2- and 3-yr-old dams were smaller than for calves from mature dams. Back fat thickness at the end of the postweaning period was lowest for calves from 3-yr-old cows, greatest for calves from mature cows, and intermediate for calves from 2-yr-old dams. Intramuscular fat percentage was similar ($P = 0.23$) at the end of the postweaning phase for all bulls, regardless of dam age.

Age of dam did not impact intake, ADG, or final BW during the finishing phase ($P \geq 0.23$), nor did it impact HCW, LMA, intramuscular fat percentage, or yield grade ($P \geq 0.15$). However, as was observed at the end of the postweaning phase, age of dam did influence carcass back fat thickness ($P < 0.01$), which was lowest for calves from 3-yr-old cows, greatest for calves from mature cows, and intermediate for calves from 2-yr-old cows.

Calves restricted during postweaning development gained more efficiently, and had similar carcass characteristics to their ad libitum-fed counterparts.

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Table 1. Postweaning treatment impacts on growth, feedlot performance, and carcass characteristics.

	Postweaning Treatment[a]				
Item	Restricted	SE	Control	SE	*P*-value
Postweaning phase					
ADG, kg	0.63	0.02	1.16	0.03	< 0.01
Final BW, kg	335	6	414	7	< 0.01
Fat thickness, cm[b]	0.25	0.02	0.38	0.02	< 0.01
Intramuscular fat percentage[b]	2.92	0.08	3.17	0.09	0.03
Finishing phase and carcass characteristics					
Feed intake (as-fed), kg/d	13.0	0.30	12.6	0.75	0.30
Final BW, kg	601	6	622	7	0.73
HCW, kg	357	4	374	5	0.67
Back fat thickness, cm	1.12	0.03	1.19	0.05	0.93
Longissimus muscle area, cm^2	86.5	1.16	88.4	1.35	0.93
Intramuscular fat percentage	5.86	0.19	5.69	0.21	0.63
Yield grade	2.69	0.07	2.81	0.08	0.70

[a]140-day postweaning treatment. Control animals fed ad libitum, Restricted animals fed 80% of ad libitum intake at similar BW.

[b]Measured via ultrasound at the end of the 140-d postweaning period.

Table 2. Dam winter supplementation treatment × postweaning treatment interactions for LM area at the end of the postweaning period ($P = 0.02$) and for average daily gain during the finishing phase ($P = 0.03$).

	Dam Winter Supplementation Treatment							
	MARG				ADEQ			
	Postweaning Treatment							
Item	Restricted	SE	Control	SE	Restricted	SE	Control	SE
End postweaning LM area, cm^2 [d]	64.5[a]	1.68	80.6[c]	1.94	69.0[b]	1.55	78.1[c]	1.74
Finishing phase ADG, kg/d	1.55[a]	0.05	1.26[c]	0.05	1.45[ab]	0.04	1.36[bc]	0.03

[a,b,c] Means in the same row with different superscripts differ $P \leq 0.05$.

[d] Measured via ultrasound at the end of the 140-d postweaning period.

Table 3. Dam age impacts on growth, feedlot performance, and carcass characteristics.

	Dam Age						
Item	2	SE	3	SE	4+	SE	*P*-value
Postweaning phase							
ADG, kg	0.96	0.04	0.85	0.04	0.87	0.02	0.08
Final BW, kg	356[a]	9.3	367[a]	9.7	400[b]	6.4	< 0.01
Back fat thickness, cm[c]	0.33[ab]	0.03	0.28[a]	0.03	0.36[b]	0.02	0.05
Ribeye area, cm^2 [c]	71.0[a]	1.81	71.0[a]	1.94	76.8[b]	1.23	< 0.01
Intramuscular fat percentage[c]	3.07	0.12	2.92	0.13	3.16	0.07	0.23
Finishing phase and carcass characteristics							
Feed intake (as-fed), kg/d	12.3	0.44	12.9	0.47	13.2	0.29	0.23
ADG, kg	1.42	0.05	1.41	0.05	1.39	0.03	0.81
Final BW, kg	611	8.9	604	9.4	619	5.6	0.38
HCW, kg	365	6.0	359	6.3	372	3.8	0.18
Back fat thickness, cm	1.14[ab]	0.05	1.04[a]	0.05	1.27[b]	0.03	< 0.01
Longissimus muscle area, cm^2	87.0	1.74	86.8	1.87	88.4	1.16	0.71
Intramuscular fat percentage	6.14	0.27	5.46	0.28	5.72	0.19	0.16
Yield grade	2.76	0.11	2.61	0.12	2.88	0.07	0.15

[a,b] Means in the same row with different superscripts differ $P \leq 0.05$.

[c]Measured via ultrasound at the end of the 140-d postweaning period.

EFFECT OF LEVEL OF WET DISTILLER GRAINS AND ORGANIC COPPER SUPPLEMENTATION ON VISCERAL ORGAN MASS, AND INTESTINAL CELLULARITY AND VASCULARITY IN FINISHING BEEF STEERS

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ABSTRACT: Thirty-two steers (401 ± 50 kg initial BW) were used to determine effects of dietary wet distillers grains (WDG) level (0, 30, and 60%, DM basis) and 30% WDG plus Cu on visceral tissue mass, intestinal cell growth, and intestinal cellularity and vascularity. Because S reduces Cu absorption in the gut, high-S distillers grains diets may cause Cu deficiency. Diets were offered daily to individual steers in an electronic feeding system. After 84 to 112 d, steers were slaughtered and individual visceral tissue weights determined. Visceral organ weights of duodenum, jejunum, small intestine, large intestine, stomach complex, and liver mass were not affected ($P \geq 0.13$) by treatment. Conversely, ileal mass tended ($P = 0.07$) to decrease linearly and mesenteric mass tended ($P = 0.07$) to increase quadratically with increasing WDG level. Expressing visceral organ mass as g/kg of empty BW, resulted in a linear ($P \geq 0.04$) increase of stomach complex and liver mass with increasing WDG level. Also, ileal and mesentery mass (g/kg of empty BW) tended ($P = 0.09$) to decrease with increasing WDG level. There were no Cu effects ($P \geq 0.24$) on the total amount of DNA, RNA, and protein in the small intestinal tissue. Total amount of ileal DNA ($P = 0.07$), ileal RNA ($P = 0.10$), and jejunal protein ($P = 0.05$) tended to decrease linearly with the increasing levels of WDG. Cell proliferation in jejunal tissue ($P = 0.50$), vascularity ($P = 0.78$) or total microvascular volume ($P = 0.95$) were not affected by treatment. Conversely, the total number of proliferating jejunal cells tended to be greater ($P = 0.09$) for steers receiving 30% WDG than those receiving 30% WDG plus organic Cu. There were not major differences of visceral tissue mass, intestinal cell growth, or intestinal cellularity. Therefore, metabolic activity and/or energy used by visceral organs most likely were not affected by treatment.

Key words: cellular growth, WDG, intestine

Introduction

Inclusion of distillers grains in feedlot diets has increased rapidly in recent years because of increased availability and price competitiveness. Although distillers grains are low in starch, this by-product is high in readily digestible fiber, protein, fat, phosphorus (Klopfenstein et al., 2008), and sulfur (Uwituze et al., 2011). Compared with corn, distillers grains increase three-fold in fiber, protein, fat, and phosphorus content (Stock et al., 2000). Wet distiller grains have more energy per kilogram of DM than corn (Firkins et al., 1985; Larson et al., 1993; Trenkle, 1996). Although greater fat absorption and greater metabolizable protein might explain some of the greater feeding value of distiller grains compared to corn, not all the variation is explained by these two factors (Klopfenstein et al., 2008). The tissue of the viscera (liver and gut) has been estimated to account for 40 to 50% of whole-animal energy expenditure (Webster, 1981) and comprise only 6 to 10% of body weight (Burrin et al., 1990). The high metabolic activity of viscera is reflected by the high rate of cell proliferation and high vascualrity (Jin et al., 1994). Also, energy intake is closely related to size of these metabolically active visceral organs (Koong et al., 1982; Ferrell and Koong, 1986; Ferrell et al., 1986). Feeding distillers grain might cause an over consumption of S which reduces Cu absorption (Kincaid, 1988; Spears, 2003). Sulfer reduces Cu absorption because of the formation of copper sulfide in the gut (Suttle, 1974). Deficiency of Cu might cause the metabolism of visceral organs to slow down (Spears, 2003). Copper is essential component of a number of enzymes including lysyl oxidize, cytochrome oxidize, superoxide dismutase, ceruloplasmin, and tyrosinase (McDowell, 1992). However, data on visceral organ size and activity of cattle consuming distiller grains are not available. It is hypothesized that feeding distiller grains might impact visceral organ size and activity. Moreover, Cu supplementation might also impact organ size and activity of cattle consuming distiller grains. Therefore the objectives of this study were to evaluate effects of wet distiller grains and copper supplementation on visceral tissue mass, intestinal growth, and intestinal cellularity and vascularity.

Materials and Methods

Animals and Diets

Thirty-two steers (401 ± 50 kg initial BW) were utilized in a completely randomized block design. Animals were used in a previous receiving study, which they were dewormed, vaccinated, dehorned as needed, and ear tagged. Steers implanted with Revalor-XS (Intervet/Scheering–Plough Animal Health, Millsboro, DE) on day 1 of the experiment. Steers were randomly assigned one of four treatments consisting of different inclusion levels of wet distillers grains (WDG). Treatments were 0, 30, and 60% WDG with copper sulfate providing supplemental copper. In addition a fourth treatment was included that contained 30% WDG with a chelated copper source providing supplemental copper rather than copper sulfate. Experimental diets were based on steam-flaked corn, yellow grease and cottonseed meal were used to balanced fat and protein contents, respectively. Steers were trained

to use Calan Gate individual feeders (American Calan Inc., Northwood, NH) over a 21-day period before the initiation of the experiment. During the training period, steers consumed a common diet of 91% concentrate:9% roughage (DM basis; 0% WDG diet in Table 1). Animal care and use protocols were approved by the WTAMU Institutional Animal Care and Use Committee. Once training was completed, steers were blocked by body weight and assigned randomly within BW grouping to 1 of 4 treatment diets. Steers were fed individually until slaughter, and blocks of cattle (8 steers per block) were slaughtered approximately 30 days apart by body weight block.

Slaughter Procedure

Steers were fed 125 to 192 days depending on body weight block. The cattle were slaughtered at 1.3 cm of back fat, visual appraisal. Cattle were removed from feed and water for 24 hours before slaughter. Steers were slaughtered within a 30-day period based on BW block. Visceral organs were obtained immediately after evisceration. Empty body weight (BW minus digesta) was determined after removal of visceral organs. According to Soto-Navarro et al. (2004), intestinal segments (duodenum, jejunum, ileum) were obtained as follows. The duodenum was identified as the segment from the pylorus to a point directly adjacent to the entry of the gastrosplenic vein into the mesenteric vein. The jejunum was the segment from the caudal end of the duodenum to the junction of jejunum and ileum. This junction was determined by measuring 40-cm from the ileocecal junction cranially to the jejunum. This 40-cm section was considered the ileum. From the convergence of the mesenteric and ileocecal veins a 15-cm segment was measured up to the mesenteric arcade to the point of intestinal intersection. From this point, a 150-cm measurement was made caudally down the jejunum. The 150-cm section was immediately removed for vascular perfusion as described below. In the same direction another 150-cm section was obtained, to estimate the sections content and mass weight.

Laboratory Analysis

Jejunal tissue was used to evaluate small intestine vascularity or amount of blood vessels in proportion to the amount of tissue. Analysis of vascularity was conducted as described by Soto-Navarro et al. (2004). Duodenal, jejunal, and ileal samples were taken for protein, DNA, and RNA analysis as previously described (Scheaffer et al., 2003).Protein, DNA, and RNA analysis were conducted as described by Soto-Navarro et al. (2004). Jejunal samples of 5 to 10 g were taken from the same location as those for DNA, RNA, and protein, and were immediately fixed in 10% formalin and placed into paraffin blocks, sectioned at 5 µm, and then mounted on glass slides for analysis of cellular proliferation as previously described (Fricke et al., 1997; Scheaffer et al., 2003).

Statistical Analysis

Data were analyzed as a completely randomized block design with the PROC Mixed procedures of SAS (SAS Inst., Inc., Cary, NC). The model included the fixed effect of treatment and random effect of body weight block. Significance for all statistical comparisons was determined at $P < 0.10$. Polynomial orthogonal contrasts were used to test for linear and quadratic effects of increasing WDG inclusion in the diet. Also, a contrast was used to compare the diets containing 30% WDG with copper sulfate to the diet containing 30% WDG with chelated copper.

Results and Discussion

There were no copper effects ($P \geq 0.16$) on digestive organ mass. Wet distillers grains levels did not ($P \geq 0.13$) alter duodenum, jejunum, small intestine, large intestine, stomach complex (rumen, reticulum, omasum, abomasums), or liver mass. Ileal mass tended (198, 172, and 123 ± 33.8 g for 0, 30 and 60% WDG, respectively; $P = 0.07$) to decrease linearly and mesenteric fat tended (7,611, 8,581, and 5,368 ± 938.3 g for 0, 30, and 60% WDG, respectively; $P = 0.07$) to increase quadratically with increasing wet distillers grains. Because only the last 40 cm of the small intestine was considered to be the ileum, this small section might not have a significant contribution to total animal energy expenditure. Even though ileal tissue is an active tissue, it is not as active as jejunal tissue (Simon et al., 1982). In general, visceral organs are active metabolically and represent a very large amount of maintenance energy consumption (Caton et al., 2000). Specifically,the liver and gut represent approximately 40% of maintenance energy demands in the body (Huntington, 1990; Reynolds et al., 1991). Metabolic activity of an organ is the result of the organ's size and metabolic activity per unit of tissue. Visceral tissues use a substantial amount of energy in comparison to their contribution to the overall mass of the body (Ferrell, 1988; Reeds et al., 1999). Expressing visceral organ mass as grams per kilogram of empty BW, resulted in linear ($P \geq 0.04$) increase of stomach complex (32.0, 35.6, and 38.2 ± 2.0 g/kg of EBW for 0, 30, and 60% WDG, respectively) and liver mass (11.5, 12.4, and 13.2 ± 0.6 g/kg of EBW for 0, 30, and 60% WDG, respectively) with increasing wet distillers grain level. This represents that the organs were becoming larger in size and therefore requiring a greater energy expenditure which results in a greater maintenance energy requirement. Because liver and gut represents approximately 40% of energy consumption (Huntington, 1990; Reynolds et. al., 1991), expressing stomach complex and liver mass as grams per kilogram of BW might imply that energy efficiency decreases with increasing WDG level. Also, ileal (0.4, 0.3, and 0.2 ± 0.06 g/kg of EBW, for 0, 30, and 60% WDG) and mesentery mass (13.4, 14.3, and 9.7 ± 1.5 g/kg of EBW) tended ($P = 0.09$) to decrease with increasing wet distillers grain level. The liner decrease in mesenteric fat mass can be attributed to a lower amount of energy retention within the mesenteric fat. The decrease in the mesenteric mass could attribute to the increase in the size of the stomach complex and liver.

There were no treatment effects ($P \geq 0.11$) in duodenal, ileal, and total small intestine DNA, RNA, and protein concentrations and RNA:DNA, and protein:DNA ratios. The RNA content was used as an indicator for protein synthetic capacity (Sainz and Bentley, 1997; Nozie`re et. al., 1999), DNA was used as a index of tissue hyperplasia (increased cell number), and the protein:DNA and RNA:DNA ratios were used as indicators for tissue

hypertrophy (increased cell size; Baserga, 1985; Reynolds et al., 1990; Jin et al., 1994).

There were no copper effects ($P \geq 0.24$) on the total amount of DNA, RNA, and protein in the small intestinal tissue. Total amount of ileal DNA (1.24, 1.04, and 0.73 ± 0.22 g for 0, 30, and 60% WDG respectively; $P = 0.07$), and total amount of ileal RNA (1.21, 0.95, and 0.74 ± 0.23 g for 0, 30, and 60% WDG, respectively; $P = 0.10$) tended to decrease linearly with increasing levels of wet distillers grains. The linear decrease in the ileal DNA represented a decrease in the number of cells that were generated with the increasing levels of wet distillers grains. Similarly, the ileal RNA decreased linearly which indicates a low protein synthetic capacity as the levels of WDG increased.

Cell proliferation (%) in jejunal tissue was not affected ($P = 0.50$) by treatment. However, the total number of proliferating jejunal cells tended to be greater ($P = 0.09$) for steers receiving 30% wet distillers grain (92.78 ± 19.83) than those receiving 30% wet distillers grain plus organic copper (47.85 ± 19.83). The 30% without copper represents a greater energy consumption which results in a lower energy efficiency for the organ when compared to the 30% with copper. Vascularity or total microvascular volume were not affected ($P \geq 0.43$) by treatment. This means that the transport capacity and activity of the jejunum was not affected by WDG or by organic copper.

The greater energy demand for the stomach complex and liver implies that animals become less efficient with increasing wet distillers grain level. Therefore, distillers grains might have a greater proportion of NE_m and lower proportion of NE_g than corn. Therefore, greater energy efficiency of distillers grains is not evident in this study. However, more research in this area to evaluate oxygen consumption and carbon dioxide production by visceral organs might improve our understanding of the greater feeding value of distiller grain as compared with corn, and of energy expenditure by visceral organs.

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Table 1. Ingredients and chemical composition of experimental diets fed to steers (DM-basis)

	Wet distillers grain, % DM			
Item	0	30	60	30 + Cu[b]
Ingredient composition[a], %, DM basis				
Steam-flaked corn	78.25	56.22	28.74	56.22
Cottonseed meal	5.58	-	-	-
Wet distiller's grains with solubles[c]	-	30.16	60.19	30.16
Supplement[d]	3.17	2.65	2.13	2.65
Copper	-	-	-	???
Yellow grease	4.02	2.00	-	2.00
Alfalfa hay	8.98	8.97	8.94	8.97
Chemical composition[e]				
CP, %	13.4	16.7	22.2	16.7
Non-protein N, % CP	2.5	1.4	0.3	1.4
ADF, %	8.3	11.7	17.0	11.7
NDF, %	13.8	20.4	28.9	20.4
EE, %	5.9	5.9	7.7	5.9
Ca, %	0.8	0.7	0.7	0.7
P, %	0.3	0.4	0.5	0.4
K, %	0.7	0.8	1.0	0.8
Mg, %	0.21	0.22	0.27	0.22
S, %	0.14	0.26	0.4	0.26
Zn, mg/kg	70.0	79.0	93.0	79.0
Fe, mg/kg	116.0	168.0	200.0	168.0
Mn, mg/kg	42.0	47.0	58.0	47.0
Cu, mg/kg	16.0	18.0	19.0	18.0

[a]Determined based on actual DM determined for each ingredient throughout the study.
[b]Treatment contained an addition of chelated Cu.
[c]Wet distiller's grains with soluble averaged 22% sorghum grain and 78% corn grain.
[d]Supplements were formulated to provide 1.06, 0.53, and 0% urea for 0, 30, and 60% wet distiller's grains with soluble.
[e]Data represent the mean of duplicate analyses for each analyte from a composite of weekly samples for each diet collected from the bunk.

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HIGH-TANNIN FORAGE UTILIZATION BY BEEF COWS I. INTAKE AND DIGESTION OF TALLGRASS PRAIRIE HAY CONTAMINATED WITH SERICEA LESPEDEZA (*LESPEDEZA CUNEATA*)

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ABSTRACT: Mature, non-pregnant, non-lactating beef cows (n = 24; initial BW = 463 ± 69 kg) were used to assess voluntary DMI of tallgrass prairie hay contaminated with sericea lespedeza in a 30-d trial. Cows were assigned randomly to 1 of 2 dietary treatments: uncontaminated tallgrass prairie hay (**UC**; 5.4% CP, 40% ADF) or tallgrass prairie hay contaminated with sericea lespedeza (**C**; 5.5% CP, 41% ADF). These forages were similar in botanical composition, except for the presence of sericea lespedeza. Sericea lespedeza constituted 19.3% of C by weight (DM basis); condensed tannin concentration in sericea lespedeza plants selected from C ranged from 200 to 250 g/kg forage DM. All cows were individually fed UC for *ad libitum* intake using a Calan-gate feeding system for 20 d. Voluntary forage DMI was not different ($P = 0.32$) between treatments during that time and averaged 113 ± 3.0 g/kg $BW^{0.75}$. On d 21, hay contaminated with sericea lespedeza was abruptly substituted for uncontaminated hay in the diets of cows assigned to C. Voluntary forage DMI was monitored for an additional 10 d. Voluntary forage DMI by cows assigned to UC remained relatively stable (112 ± 2.8 g/kg $BW^{0.75}$) during that time, while voluntary forage DMI by cows assigned to C decreased (treatment x time, $P < 0.01$) sharply and averaged 61 ± 8.9 g/kg $BW^{0.75}$. Nutrient digestion was assessed during the last 6 d of the trial using ADIA as an internal marker. Total-tract DM, CP, and NDF digestibilities were not different ($P \geq 0.29$) between C and UC. In contrast, total digestible DMI by cows fed UC was more than 2-fold greater ($P < 0.01$) than by cows fed C (64 vs. 29 ± 6.2 g/kg $BW^{0.75}$ for UC and C, respectively). Our results were interpreted to indicate that tallgrass prairie hay contaminated with sericea lespedeza may be a useful model for the study of the appetite-suppressing effects of that plant. Furthermore, differences in voluntary DMI between contaminated and uncontaminated hays of similar chemical composition were manifested rapidly after introduction of C into beef cow diets.

Key Words: condensed tannins, forage intake, *Lespedeza cuneata*

Introduction

Sericea lespedeza (*Lespedeza cuneata*) is classified as an invasive plant throughout the Great Plains, the eastern United States, and eastern Canada (USDA, 2010). It infests approximately 600,000 acres of native Tallgrass range in the Kansas Flint Hills (Eddy et al., 2003). Prolific seed production has contributed to the spread of sericea lespedeza on rangelands (Wang et al., 2008). The aggressive nature of the plant reduces native grass production by up to 92% (Eddy et al., 2003). Herbicides retard the spread of sericea lespedeza but application is difficult and expensive under the best of circumstances and impossible in steep or rocky terrain (Eddy et al., 2003).

Intake of sericea lespedeza by grazing beef cattle is poor, due presumably to the presence of tannins in the plant (Terrill et al., 1989; Mantz et al., 2009). Condensed tannins reduce protein digestion by ruminants (Jones and Mangan, 1977) and may also decrease plant palatability.

Increasing grazing pressure on sericea lespedeza may reduce seed production and slow the spread of the plant; however, the difficulties associated with measurement of intake by grazing beef cattle have hampered development of workable research models. Detailed study of the appetite-suppressing effects of sericea lespedeza under controlled conditions is essential in order to develop appropriate strategies to increase grazing pressure on this plant. Such information could lead to a degree of biological control of this noxious weed using the most economically-important grazer (i.e., beef cattle) in the Kansas Flint Hills.

Feeding sericea lespedeza as sun-cured hay to confined beef cattle would be a convenient way to study the intake-limiting properties of this plant. This approach has not been attempted to date because previous research indicated that allowing sericea lespedeza to sun cure after harvest sharply decreased the amount of extractable condensed tannins in the plant and the capability of condensed tannins to bind proteins (Terrill et al., 1989, 1990, and 1994; Eckerle et al., 2010). Based on these reports, it was doubtful whether sun-cured prairie hay containing sericea lespedeza would produce the aversion in confined beef cattle that is commonly observed in free-ranging beef cattle exposed to the fresh plant. Therefore, the objective of our study was to compare intakes of tallgrass prairie hay by beef cows when hay was either uncontaminated or heavily contaminated by sericea lespedeza.

Materials and Methods

All procedures used in the care, handling, and sampling of animals in our study were reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee (Protocol # 2650.5).

Tallgrass prairie hay contaminated by sericea lespedeza was harvested from a single pasture in Greenwood County,

Kansas, cut, sun-cured, packaged into 0.75 × 0.5 × 0.5 m bales (approximately 35 kg), and stored at the Kansas State University Commercial Cow-Calf Unit. Forage was harvested in late July, which corresponded to the budding stage of sericea lespedeza and maximal concentration of extractable condensed tannins (Eckerle et al., 2010).

Plant-species composition within the study area was estimated using a modified step-point technique (Owensby, 1973); sericea lespedeza comprised 19.3% of all plants encountered during the procedure. Above-ground biomass of sericea lespedeza averaged 1,001 kg/acre. Concentrations of extractable condensed tannins in individual sericea lespedeza plants ranged from 200 to 250 g/kg forage DM.

Uncontaminated tallgrass prairie hay was harvested in Pottawatomie County, KS also late in July. Uncontaminated hay was verified to be free of sericea lespedeza, cut, sun-cured, packaged into 1.5 × 1.5 m cylindrical bales (approximately 450 kg), and stored at the Kansas State University Commercial Cow-Calf Unit. Species compositions of contaminated and uncontaminated forages were similar in all respects, except for the presence of sericea lespedeza.

Bales of each forage type were sampled to determine CP and ADF concentration and were paired based on similarity in those values. Average CP and ADF concentrations of contaminated and uncontaminated hay are shown in Table 1. Purposeful selection for similarity in protein and fiber concentrations between forage types was intended to prevent confounding forage quality with effects on intake. Bales of contaminated and uncontaminated hay selected for the study were ground separately to a 10-cm particle size.

Table 1. Chemical composition of tallgrass prairie hay contaminated or uncontaminated by sericea lespedeza (DM basis).

Item	Forage CP (%)	Forage ADF (%)
Uncontaminated	5.4	39.8
Contaminated	5.5	41.0

Mature, non-pregnant, non-lactating beef cows (n = 24; initial BW = 463 ± 69 kg; initial BCS 4.2 ± 0.76) were housed in a single pen (40 × 80 m) equipped with a Calan-gate feeding system. Gated feed bunks were covered but the remaining area of the pen was open to ambient air and wind. Cows were fitted with a single transponder capable of opening 1 gated feed bunk only and trained to use the Calan-gate feeding system over a period of 30 d. During this time, cows were fed uncontaminated prairie hay for *ad libitum* intake. Cows were offered forage twice daily at 0600 and 1800 (130% of rolling 5-d average DMI). Daily forage refusals were collected and weighed at 0530. Daily voluntary DM intakes were determined by subtracting daily refusals from the total amount of hay offered. Intakes were expressed in g/kg $BW^{0.75}$. Once forage DMI stabilized at approximately 2.5% BW, the trial was initiated.

Cows were stratified by age and BCS and assigned randomly to 1 of 2 dietary treatments: uncontaminated tallgrass prairie hay (**UC**) or tallgrass prairie hay contaminated with sericea lespedeza (**C**). All cows were individually fed UC hay for *ad libitum* intake for an additional 20 d to initiate the trial. Voluntary forage DMI was not different (P = 0.32; data not shown) between treatments during that time and averaged 113 ± 3.0 g/kg $BW^{0.75}$. On d 21, hay contaminated with sericea lespedeza was abruptly substituted for uncontaminated hay in the diets of cows assigned to C. Voluntary forage DMI was monitored for an additional 10 d.

Total-tract diet digestion was assessed from d 26 to 30 according to Olson et al. (2008). Forage samples were collected from d 25 to 29. Fecal grab samples were collected every 4 h on d 26 to 30. The collection interval was staggered 2 h each day to account for diurnal variation in fecal output and composition.

Daily forage, ort, and fecal samples were dried in a forced air-over (96 h; 50 °C), weighed, and ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1-mm screen. Forage samples were composited within forage type; fecal and ort samples were composited across days within animal.

Forage, orts, and feces were analyzed for DM (16 h; 105 °C), OM (8 h; 450 °C), and N (FP-528, LECO, St. Josephs, Michigan). These samples were also analyzed for NDF, ADF, and acid detergent insoluble ash (ADIA) using procedures described by Van Soest et al. (1991). Total tract nutrient digestion coefficients were calculated using ADIA as an internal marker (Cochran and Galyean, 1994; Olson et al., 2008).

Intake data were analyzed as a completely randomized design with repeated measures (PROC MIXED; SAS Inst. Inc., Cary, NC). The model included terms for treatment, day, and treatment × day. Fixed effects were tested using type-3 error rates. Treatment × day effects were detected (P < 0.01); therefore, interaction Least Squares means were reported with pooled standard errors.

Digestibility data were analyzed as a completely randomized design (PROC GLM; SAS Inst. Inc., Cary, NC). Class variables included animal and treatment. The model included a term for treatment only. Fixed effects were tested using type-3 error rates. Least Squares means were separated by the method of Least Significant Difference and reported with pooled standard errors. Means were considered to be different when $P \leq 0.05$.

Results and Discussion

Voluntary forage DMI by cows assigned to UC remained relatively stable (112 ± 2.8 g/kg $BW^{0.75}$) during the last 10 d of our trial. Conversely, voluntary forage DMI by cows assigned to C decreased (treatment x time, P < 0.01) sharply and averaged 61 ± 8.9 g/kg $BW^{0.75}$ during that period (Figure 1). Abrupt introduction of prairie hay contaminated by sericea lespedeza seemed to exert an immediate but relatively minor effect on forage DMI from d 21 to d 24 (average difference between UC and C = 21 g DM/kg $BW^{0.75}$). Between d 25 and 30, average voluntary DMI of cows assigned to C declined dramatically (average difference between UC and C = 73 g DM/kg $BW^{0.75}$). This occurred even though both C and UC had similar CP and ADF concentrations. We speculated that the immediate

decline in voluntary DMI that occurred between d 21 and 24 may be indicative of a relatively minor flavor aversion. Conversely, the precipitous drop in intake of C that followed (d 25 to 30) appeared to be driven by significant post-ingestive consequences of sericea lespedeza ingestion. This effect may have been associated with a ruminal build-up of tannin-protein complex that caused a general decrease in the activity of ruminal microorganisms.

Total-tract DM, CP, and NDF digestibilities were not different ($P \geq 0.29$) between C and UC (Table 2). In contrast, total digestible DMI by cows fed UC was more than 2-fold greater ($P < 0.01$) than that by cows fed C (64 vs. 29 g/kg $BW^{0.75}$ for UC and C, respectively). Clearly, beef cows that were offered C experienced a significant nutrient deficit compared to beef cows fed UC.

Implications

Our results were interpreted to indicate that tallgrass prairie hay contaminated with sericea lespedeza may be a useful model for the study of the appetite-suppressing effects of that plant. Furthermore, differences in voluntary DMI between contaminated and uncontaminated hays of similar chemical composition were manifested rapidly after introduction of C into beef cow diets. Palatability of tannins may be responsible for an initial but relatively minor flavor aversion; however, continued intake of contaminated forage may have resulted in a build-up of tannin-protein complexes in the rumen that suppressed forage DMI substantially. Using this model to investigate methods to mitigate the negative effects of consuming high-tannin forages appears promising.

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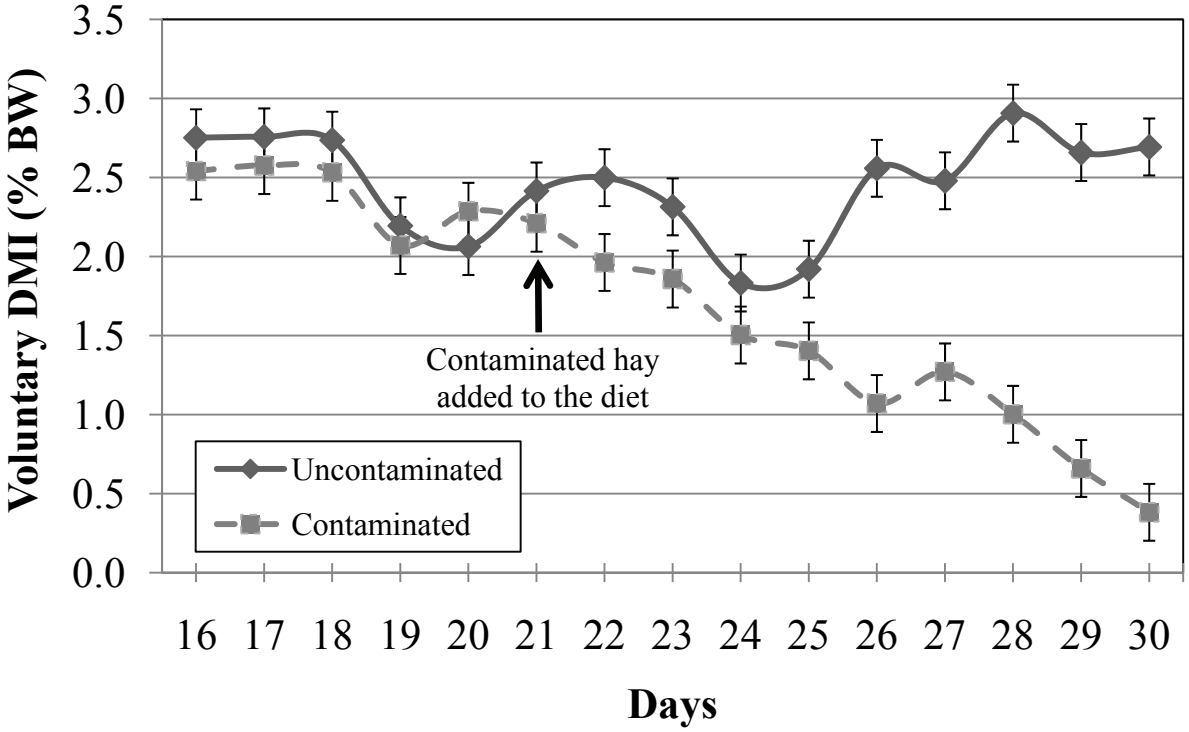
Table 2. Total-tract nutrient digestion and total digestible DMI by beef cows fed Tallgrass prairie hay contaminated by sericea lespedeza.

Item	Uncontaminated Forage	Contaminated Forage	SEM	*P*-Value
Total -tract DM digestibility, %	51.9	50.4	1.82	0.59
Total-tract CP digestibility, %	24.8	26.6	1.98	0.53
Total-tract NDF digestibility, %	59.7	56.9	1.84	0.29
Total digestible DMI, g/kg $BW^{0.75}$	64.0[a]	29.0[b]	6.17	< 0.01

[a, b] Means within a row lacking common superscripts are different.

Figure 1. Effects of sericea lespedeza contamination on voluntary DMI of tallgrass prairie hay by beef cows.



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HIGH-TANNIN FORAGE UTILIZATION BY BEEF COWS II. EFFECTS OF CORN STEEP LIQUOR SUPPLEMENTATION ON INTAKE AND DIGESTION OF TALLGRASS PRAIRIE HAY CONTAMINATED WITH SERICEA LESPEDEZA (*LESPEDEZA CUNEATA*)

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ABSTRACT: Mature, non-pregnant, non-lactating beef cows (n = 24; initial BW = 546 ± 131 kg) were used to evaluate the effects of corn steep liquor (**CSL**; 14.7% CP, 45% DM) supplementation on voluntary DMI of tallgrass prairie hay contaminated with sericea lespedeza during a 29-d trial. Sericea lespedeza was 19.3% of forage DM by weight; condensed-tannin concentration in sericea lespedeza plants selected from contaminated hay ranged from 200 to 250 g/kg forage DM. Cows were assigned randomly to 1 of 4 feeding levels of CSL: 0, 0.6, 1.2, or 1.8 kg DM/d. Cows were individually fed contaminated hay for *ad libitum* intake using a Calan-gate feeding system. Cows were offered contaminated hay only during the first 14 d of the trail. Voluntary forage DMI was not different ($P = 0.52$) between treatments during that time and averaged 83 ± 2.2 g/kg $BW^{0.75}$. Beginning on d 15, supplemental CSL was abruptly introduced into cow diets at assigned feeding levels; it was offered once daily and was consumed by cows within 30 min. Cows supplemented with CSL ate more ($P \leq 0.01$) forage DM from d 15 to d 29 than unsupplemented cows; however, there was no difference ($P \geq 0.38$) in forage DMI between CSL feeding levels. Diet digestion was monitored using ADIA as an internal marker from d 23 to d 29. Total-tract DM digestibility was greater ($P < 0.01$) for cows fed 1.2 or 1.8 kg CSL than for cows fed 0 or 0.6 kg CSL. Total-tract CP digestion was least ($P < 0.01$) in cows fed no CSL (-1.5%), was slightly higher ($P < 0.01$) in cows fed 0.6 kg CSL (18.6%), and was greatest ($P < 0.01$) in cows fed either 1.2 or 1.8 kg CSL (51.7 and 52.3%, respectively). Total digestible DM intake by cows fed 1.2 or 1.8 kg CSL was greater ($P \leq 0.03$; 75 and 88 g/kg $BW^{0.75}$, respectively) than that by cows fed 0 or 0.6 kg CSL (41 and 55 g/kg $BW^{0.75}$, respectively). Under the conditions of our study, CSL ameliorated the effects of condensed tannins on forage DMI and digestion in cows fed Tallgrass prairie hay contaminated with sericea lespedeza.

Key words: condensed tannins, forage intake, *Lespedeza cuneata*

Introduction

Sericea lespedeza (*Lespedeza cuneata*) is a noxious weed that infests approximately 600,000 acres of native Tallgrass range in the Kansas Flint Hills (Eddy et al., 2003). Intake of sericea lespedeza by grazing livestock is poor, presumably due to the presence of tannins in the plant (Terrill et al., 1989; Mantz et al., 2009). Condensed tannins significantly reduce dietary protein and DM digestion by ruminants (Jones and Mangan, 1977; Terrill et al., 1989). These circumstances create negative post-ingestive consequences which deter consumption (Mantz et al., 2009). Poor intake of sericea lespedeza translates to negligible grazing pressure, which ensures that sericea lespedeza will be able to produce seed and continue to spread.

Increasing grazing pressure on sericea lespedeza may slow its advance and allow a measure of biological control using the most economically-important grazer (i.e., beef cattle) in the Flint Hills. Feedstuffs or feed additives with tannin-binding properties may allow grazing beef cattle to comfortably and voluntarily select this plant.

Jones and Mangan (1977) reported that feed-grade polyethylene glycol may inhibit the formation of tannin protein complexes in the rumen. Mantz et al. (2009) reported that confined beef cattle fed 454 g of polyethylene glycol (**PEG**) daily ate more sericea lespedeza than cattle that were not fed PEG. Use of PEG by commercial beef producers has not been widely adopted for two reasons: 1) feeding PEG at the rates necessary to increase intake of sericea lespedeza is cost prohibitive and 2) feeding PEG at the rates necessary to increase intake of sericea lespedeza is disallowed from a regulatory standpoint (AAFCO, 2008).Therefore, it is advantageous to identify substances that are generally regarded as safe (GRAS; FDA, 2011), cost effective, and that mitigate the consequences of consuming a diet high in tannins.

Preliminary research in our laboratory indicated that corn steep liquor (**CSL**) has binding affinity for condensed tannins that equals or exceeds that of PEG. The objective of our study was to determine the effects of CSL supplementation on intake and digestion of Tallgrass prairie hay contaminated with sericea lespedeza.

Materials and Methods

All procedures used in the care, handling, and sampling of animals in our study were reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee (Protocol # 2650.5).

Preliminary Data. We developed an *in vitro* method for estimating the binding affinity of various compounds for condensed tannins and the degree of protection from tannin binding that these compounds bestowed on true proteins. This method involved mixing purified tannin with a purified source of true protein (bovine serum albumin; **BSA**) in the presence or absence of potential tannin-

mitigating compounds. The ratio of tannin to true protein in solution was held at 4:1 purified tannin to true protein, approximating the tannin:true protein ratio in the diets of beef cattle consuming sericea lespedeza only (Mantz et al., 2009). After the reaction was allowed to take place in the presence or absence of a mitigating agent, BSA in the solution that remained unbound by tannin was measured using a spectrophotometric technique (Sprint Rapid Protein Analyzer, CEM USA, Matthews, NC).

Among the mitigating agents that we evaluated were polyethylene glycol (**PEG**) and corn steep liquor (**CSL**) at doses of 16 mg / mg of true protein in the original sample. This dose of the mitigating agents created a ratio of mitigator to:true protein that was approximately equal to the feeding rate of PEG recommended by Mantz et al. (2009) to increase consumption of sericea lespedeza by beef cattle.

In untreated samples, an average of 57.3% of the true protein was bound by tannins and would have been resistant to microbial protein digestion in the rumen (Table 1). The average amount of tannin-bound protein in the PEG-treated samples declined by approximately 16% relative to the untreated samples, indicating that PEG provided a modest degree protection from tannin. Conversely, an equivalent dose of CSL appeared to fully protect BSA in the reaction vessel from interacting negatively with tannins. True protein availability of the CSL-treated samples was greater than the original amount of BSA placed in the reaction vessels. This was due to the fact that CSL contained a significant amount of true protein (Table 1).

These data were interpreted to suggest that CSL bound condensed tannins and protected true proteins to a greater degree than PEG. We hypothesized that beef cattle supplemented with a proper dose of CSL may be able to safely consume greater quantities of high-tannin forages than unsupplemented beef cattle.

Forage. Tallgrass prairie hay contaminated by sericea lespedeza was harvested from a single pasture in Greenwood County, Kansas, cut, sun-cured, packaged into 0.75 × 0.5 × 0.5 m bales (approximately 35 kg), and stored at the Kansas State University Commercial Cow-Calf Unit (Table 2). Forage was harvested in late July, which corresponded to the budding stage of sericea lespedeza and maximal concentration of extractable condensed tannins (Eckerle et al., 2010). Bales of contaminated hay used in the study were ground to a 10-cm particle size.

Table 2. Chemical composition of tallgrass prairie hay contaminated by sericea lespedeza and corn steep liquor (CLS; DM basis).

Item	CP (%)	ADF (%)
Hay	5.5	41.0
CSL	14.7	41.0

Plant-species composition within the study area was estimated using a modified step-point technique (Owensby, 1973); sericea lespedeza comprised 19.3% of all plants encountered during the procedure. Above-ground biomass of sericea lespedeza averaged 1,001 kg/acre. Concentrations of extractable condensed tannins in individual sericea lespedeza plants ranged from 200 to 250 g/kg forage DM.

Supplement. Corn steep liquor was purchased from Archer Daniels Midland in Columbus, NE, transported to the Kansas State University Commercial Cow-Calf Unit, and stored in a polyvinyl chloride container (Table 2).

Intake and Digestibility Measurements. Mature, non-pregnant, non-lactating beef cows (n = 24; initial BW = 546 ± 131 kg) were housed in a single pen (40 × 80 m) equipped with a Calan-gate feeding system. Gated feed bunks were covered but the remaining area of the pen was open to ambient air and wind. Cows were fitted with a single transponder capable of opening 1 gated feed bunk only and trained to use the Calan-gate feeding system over a period of 30 d. Cows were then stratified by body weight and BCS and assigned randomly to be fed 1 of 4 supplemental levels of CSL: 0, 0.6, 1.2, and 1.8 kg DM/d. All cows were individually fed tallgrass prairie hay contaminated with sericea lespedeza for *ad libitum* intake for 14 d (130% of rolling 5-d average DMI). Cows were offered forage twice daily at 0600 and 1800. Daily forage refusals were collected and weighed at 0530. Daily voluntary DM intakes were determined by subtracting daily refusals from the total amount of hay offered. Intakes were expressed in g/kg $BW^{0.75}$.

Beginning on d 15, supplemental CSL was abruptly introduced into cow diets at assigned feeding levels; it was offered once daily and was consumed by cows within 30 min. Forage and supplement intake were monitored during the following 14 d. The purpose of the abrupt introduction of CSL into cow diets was to minimize the opportunity for ruminal microbes to adapt to nutrients in CSL.

Total-tract diet digestion was assessed from d 23 to 29 according to Olson et al. (2008). Forage samples were collected from d 23 to 28. Fecal grab samples were collected every 4 h on d 24 to 29. The collection interval was staggered 2 h each day to account for diurnal variation in fecal output and composition.

Daily forage, ort, and fecal samples were dried in a forced air-over (96 h; 50 °C), weighed, and ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1-mm screen. Forage and CSL samples were composited across days; ort and fecal samples were composited across days within animal.

Forage, CSL, orts, and feces were analyzed for DM (16 h; 105 °C), OM (8 h; 450 °C), and N (FP-528, LECO, St. Josephs, Michigan). These samples were also analyzed for NDF, ADF, and acid detergent insoluble ash (ADIA) using procedures described by Van Soest et al. (1991). Total tract nutrient digestion coefficients were calculated using ADIA as an internal marker (Cochran and Galyean, 1994; Olson et al., 2008).

Statistical analyses. Intake data were analyzed as a completely randomized design with repeated measures (PROC MIXED; SAS Inst. Inc., Cary, NC). The model included terms for treatment, day, and treatment × day. Fixed effects were tested using type-3 error rates. Treatment × day effects were not detected ($P \geq 0.96$); therefore, main effects of treatment were reported as Least Squares means. Means were considered to be different when $P \leq 0.05$.

Digestibility data were analyzed as a completely randomized design (PROC GLM; SAS Inst. Inc., Cary,

NC). Class variables included animal and treatment. The model included a term for treatment only. Type-3 error rates were used test for differences in DM and CP digestibilities, as well as total-digestible DM intake. Least Squares means were separated by the method of Least Significant Difference and reported with pooled standard errors. Means were considered to be different when $P \leq 0.05$.

Results and Discussion

Prior to introduction of CSL, voluntary forage DMI did not differ ($P = 0.52$) between treatments and averaged 83 ± 2.2 g/kg $BW^{0.75}$. After introduction of CSL (d 15 to 29), supplemented cows ate more ($P \leq 0.01$) forage DM than unsupplemented cows; however, there was no difference ($P \geq 0.38$) in forage DMI between CSL feeding levels (Table 3). The smallest dose of CSL used in our trial (i.e., 0.6 kg DM/d) stimulated maximum intake of tallgrass prairie hay contaminated with sericea lespedeza in a short-term experiment.

It is possible that corn steep liquor could have stimulated intake of the protein-poor prairie hay by simply supplying more CP to the rumen; however, the purpose of the abrupt introduction of CSL into cow diets was to minimize the opportunity for ruminal microbes to adapt to the presence of nutrients in CSL. The amount of supplemental CP provided by CSL was modest (88, 176, and 265 g DM for 0.6, 1.2, and 1.6 kg CSL DM/d, respectively). Köster et al. (1996) estimated that approximately 540 g of supplemental CP was needed to maximize intake of low-quality tallgrass prairie hay in fully-adapted beef steers. The vigorous, immediate increase in voluntary consumption of tallgrass prairie hay contaminated by sericea lespedeza that we observed may have been the result of rapid complexing between CSL and tannins within the rumen.

Total-tract DM digestibility was greater ($P < 0.01$) for cows fed 1.2 or 1.8 kg CSL than for cows fed 0 or 0.6 kg CSL (Table 3). Total-tract CP digestion was least ($P < 0.01$) in cows fed no CSL, was slightly greater ($P < 0.01$) in cows fed 0.6 kg CSL, and was greatest ($P < 0.01$) in cows fed either 1.2 or 1.8 kg CSL. Total digestible DM intake by cows fed 1.2 or 1.8 kg CSL was greater than that by cows fed 0 or 0.6 kg CSL. In contrast to our intake data, it appeared that the CSL dose needed to optimize digestion characteristics of the diet was equal to or greater than 1.2 kg DM/d. Further research is warranted to evaluate the optimal CSL dose needed to mitigate the consequences of consuming a diet high in tannins.

Implications

Supplementation of corn steep liquor may increase tolerance of beef cows for high tannin-forages. In our study, supplemental corn steep liquor ameliorated the negative consequences of tannin consumption in a dose-dependent manner when fed to beef cows in confinement. The beef cows in our study had only limited opportunity to selectively avoid sericea lespedeza because it was offered in chopped form and in a mixture with other forage species. It is unknown if supplemental CSL can influence forage selection preference when cattle have the opportunity to eat either uncontaminated forage or forage contaminated by sericea lespedeza.

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Table 1. Binding affinity of condensed tannins for bovine serum albumin (BSA) in the presence of polyethylene glycol or corn steep liquor.

Sample	Mitigator	Mitigator Dose[a]	True Protein Availability (%)	Tannin-Bound Protein (%)
Tannin + BSA	None	0	42.7	57.3
Tannin + BSA	PEG	16	59.0	41.0
Tannin + BSA	CSL	16	155.7	-

[a] Mitigator dose is expressed as mg/mg BSA in the original sample.
[b] True protein availability was expressed as % BSA in the original sample, and tannin-bound protein was expressed as the inverse of true protein availability.

Table 3. Effects of increasing dose of corn steep liquor on intake and digestion of tallgrass prairie hay contaminated by sericea lespedeza.

	Corn steep liquor intake, (kg DM /d)				
Item	0	0.6	1.2	1.8	SEM
Forage DMI, g/kg $BW^{0.75}$	69.9[a]	80.7[b]	80.9[b]	84.6[b]	3.09
Total -tract DM digestibility, %	52.6[a]	55.6[a]	65.6[b]	66.3[b]	2.08
Total-tract CP digestibility, %	- 1.5[a]	18.6[b]	51.7[c]	52.3[c]	4.53
Total digestible DMI, g/kg $BW^{0.75}$	40.9[a]	55.0[ab]	75.2[bc]	87.6[c]	6.20

[a, b, c] Means within a row lacking common superscripts are different.

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HIGH-TANNIN FORAGE UTILIZATION BY BEEF COWS III. EFFECTS OF CORN STEEP LIQUOR SUPPLEMENTATION ON VOLUNTARY SELECTION OF TALLGRASS PRAIRIE HAY CONTAMINATED WITH SERICEA LESPEDEZA (*LESPEDEZA* CUNEATA) AND UNCONTAMINATED TALLGRASS PRAIRIE HAY

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ABSTRACT: Mature, non-pregnant, non-lactating beef cows (n = 16; initial BW = 525 ± 76 kg) were used to evaluate the effects of corn steep liquor (**CSL**; 44.7% DM, 14.7% CP) supplementation on voluntary selection of uncontaminated tallgrass prairie hay (**UC**; 5.4% CP, 40% ADF) and tallgrass prairie hay contaminated with sericea lespedeza (**C**; 5.5% CP, 41% ADF) during a 24-d trial. These forages were similar in botanical composition with the exception of sericea lespedeza. Sericea lespedeza was 19.3% of forage DM by weight; condensed-tannin concentration in sericea lespedeza plants selected from C ranged from 200 to 250 g/kg forage DM. Cows were assigned randomly to be fed either 0 or 0.6 kg CSL/d (DM basis). Cows were individually penned and fed UC and C hay in separate feed bunks for *ad libitum* intake. Access to both forages was simultaneous. Supplement was offered once daily and was consumed by cows within 30 min. Cows were allowed to adapt to supplementation treatments for 10 d before forage intake measurements began. Uncontaminated hay DMI was not different (P = 0.65) between supplemented and unsupplemented cows from d 11 to 24. Conversely, cows supplemented with CSL ate 25% more ($P < 0.01$; 63 g/kg $BW^{0.75}$) C from d 11 to d 24 than unsupplemented cows (50 g/kg $BW^{0.75}$). In addition, cows supplemented with CSL ate more ($P = 0.05$; 105 g/kg $BW^{0.75}$) total forage DM from d 11 to d 24 than unsupplemented cows (94 g/kg $BW^{0.75}$). Diet digestion was monitored using ADIA as an internal marker from d 19 to 24. Total-tract DM and CP digestibilities were not different ($P \geq 0.17$) between treatments. Total digestible DM intake by cows fed CSL was 23% greater ($P < 0.01$; 64 g/kg $BW^{0.75}$) than that by unsupplemented cows (49 g/kg $BW^{0.75}$). Under the conditions of our study, a low level of supplemental CSL was associated with increased beef cow selection of tallgrass prairie hay contaminated with sericea lespedeza. We interpreted these data to suggest that supplemental CSL may increase beef cow tolerance for high-tannin forages.

Key words: condensed tannins, forage intake, *Lespedeza cuneata*

Introduction

Sericea lespedeza (*Lespedeza cuneata*) is classified as a noxious weed throughout the Great Plains (USDA, 2010). It produces copious amounts of seed annually (Eddy et al., 2003). In addition, sericea lespedeza contains high levels of condensed tannins during much of the growing season, which deters grazing by large domestic herbivores (Eckerle et al., 2010). In the Kansas Flint Hills alone, this plant infests approximately 600,000 acres of native tallgrass range, reducing native grass production by up to 92% (Eddy et al., 2003). Increased grazing pressure on sericea lespedeza by beef cattle may slow its spread and facilitate some measure of biological control. Feedstuffs or feed additives with tannin-binding properties may promote voluntary consumption of this plant by grazing beef cattle by beef cattle.

Mantz et al. (2009) reported that confined beef cattle fed polyethylene glycol (**PEG**) daily ate more sericea lespedeza than cattle that were not fed PEG; however, use of PEG by commercial beef producers has been problematic. Feeding PEG at the rates necessary to increase intake of sericea lespedeza is cost prohibitive and disallowed from a regulatory standpoint (AAFCO, 2008). Eckerle et al. (2011) reported that low to moderate amounts of supplemental corn steep liquor (i.e., 0.6 to 1.8 kg/d) increased intake of tallgrass prairie hay contaminated with sericea lespedeza by beef cows fed in confinement. Corn steep liquor is an inexpensive, palatable, and abundant byproduct of wet-corn milling and is *Generally Regarded as Safe* (GRAS) by the U.S. Food and Drug Administration (FDA, 2011). It is unknown if beef cattle supplemented with corn steep liquor will readily consume forage contaminated by sericea lespedeza when uncontaminated forage is available simultaneously. Therefore, the objective of our study was to determine the effects of a low level of corn steep liquor fed to beef cows on voluntary selection of tallgrass prairie hay contaminated by sericea lespedeza when uncontaminated tallgrass prairie hay was also available.

Materials and Methods

All procedures used in the care, handling, and sampling of animals in our study were reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee (Protocol # 2650.5).

Tallgrass prairie hay contaminated with sericea lespedeza was harvested from a single pasture in Greenwood County, Kansas, cut, sun-cured, packaged into 0.75 × 0.5 × 0.5 m bales (approximately 35 kg), and stored at the Kansas State University Commercial Cow-Calf Unit. Forage was harvested in late July, which corresponded to

the budding stage of sericea lespedeza and maximal concentration of extractable condensed tannins (Eckerle et al., 2010).

Plant-species composition within the study area was estimated using a modified step-point technique (Owensby, 1973); sericea lespedeza comprised 19.3% of all plants encountered during the procedure. Above-ground biomass of sericea lespedeza averaged 1,001 kg/acre. Concentrations of extractable condensed tannins in individual sericea lespedeza plants ranged from 200 to 250 g/kg forage DM.

Uncontaminated tallgrass prairie hay was harvested in Pottawatomie County, KS also late in July. Uncontaminated hay was verified to be free of sericea lespedeza, cut, sun-cured, packaged into 1.5 × 1.5 m cylindrical bales (approximately 450 kg), and stored at the Kansas State University Commercial Cow-Calf Unit. Species compositions of contaminated and uncontaminated forage were similar in all respects, except for the presence of sericea lespedeza.

Bales of each forage type were sampled to determine CP and ADF concentrations and were paired based on similarity in those values. Average CP and ADF concentrations of contaminated and uncontaminated hays are shown in Table 1. Purposeful selection for similarity in protein and fiber concentrations between forage types was intended to prevent confounding forage quality with effects on intake. Bales of contaminated and uncontaminated hay selected for the study were ground separately to a 10-cm particle size.

Table 1. Chemical composition of tallgrass prairie hay contaminated or uncontaminated by sericea lespedeza and corn steep liquor (CLS) fed to beef cows (DM basis).

Item	CP (%)	ADF (%)
Uncontaminated hay	5.4	39.8
Contaminated hay	5.5	41.0
CSL	14.7	-

Mature, non-pregnant, non-lactating beef cows (n = 16; initial BW = 526 ± 76 kg; initial BCS = 4.8 ± 0.45) were assigned randomly to 1 of 2 dietary treatments: 0.0 or 0.6 kg/d corn steep liquor (**CSL**; DM basis; Table 1). Cows were individually confined in 5 × 20 m pens and offered uncontaminated hay (**UC**) and contaminated hay (**C**) in separate feed bunks for *ad libitum* intake. Pens were arranged in 2 blocks of 8; feed bunks were covered but the remaining area of pens was open to ambient air and wind. Access to both C and UC was simultaneous and allowed cows to the opportunity to display preference for one forage type over the other.

Supplemental CSL was offered to treated cows on d 1 to 24 at 0530; it was consumed completely within 30 min. During the entire study, cows had access to both forages offered at 130% of the 5-d rolling average DMI. Forages were fed twice daily at 0600 and 1800. Daily forage refusals were collected and weighed at 0530. Daily voluntary DM intakes of C and UC were determined by subtracting daily refusals from the total amount of hay offered. Intakes were expressed in g/kg $BW^{0.75}$. Voluntary intakes of C and UC were measured from d 1 to 24 and analyzed separately from d 1 to 14 (unadapted to CSL) and from d 15 to 24 (adaped to CSL).

Total-tract diet digestion was assessed from d 18 to 24 according to Olson et al. (2008). Forage samples were collected from d 18 to 23. Fecal grab samples were collected every 4 h from d 19 to 24. The collection interval was staggered 2 h each day to account for diurnal variation in fecal output and composition. Samples of refused C and UC were also collected on those days.

Daily forage, ort (for both hay types), and fecal samples were dried in a forced air-over (96 h; 50 °C), weighed, and ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1-mm screen. Samples of C, UC, and CSL were composited across days on an equal weight basis; ort and fecal samples were composited across days within animal on an equal weight basis.

Forage, CSL, ort, and fecal composite samples were analyzed for DM (16 h; 105 °C), OM (8 h; 450 °C), and N (FP-528, LECO, St. Josephs, Michigan). These samples were also analyzed for NDF, ADF, and acid detergent insoluble ash (ADIA) using procedures described by Van Soest et al. (1991). Total tract nutrient digestion coefficients were calculated using ADIA as an internal marker (Cochran and Galyean, 1994; Olson et al., 2008).

Intakes of C and UC from d 1 to 14 (unadapted to CSL) and from d 15 to 24 (adapted to CSL) were analyzed as a randomized complete block with repeated measures (PROC MIXED; SAS Inst. Inc., Cary, NC). The model included terms for treatment, day, block, all 2-way interactions, and treatment × day × block. Day was the repeated measures term and animal within treatment and day was used as the error term (CS option; SAS Inst. Inc., Cary, NC). Fixed effects were tested using type-3 error rates. Interactions were not detected ($P \geq 0.96$) for any of the variables we examined; therefore, main effects of treatment were reported as Least Squares means. Means were considered to be different when $P \leq 0.05$. Tendencies were discussed when $P > 0.05$ and < 0.10.

Digestibilities of DM and CP, as well as total digestible DM intake, were analyzed as a randomized complete block (PROC GLM; SAS Inst. Inc., Cary, NC). Class variables included animal, treatment, and block. The model included a term for treatment only. Fixed effects were tested using type-3 error rates. Least Squares means were separated using the method of Least Significant Difference and reported with pooled standard errors. Means were considered to be different when $P \leq 0.05$.

Results and Discussion

Intakes of C and UC were monitored from d 1 to 14 to ascertain how supplemented and unsupplemented cows were using these forages before ruminal microorganisms became fully adapted to CSL. Unsupplemented cows ate more ($P = 0.04$) UC than cows fed CSL from d 1 to 14 (Table 2). Conversely, unsupplemented cows tended to eat less ($P = 0.09$) C than cows fed CSL. Eckerle et al. (2011) reported also that beef cows increased intake of tallgrass prairie hay contaminated by sericea lespedeza immediately after CSL was introduced into the diet. These data were interpreted to suggest that beef cows may respond to

supplemental CSL by increasing intake of hay contaminated with sericea lespedeza even when ruminal microorganisms are unadapted or minimally adapted to CSL.

Uncontaminated hay DMI was not different ($P = 0.65$) between supplemented and unsupplemented cows from d 11 to 24 (Table 2). Conversely, cows supplemented with CSL ate 25% more ($P < 0.01$) C from d 11 to d 24 than unsupplemented cows. Cows supplemented with CSL also ate more ($P = 0.05$) total forage DM from d 11 to d 24 than unsupplemented cows.

Beef cows supplemented with CSL voluntarily consumed more tallgrass prairie hay contaminated with sericea lespedeza than unsupplemented beef cows, even when uncontaminated hay was available concurrently. These data were interpreted to indicate that low levels of supplemental CSL may increase beef cow acceptance of and tolerance for high-tannin forages.

Total-tract DM and CP digestibilities were not different ($P \geq 0.17$) between treatments. Eckerle et al. (2011) reported that total-tract DM and CP digestibilities by beef cows fed tallgrass prairie hay contaminated with sericea lespedeza were maximized at CSL supplementation levels of 1.2 kg DM/d or greater. Further research is warranted to evaluate feeding rates of CSL that are needed for optimal digestion of low-quality tallgrass prairie hay contaminated with sericea lespedeza.

Total digestible DMI by cows fed CSL was 23% greater ($P < 0.01$) than that by unsupplemented cows. Eckerle et al. (2011) reported that unadapted cattle fed 1.2 to 1.8 kg of CSL/d (DM) had comparable total digestible DMI. Cows supplemented with CSL in our study appeared to have greater dietary energy availability than unsupplemented cows, even though the estimated increase in NE_m supply associated with supplementing CSL at 0.6 kg DM/d was only 1.1 Mcal/d (Kalscheur et al., 2008). Over a longer feeding period, this may have translated to improved performance.

Implications

Low-level supplementation of corn steep liquor may increase both acceptance of and tolerance for high tannin-forages by beef cows. Corn steep liquor fed at 0.6 kg DM/d ameliorated some of the negative consequences of tannin consumption on digestible DM intake. In addition, voluntary consumption of high-tannin forage increased by 25% in supplemented vs. unsupplemented beef cows. It is unknown if supplemental CSL can promote voluntary selection of actively-growing sericea lespedeza by beef cattle grazing native rangeland in the Kansas Flint Hills.

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Table 2. Effects of low-level corn steep liquor supplementation on forage intake and digestion by beef cows simultaneously offered tallgrass prairie hay that was contaminated with sericea lespedeza and that was uncontaminated by sericea lespedeza.

	Corn steep liquor intake, (kg DM /d)			
Item	0	0.6	SEM	*P*-Value
Before adaptation to CSL, d 1 to 14[a]				
Uncontaminated forage DMI, g/kg $BW^{0.75}$	68.2	62.2	2.02	0.04
Contaminated forage DMI, g/kg $BW^{0.75}$	28.9	33.6	1.94	0.09
Total forage DMI, g/kg $BW^{0.75}$	97.1	95.8	2.14	0.67
After adaptation to CSL, d 15 to 24[a]				
Uncontaminated forage DMI, g/kg $BW^{0.75}$	43.6	41.6	3.10	0.65
Contaminated forage DMI, g/kg $BW^{0.75}$	50.3	63.0	2.48	< 0.01
Total forage DMI, g/kg $BW^{0.75}$	93.9	104.7	3.90	0.05
Total -tract DM digestibility, %	50.5	53.9	1.66	0.17
Total-tract CP digestibility, %	17.1	18.5	2.15	0.64
Total digestible DMI, g/kg $BW^{0.75}$	48.7	63.7	3.49	< 0.01

[a] Supplemental CSL was fed from d 1 to 24. The first 14 d of the experiment were considered to represent an adaptation period to supplemental CSL. Animals were considered fully adapted to CSL after d 15.

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POTENTIAL USE OF A NEW FORAGE BARLEY VARIETY FOR RUMINANT LIVESTOCK DIETS

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ABSTRACT: A new forage barley variety (Hooded; HB) was compared to grain barley (Strider; SB), forage oats (Everleaf; EO), and grain oats (Gray; GO) for use in ruminant livestock diets. Use of interseeded legumes (Austrian field peas; PEAS) with each variety was also evaluated. Varieties were fall planted in a randomized complete block design using 4 field replicates of 20 plots. Within replicate each variety (including PEAS) was planted in 4 randomly allotted 37m^2 plots, with 2 plots containing interseeded peas, and harvested when seed maturity reached soft dough stage. Four subplots (0.5m^2) were harvested within each plot to evaluate hay or silage production (DM/hectare). Hay subplots were allowed to wilt in a covered area for 30 d, while silage subplots were chopped and sealed in oxygen-limiting bags within 8 h of harvest and allowed to ferment for 60 d. Subplots were analyzed for nutrient content, with composites of each variety evaluated for DM and fiber digestibility (24 h) using four ruminally-fistulated steers. Comparing barley varieties for either harvest method, CP was similar ($P >$ 0.10) while ADF content was greater ($P < 0.01$) in HB resulting in a lower ($P < 0.01$) TDN value. Digestibility of DM was lower (P = 0.02) for HB when harvested as hay (40.0%), but greater ($P = 0.05$) when harvested as silage (41.2%) versus SB (41.5 and 37.9% for hay and silage, respectively). In comparison to EO, HB had greater ($P < 0.01$) CP and TDN, but reduced ($P < 0.01$) DM production across harvest methods. The HB variety had greater ($P < 0.05$) digestibility versus EO across harvest methods. Barley varieties had greater ($P < 0.01$) CP, TDN, and overall digestibility; but lower ($P < 0.01$) DM production versus oat varieties regardless of harvest method. Use of PEAS increased ($P < 0.05$) CP content and DM production across all varieties with a greater impact on silage values versus hay, while impact on digestibility was inconsistent across varieties. In conclusion, HB was similar to SB in nutrient content and rumen digestibility, and both barley varieties were better than the oat varieties in nutrient comparisons, but had lower DM production/hectare.

Keywords: Hooded barley, forage grains, ruminants

Introduction

The increasing cost of both grain and forage sources over the past 10 years (USDA, 2010) have resulted in increased feed expenses for livestock producers. In regards to beef cattle production, the Northwest region of the U.S. (PNW) typically has the highest feed costs and therefore, highest cost of production on an annual basis (Cattle Fax, 2009). Research into alternative and new feed sources will be paramount in reducing or minimizing future feed expenditures.

Barley and many other small grains have been primarily used as grain (or starch) sources in livestock feeds. As forage, most small grains can be harvested early in development for silage production or allowed to wilt and stored as hay. Typically small grain silages and hays have lower protein levels compared to cool-season grasses, but have comparable digestible energy levels (NRC, 1996). The advantage of using small grains for forage production, especially in the western U.S., is that they can be planted in the fall, receive abundant moisture during the winter, and be ready for harvest in mid-to-late spring. The objective of this study was to evaluate the potential use of a new forage barley variety (Hooded) compared to a common grain barley variety and both forage and grain oat varieties in regards to nutritional quality and agronomic production per area.

Materials and Methods

Planting and pre-harvest plot management. Four field replicates of 20 plots (37m^2 per plot) were fall planted with four small grain varieties to evaluate both hay and silage production the following spring. Plots (n = 4 plots·variety^{-1}·field replicate^{-1}) were randomly assigned to: 1) Hooded barley (**HB**; forage variety), 2) Strider barley (**SB**; grain variety), 3) Everleaf oats (**EO**; forage variety), or 4) Gray oats (**GO**; grain variety). The impact of inter-mixed legumes was also evaluated by interseeding Austrian winter peas (*Pisum sativum* L.; **PEAS**) in half of the small grain variety plots (n = 2 plots·variety^{-1}·field replicate^{-1}), plus individually in four additional plots per field replicate. Small grain varieties were planted at a density of 185 seeds/m^2 (68 kg/ha), while PEAS were planted at a density of 80 seeds/m^2. The PEAS were not inoculated prior to planting. Nitrogen fertilizer was applied to all plots in the spring (March), with the exception of two PEAS plots per field replicate (**PEAS-N**).

Harvest. At time of harvest, two subplots (0.5 m^2 per subplot) within each plot were harvested to determine hay production (**HAY**) and two were harvested to determine silage (**SILAGE**) production characteristics. Harvest of all plots within a variety occurred when average kernel maturity reached soft dough stage (based on visual observation). Maturity of interseeded PEAS

were different due to small grain varieties maturing at different periods. To account for differences in PEAS maturity, one fertilized (**PEAS+N**) and one PEAS-N plot per field replicate was harvested during each small grain variety harvest period.

Each HAY subplot was bundled into permeable plastic bags and suspended in a sheltered facility to limit exposure to sunlight and precipitation. Bundles were allowed to wilt for 45 d, after which they were weighed, coarsely ground, and composited (2 bundles per plot) for further analysis. Composited samples were dried at 60°C for 24 h to determine DM, then ground to 1 mm using a Wiley mill. Samples were analyzed for CP (FP-528 N analyzer; LECO Corp., St. Joseph, MI), NDF and ADF (ANKOM methods 8 and 9; ANKOM Technology, Macedon, NY), and ash content (AOAC, 2010). Small grain and legume energy prediction equations published by Bull (1981) were used to estimate TDN and NE_l. The TDN values were then converted into ME, NE_m, and NE_g for beef cattle using energy equations published in NRC (1996). Relative feed value (RFV) was predicted using equation published by Bolsen (1991).

Each SILAGE subplot was weighed and coarsely chopped (average particle length = 2.54 to 3.81 cm) within 8 h of harvest. Chopped material from each subplot was split into two equal portions and vacuum sealed in oxygen impermeable polyethylene bags (FoodSaver Vacuum Sealer; Jarden Corp, Rye, NY) fitted with a one-way pressure valve. Sealed bags were stored for 120 d inside a storage container to reduce environmental temperature fluctuations and direct sunlight. After the ensiling period bags were unsealed, pH was measured, and silage was evaluated for mold and discoloration. Samples were dried at 60°C for 48 h, ground to 1 mm, and analyzed for nutrient content similar to HAY samples. Energy density (TDN and NE_l) was predicted using corn silage and legume equations published by Bull (1981).

In-situ rumen digestibility. Four ruminally-fistulated beef steers were used to determine rumen digestibility of both HAY and SILAGE samples. Two steers were designated for SILAGE samples and 2 were designated for HAY samples, with each steer within harvest method receiving all variety and PEAS combinations. Steers were fed ad libitum grass hay (8.1% CP, DM basis) supplemented with 0.8 kg of a 19% CP (DM basis) commercial pellet for 7 d prior to collections, and during the collection period. Dacron bags (3 bags·variety^{-1}·collection time^{-1}) containing approximately 0.5 g of sample were placed in representative steers just prior to supplement feeding (0800 h), with 0 h bags being rinsed and stored for analysis. Subsequent bags were removed at 6, 12, and 24 h post-feeding, rinsed with tap water until clear, and frozen (-20°C) for later analysis. Bags were dried at 60°C for 48 h, and then analyzed for NDF and ADF content (ANKOM methods 8 and 9).

Statistical analysis. All nutrient analysis data were analyzed as a randomized complete block design with field replication as the blocking factor and plot as the experimental unit. Small grain variety and PEAS were considered fixed effects, while field replicate was considered a random effect and the error term was plot within variety x field replicate. Mean separation using Student t-test analysis was used to compare: 1) HB versus SB, 2) HB versus EO, and 3) barley varieties (HB and SB) versus oat varieties (EO and GO). In-situ digestibility data was analyzed as repeated measures in time using variety as the fixed effect, and plot within variety x field replicate as the error term. All models were evaluated using MIXED procedures of SAS (SAS Inst., Cary, NC)

Results and Discussion

HAY analysis.

When comparing barley varieties, CP and ash content was similar ($P > 0.10$) between HB and SB (table 1). Fiber content (both NDF and ADF) was greater ($P \leq 0.01$) in HB samples, resulting in lower ($P < 0.01$) energy estimates (TDN, NE_m, NE_l, and NE_g) and RFV for HB compared to SB. There were no differences ($P > 0.10$) in DM, CP, or TDN production per hectare between the barley varieties. Both varieties were similar ($P > 0.10$) in NDF and ADF digestibility (table 3) after 24 h, while DM digestibility tended ($P = 0.09$) to be lower in HB at 12 h and was lower ($P = 0.02$) at 24 h. The digestibility data would support the lower estimated energy values (table 1) for the HB variety compared to SB.

The HB variety had greater ($P < 0.01$) CP, estimated energy density (TDN, NE_m, NE_l, and NE_g), and RFV; along with lower ($P < 0.01$) NDF and ADF content versus the EO variety (table 1). Though nutrient content was lower in the EO variety, DM and TDN production per hectare was greater ($P < 0.01$) and CP production per hectare was similar ($P = 0.46$) compared to the HB variety. Though a cost analysis was not completed, the production per hectare data should be included when determining actual value of a small grain forage source. In-situ digestibility (table 3) of DM, NDF, and ADF was consistently greater ($P < 0.01$) for the HB variety at all collection periods compared to the EO variety. The differences in DM and fiber digestibility must also be taken into account when comparing small grain forage varieties due to the potential impact on nutrient value and production per hectare.

When comparing barley varieties (HB and SB) to oat varieties (EO and GO), the oat varieties had lower ($P < 0.01$) CP, estimated energy density (TDN, NE_m, NE_l, and NE_g), and RFV (table 1). The lower energy density values were a result of greater ($P < 0.01$) amounts of NDF and ADF content in the oat varieties. The higher fiber content of the oat varieties translated into greater ($P < 0.01$) amounts of DM production per hectare, which subsequently resulted in greater ($P < 0.01$) TDN

production per hectare. The lower CP content of the oat varieties was offset by the higher DM production per hectare, resulting in similar ($P = 0.49$) amounts of CP produced per hectare. From a digestibility standpoint (table 3), the barley varieties had greater ($P < 0.01$) DM, NDF, and ADF digestibility at all collection times. The digestibility component must be taken into accounted when evaluating the production per hectare and nutrient values.

Across varieties PEAS increased ($P < 0.01$) CP, ash, and DM, CP, and TDN production per hectare; and decreased ($P < 0.05$) NDF content (table 1). Interseeded PEAS resulted in depressed ($P < 0.05$) in-situ DM, NDF, and ADF digestibility at 12 h, but had no effect ($P > 0.10$) at 24 h.

SILAGE analysis.

Comparison of barley varieties when harvested for silage (table 2) indicate similar ($P > 0.10$) concentrations of CP and NDF, but the SB variety had lower ($P < 0.01$) ADF content resulting in greater ($P < 0.01$) estimated energy (TDN, NE_m, NE_l, and NE_g) density. The RFV was similar ($P = 0.24$) between the barley varieties. The SB variety tended ($P < 0.10$) to have greater production of DM and CP per hectare, and greater ($P = 0.04$) TDN production per hectare compared to the HB variety. In-situ DM digestibility was greater ($P \leq 0.05$) for HB at all collection times (table 3). Both NDF and ADF digestibility was greater ($P < 0.01$) at 6 h for HB, but similar ($P > 0.10$) to SB at 24 h.

When comparing forage varieties (table 2), HB had greater ($P < 0.01$) CP, estimated energy (TDN, NE_m, NE_l, and NE_g) density, and RFV compared to the EO variety. The higher estimated energy values and RFV are a result of lower ($P < 0.01$) NDF and ADF content observed in the HB samples. Similar to the HAY results, the lower nutrient values in the EO variety were offset by greater production per hectare, as indicated by the greater ($P < 0.01$) amounts of DM and TDN per hectare reported in table 1. The increased production per hectare also offset the lower CP value in EO, with neither forage variety being different ($P = 0.65$) in CP production per hectare. In-situ DM, NDF, and ADF digestibility was greater ($P < 0.01$) for the HB variety compared to EO at all collection times (table 3).

Similar to the HAY values, the SILAGE barley varieties (HB and SB) had greater ($P < 0.01$) protein and estimated caloric value compared to the oat varieties (EO and GO; table 2). The higher predicted energy values for the barley varieties was a result of lower ($P < 0.01$) NDF and ADF content versus the oat varieties. Dry matter and TDN production per hectare was greater ($P < 0.05$) for the oat varieties; while CP production per hectare was lower ($P < 0.01$). In-situ DM and NDF digestibility was greater ($P < 0.05$) for the barley varieties during all collection times (table 3), while ADF digestibility was greater in barley varieties during the first 12 h and tended ($P = 0.08$) to be greater at 24 h.

Across varieties PEAS increased ($P < 0.05$) CP, TDN, NE_m, NE_l, NE_g, RFV, and DM, CP, and TDN production per hectare; and decreased ($P < 0.05$) NDF and ADF content. Regarding ADF and energy density estimates, PEAS reduced ADF content to a greater degree in the oat varieties resulting in a variety x PEAS interaction ($P < 0.10$) for ADF and all energy estimates. Interseeded PEAS depressed ($P < 0.05$) In-situ DM, NDF, and ADF digestibility at 12 h, but increased ($P < 0.05$) DM and ADF digestibility at 24 h.

Implications

The use of HB as a small grain forage source for ruminants, especially cattle, is comparable to SB and has greater nutrient density compared to both forage and grain oat varieties. The data presented also indicates that proper evaluation of a new forage variety must take into account nutrient content, production per hectare and, if possible, evaluation of digestibility. The compilation of these three evaluation processes indicates that either HB or SB variety would be superior to the oat varieties, and SB would be marginally superior to HB. The use of interseeded PEAS had variable effect on nutrient content, but consistently increased production per acre; and had a bigger impact on SILAGE production versus HAY production.

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Table 1. Hay production: Agronomic and nutrient analyses of wilted[1] forage and grain barley and oat varieties, with and without interseeded Austrian winter peas.

	Small grain variety[2]					Contrasts; *P* =		
Item	HB	SB	EO	GO	SEM	HB vs SB	HB vs EO	Barley vs Oats
Primary use	*Forage*	*Grain*	*Forage*	*Grain*	---	---	---	---
Growing days	229	225	243	236	---	---	---	---
Agronomic production[3,4]								
DM, tons/hectare[a]	26.5	26.9	31.7	33.0	1.17	0.74	<0.01	<0.01
CP, tons/hectare[a]	1.14	1.14	1.19	1.16	0.15	0.96	0.46	0.49
TDN, tons/hectare[a]	1722.7	1783.5	2017.7	2128.9	76.0	0.43	<0.01	<0.01
Nutrient analysis[4]								
Dry matter, %	89.6	90.3	89.1	89.1	0.50	0.16	0.39	0.03
Crude protein, %[a]	10.7	10.5	9.3	8.6	0.30	0.40	<0.01	<0.01
NDF, %[a]	62.6	59.7	67.6	64.1	1.12	0.01	<0.01	<0.01
ADF, %	36.3	32.0	40.3	37.5	0.81	<0.01	<0.01	<0.01
Ash, %[a]	6.9	6.6	7.9	6.4	0.19	0.23	<0.01	<0.01
TDN, %[5]	64.9	66.3	63.7	64.6	0.25	<0.01	<0.01	<0.01
NE_m, Mcal/kg[6]	1.47	1.51	1.43	1.46	0.01	<0.01	<0.01	<0.01
NE_l, Mcal/kg[5]	1.47	1.51	1.44	1.47	0.01	<0.01	<0.01	<0.01
NE_g, Mcal/kg[6]	0.88	0.92	0.84	0.87	0.01	<0.01	<0.01	<0.01
RFV[7]	90.5	100.7	79.5	87.0	2.44	<0.01	<0.01	<0.01

[1]Varieties were harvested when grain fraction achieved soft dough stage. Samples were allowed to wilt for approximately 45 d prior to nutrient analysis.
[2]Small grain varieties with and without interseeded Austrian winter peas (PEAS). HB = Hooded barley, SB = Strider barley, EO = Everleaf oats, GO = Gray oats.
[3]Calculated based on $0.5m^2$ clipping areas within plots.
[4]Dry matter basis.
[5]Based on published prediction equations for small grain forages, silage, and legumes (Bull, 1981).
[6]Based on published dietary energy equations (NRC, 1996).
[7]Relative Feed Value. Based on equations published by National Feed Ingredients Association (Bolsen, 1991).
[a]PEAS effect ($P < 0.05$).

Table 2. Silage production: Agronomic and nutrient analyses of post-ensiled[1] forage and grain barley and oat varieties, with and without interseeded Austrian winter peas.

	Small grain variety[2]					Contrasts; *P* =		
Item	HB	SB	EO	GO	SEM	HB vs SB	HB vs EO	Barley vs Oats
Primary use	*Forage*	*Grain*	*Forage*	*Grain*	---	---	---	---
Growing days	229	225	243	236	---	---	---	---
Agronomic production[3,4]								
DM, tons/hectare[b]	11.9	12.9	15.0	13.8	0.59	0.10	<0.01	<0.01
CP, tons/hectare[a]	1.54	1.66	1.57	1.28	0.07	0.09	0.65	<0.01
TDN, tons/hectare[a]	780.2	866.9	930.8	847.7	39.5	0.04	<0.01	0.03
Nutrient analysis[4]								
Dry matter, %[c]	39.8	44.5	43.1	37.1	0.71	<0.01	<0.01	<0.01
Crude protein, %[a]	12.9	12.8	10.4	9.3	0.32	0.80	<0.01	<0.01
NDF, %[a]	58.0	58.4	65.4	68.0	1.04	0.72	<0.01	<0.01
ADF, %[a,d]	32.5	30.1	37.6	38.4	0.52	<0.01	<0.01	<0.01
Ash, %	6.9	6.6	7.8	6.6	0.13	0.04	<0.01	<0.01
TDN, %[5,a,d]	65.4	67.0	62.0	61.5	0.34	<0.01	<0.01	<0.01
NE_m, Mcal/kg[6,a,d]	1.48	1.53	1.37	1.36	0.01	<0.01	<0.01	<0.01
NE_l, Mcal/kg[5,a,d]	1.41	1.48	1.27	1.25	0.01	<0.01	<0.01	<0.01
NE_g, Mcal/kg[6,a,d]	0.89	0.94	0.79	0.78	0.01	<0.01	<0.01	<0.01
RFV[7,a,d]	102.3	104.7	85.2	81.0	2.08	0.24	<0.01	<0.01

[1]Varieties were harvested when grain fraction achieved soft dough stage. Samples were allowed to ferment for approximately 120 d prior to nutrient analysis (post-ensiled).
[2]Small grain varieties with and without interseeded Austrian winter peas (PEAS). HB = Hooded barley, SB = Strider barley, EO = Everleaf oats, GO = Gray oats.
[3]Calculated based on 0.5m^2 clipping areas within plots.
[4]Dry matter basis.
[5]Based on published prediction equations for small grain forages, silage, and legumes (Bull, 1981).
[6]Based on published dietary energy equations (NRC, 1996).
[7]Relative Feed Value. Based on equations published by National Feed Ingredients Association (Bolsen, 1991).
[a]PEAS effect ($P < 0.01$).
[b]PEAS effect ($P < 0.05$).
[c]Variety x PEAS interaction ($P < 0.05$).
[d]Variety x PEAS interaction ($P < 0.10$).

Table 3. In-situ rumen digestibility of forage and grain barley and oat varieties, with and without interseeded Austrian winter peas.

	Small grain variety[1,2]					Contrasts; *P* =		
Item	HB	SB	EO	GO	SEM	HB vs SB	HB vs EO	Barley vs Oats
	HAY[3]							
DM digestibility[5]								
6 h	25.1	25.6	21.5	21.8	0.68	0.44	<0.01	<0.01
12 h[a]	28.3	29.3	24.6	23.9	0.61	0.09	<0.01	<0.01
24 h	40.0	41.5	35.1	34.1	0.61	0.02	<0.01	<0.01
NDF digestibility[5]								
6 h[b]	28.6	28.1	24.9	23.4	1.28	0.78	0.03	<0.01
12 h[a]	36.9	34.7	31.1	28.5	1.45	0.22	<0.01	<0.01
24 h	54.0	55.1	47.6	44.8	1.08	0.41	<0.01	<0.01
ADF digestibility[5]								
6 h	27.3	27.2	24.9	23.4	1.32	0.96	0.15	0.01
12 h[b]	35.1	35.1	30.1	28.7	1.21	0.99	<0.01	<0.01
24 h	53.2	54.4	47.5	44.1	0.98	0.32	<0.01	<0.01
	SILAGE[4]							
DM digestibility[5]								
6 h	31.2	28.1	24.2	29.6	1.40	0.03	<0.01	<0.01
12 h[a]	32.8	31.0	27.1	30.1	0.65	<0.01	<0.01	<0.01
24 h[a]	41.2	37.9	35.2	37.7	1.65	0.05	<0.01	0.01
NDF digestibility[5]								
6 h	34.7	29.0	26.8	30.2	1.06	<0.01	<0.01	<0.01
12 h[b,c]	39.2	36.1	33.4	36.3	1.50	0.10	<0.01	0.05
24 h	54.2	49.9	46.0	49.1	2.12	0.11	<0.01	0.02
ADF digestibility[5]								
6 h[a]	33.2	28.8	26.7	29.7	0.90	<0.01	<0.01	<0.01
12 h[b]	38.4	34.8	32.5	35.6	1.41	0.05	<0.01	0.05
24 h[b,d]	52.8	48.5	45.5	48.6	2.24	0.13	0.01	0.08

[1]Small grain varieties with and without interseeded Austrian winter peas (PEAS). HB = Hooded barley, SB = Strider barley, EO = Everleaf oats, GO = Gray oats.
[2]Values are on dry matter basis.
[3]Varieties were harvested when grain fraction achieved soft dough stage. Samples were allowed to wilt for approximately 45 d prior to nutrient analysis.
[4]Samples were allowed to ferment for approximately 120 d prior to nutrient analysis (post-ensiled).
[5]Hours post-feeding.
[a]PEAS effect ($P < 0.01$).
[b]PEAS effect ($P < 0.05$).
[c]Variety x PEAS interaction ($P < 0.05$).
[d]Variety x PEAS interaction ($P < 0.10$).

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INFLUENCE OF RUMINALLY-UNDEGRADABLE PROTEIN SUPPLEMENTATION AND ADVANCING GESTATION ON FORAGE USE AND PERFORMANCE BY BEEF COWS CONSUMING LOW-QUALITY, WARM SEASON FORAGE

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ABSTRACT: Concurrent experiments were conducted to evaluate the effects of ruminally-undegradable protein (**RUP**) supply and advancing gestation on low-quality forage use by spring-calving beef cows. Cows were fed 1 of 3 supplements daily that supplied similar amounts of ruminally-degradable protein (**RDP**; 0.09% BW) and increasing amounts of RUP: 0.05% BW (**LOW**), 0.07% BW (**MOD**), or 0.09% BW (**HI**). Supplements were formulated such that RDP was equal to 11% of predicted digestible OM intake and were fed for 15-wk before expected onset of calving. In trial 1, 18 pregnant cows (initial BW = 426 ± 32 kg) were fed tallgrass prairie hay (2.1% CP, 54% ADF) for ad libitum intake. Intake and digestion (via ADIA) were measured weekly between wk 14 and 4 pre-partum. Forage DMI, total DMI, and total digestible DMI of cows fed LOW was greater ($P < 0.01$) than that of cows fed MOD and HI. Total tract DM digestibility by cows fed HI (52.4%) was greater ($P < 0.01$) than by cows fed LOW (51.8%), although the difference was not large; MOD was intermediate. Forage DMI, total DMI, and total digestible DMI increased ($P < 0.03$) cubically between 14 and 4 wk pre-partum, while total tract DM digestibility decreased ($P = 0.01$) linearly over time. In trial 2, 117 pregnant beef cows (initial BW = 526 ± 52 kg) grazing dormant native tallgrass pastures (2.3% CP, 54% ADF) were assigned randomly to the same supplements and feeding rates used in trial 1. Cow ADG ($P = 0.12$) and BCS change ($P = 0.14$) did not differ among treatments during the pre-partum period. Subsequent pregnancy rate ($P = 0.62$) and calving interval ($P = 0.72$) did not differ among treatments. Under the conditions of our study, pregnant cows consuming low-quality warm-season forage and fed supplemental RDP at 0.09% of BW daily and RUP at 0.05% of BW daily appeared to receive sufficient MP to maximize performance within the constraints of energy supply. Cows appeared to compensate for the nutritional demands of pregnancy by increasing forage intake between 14 and 4 wk pre-partum but DM digestibility decreased as DMI increased.

Key Words: forage, ruminally-undegradable protein, supplementation

Introduction

Pre- and postpartum deficiencies of MP have been identified as potentially limiting to productivity of the beef cow and calf. Pre-partum supplementation of forage-based diets with RUP increased weight gain (Dhuyvetter et al., 1993; Lalman et al., 1993) by beef cows and heifers. Similarly, postpartum RUP supplementation of forage-fed beef cows increased breeding performance (Wiley et al., 1991; Triplett et al., 1995). Feeding levels of RDP were not clearly defined in these studies. It is unclear if RDP supply was sufficient to maximize ruminal OM digestion among animals fed low-levels of supplemental MP.

Forbes (1970) reported that advancing gestation was associated with changes in nutrient requirements, dietary intake, ruminal passage rate, and ruminal nutrient digestion. The nature of these relationships may be specific to particular diet types and supplementation regimens.

Our objectives were 1) to determine the value of supplementing RUP when dietary RDP supply was estimated to be adequate to support normal ruminal fermentation and 2) to monitor the changes in intake and digestion that precede parturition in beef cows fed low-quality, warm-season forage.

Materials and Methods

Two concurrent experiments were conducted at the beef cattle research facilities located adjacent to the campus of Kansas State University, Manhattan. Animal care practices were approved by the Kansas State University Animal Care and Use Committee. Water and a commercial salt-based, trace-mineral supplement (not less than 98% NaCl, 0.2% Mn, 0.1% Fe, 0.1% Mg, 0.05% Cu, 0.01% Co, 0.008% Zn, and 0.007% I) were available to animals ad libitum. Animals were maintained in enclosures open to the external environment (Trial 1) or on native tallgrass pasture (Trial 2).

Cows used in both experiments were fed 1 of 3 supplements daily that supplied similar amounts of ruminally-degradable protein (**RDP**; 0.09% BW) and increasing amounts of RUP: 0.05% BW (LOW), 0.07% BW (MOD), or 0.09% BW (HI). To estimate appropriate feeding rates for supplements, OM intake and OM digestion of tallgrass prairie forage during gestation were estimated to average 1.8% of body weight and 54%, respectively, based on observations by Vanzant et al. (1991). In addition, Köster et al. (1996) determined that a RDP intake equal to 11% of total digestible OM intake (TDOMI) resulted in maximal TDOMI by non-pregnant, dry cows consuming forage similar to that used in our study. Therefore, supplements were all formulated to supply RDP in such a way as to to maintain approximately the ratio of RDP intake:TDOMI suggested by Köster et al. (1996; i.e., 0.11).

Trial 1. Pregnant Angus × Hereford cows (n = 18; initial BW = 426 ± 32 kg; initial BCS = 4.5 ± 0.45) were used in a 3-treatment, randomized complete block

experiment to examine the effects of supplemental RUP and advancing gestation on forage intake and digestion. Cows were blocked on the basis of BCS and assigned randomly to be fed 1 of the 3 supplements described previously (Table 1). Cows were housed in 24 individual, adjacent pens (1.5 X 6.7 m) equipped with feed bunks and automatic watering cups. Pens were covered partially but exposed to ambient air and wind.

Tallgrass prairie hay was harvested in late summer from a stand of native prairie dominated by big bluestem (*Andropogon gerardii* Vitman), indiangrass (*Sorghastrum nutans* [L.] Nash), little bluestem (*Schizachyrium scoparium* [Michx.] Nash), sideoats grama (*Bouteloua curtipendula* [Michx.] Torr.), and switchgrass (*Panicum virgatum* L.). Hay was chopped to 15-cm particle length before feeding (Table 2).

Table 1. Supplement composition (Exp. 1 & 2)

	RUP Level[a]		
Ingredient	LOW	MOD	HI
Dry matter, %	90.8	91.5	93.4
--------- Feed composition, % DM ---------			
Blood meal	0.1	4.7	9.3
Corn Gluten Meal	0.2	6.5	12.9
Soybean meal	71.5	63.3	55.1
Sorghum grain	23.8	21.1	18.3
Molasses	4.4	4.4	4.4
---------- Chemical composition, % DM ----------			
OM	93.4	94.4	93.4
NDF	20.3	13.2	12.7
ADF	6.6	6.5	6.8
ADIA	0.44	0.31	0.32
CP	41.25	45.63	48.75
---------- Protein composition, % CP ----------			
RDP	63.4	57.4	52.4
RUP	36.6	42.6	47.6

[a] Supplements contained similar amounts of RDP (0.09% BW) and increasing amounts of RUP: 0.05% BW (LOW), 0.07% BW (MOD), or 0.09% BW (HI).

Table 2. Forage composition (Exp 1 & 2)

		Chemical composition, % DM				
Forage	DM	OM	NDF	ADF	ADIA	CP
Exp. 1	94.7	93.1	79.0	53.8	5.15	2.13
Exp. 2	95.0	92.7	79.2	54.3	5.67	2.31

Supplements were fed during the 15-wk period preceding the average date of parturition (March 9 $\pm$ 13 d). Feeding commenced on November 24 and concluded when all cows had calved. Supplements were fed to cows at 0600 daily. Tallgrass prairie hay was offered at 0700 at 130% of the previous 5-d average intake. Beginning on December 1 and continuing until February 8, subsamples of hay, supplement, and orts were collected daily (approximately 3% of daily total). Fecal grab samples were also collected (0800 daily). Sample collection corresponded to the period spanning 14 through 5 weeks pre-partum.

Cows were weighed and evaluated for body condition (1 to 9 scale) at monthly intervals during the trial. On February 9, cows were removed from pens and placed onto a native tallgrass prairie pasture. Measurement of intake was discontinued at that time; however, supplementation continued until calving.

Forage, supplement, and fecal samples were dried in a forced-air oven (96 h; 50 °C), weighed, and ground (No. 4 Wiley mill, Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen. Daily samples of hay and supplement were composited over the entire trial. Ort and fecal grab samples were composited on an equal-weight basis within animal and week of the trial. Samples were analyzed for DM (12 h; 105 °C), OM (8 h; 450 °C), Kjeldahl N. Samples were analyzed also for NDF (without amylase or sulfite), ADF, and acid detergent insoluble ash (ADIA) using procedures described by Van Soest et al. (1991).

Hay intakes by individual animals were summarized as 10 weekly means beginning on December 1 and ending on February 8. Proportional intakes (% BW) were expressed using individual animals' average BW for each month of the trial. Fecal output estimates for individual cows, corresponding to weekly intake averages, were made using ADIA as an internal marker.

Forage intake and total-tract digestion data were analyzed using models appropriate for a randomized complete block experiment with repeated measures (Proc MIXED; SAS Inst. Inc., Cary, NC). Models included terms for treatment, block, period, and appropriate interactions. No interaction effects were detected; therefore, main effects of treatment and advancing gestation were reported. When protected by a significant F test ($P < 0.05$), least squares treatment means were separated using the method of Least Significant Difference. Trends in intake and digestion that occurred with advancing gestation were evaluated using orthogonal contrasts.

Trial 2. Pregnant Angus × Hereford cows (n =117; initial BW = 526 ± 52 kg; initial BCS = 5.2 ± 0.60) were used in a randomized complete block experiment to examine the effects of supplemental RUP on animal performance. Cows were stratified by weight and BCS and assigned randomly to receive 1 of the 3 supplements evaluated in trial 1 (Table 1). Feeding rates of supplements were identical to those in trial 1.

Within treatment, cows were assigned randomly to graze one of three native tallgrass pastures. Dominant pasture plants were big bluestem (*Andropogon gerardii* Vitman), indiangrass (*Sorghastrum nutans* [L.] Nash), little bluestem (*Schizachyrium scoparium* [Michx.] Nash), sideoats grama (*Bouteloua curtipendula* [Michx.] Torr.), and switchgrass (*Panicum virgatum* L.). Stocking rate was approximately 3.1 ha/cow.

Standing forage biomass and chemical composition of pasture forage were estimated from hand-clipped samples collected on November 29 and April 3. Chemical composition was measured using methods described for trial 1 and expressed as an average of the two clipping dates (Table 2). Standing forage biomass averaged 3,303 kg/ha during the trial. This was interpreted to indicate that forage availability did not limit forage intake at the stocking rates used during our trial.

Cows were gathered from the pastures each morning (0700 to 1000) and sorted into respective treatment groups. Supplements were group-fed and cows were returned immediately to their assigned pastures. This process was

repeated daily from November 25 until all cows had calved (average calving date = March 7 $\pm$ 13 d). Once calving occurred, treatments were discontinued and all cows were moved to a separate pasture.

Cows were weighed and assigned a BCS (1 to 9 scale) at 4-wk intervals until calving was complete. Performance of calves was monitored from birth until weaning the following fall. Pregnancy status of each cow was ascertained by rectal palpation the following October.

Cow and calf performance were analyzed as a randomized complete block (Proc MIXED; SAS Inst. Inc., Cary, NC). Models included terms for treatment, pasture, period, and treatment within pasture. Treatments within individual pastures were the experimental unit. When protected by a significant F test ($P < 0.05$), least squares treatment means were separated using the method of Least Significant Difference.

Pregnancy rates were analyzed using PROC CATMOD (SAS Inst. Inc., Cary, NC). Least Squares means for pregnancy rates were reported.

Results and Discussion

Trial 1. Forage DMI, total DMI, and total digestible DMI of cows fed LOW was greater ($P < 0.01$; Table 4) than that of cows fed MOD and HI. Total tract DM digestibility did not differ ($P > 0.10$) between treatments; however, total-tract NDF digestibility by cows fed LOW and HI was greater ($P \leq 0.05$) than that by cows fed MOD.

The likelihood of a response to supplemental RUP supplementation seems dependent upon adequacy of RDP supply. Among pregnant mature cows fed at least 200 g RDP/d, the addition of supplemental RUP had no effect on intake during the last third of gestation (Hunter and Magner, 1988; Van Saun et al., 1993; Sletmoen-Olson et al., 2000). Conversely, intake decreased with increasing RUP supplementation when RDP supply was inadequate (Hibberd and Martin, 1990).

Forage DMI, total DMI, and total digestible DMI increased ($P \leq 0.03$) cubically between 14 and 4 wk pre-partum, while total tract DM and NDF digestibilities decreased ($P \leq 0.03$) linearly over time (Table 5). Forbes (1970) noted that increased body weight and nutrient requirements coincident with advancing gestation stimulated dietary intake until fetal tissues reach sufficient size to begin to compress the rumen. Bauman and Currie (1980) indicated that fetal size in the bovine increased logarithmically, with approximately 50% of fetal growth occurring during the last 35 d of pregnancy. Simultaneously, ruminal digesta content and capacity decrease (Stanley et al., 1993).

The scenario suggested by Forbes (1970) has been supported by some work (Campling, 1966; Penzhorn and Meintjes, 1972; Vanzant et al., 1991); however, conflicting data exist. Hunter and Siebert (1986) reported that forage DM intake was similar in pregnant and non-pregnant cows from 12 to 1 wk pre-partum. Conversely, pregnant shorthorn cows consistently ate less grass hay or silage DM than non-pregnant counterparts during the last 4 months of gestation (Jordan et al., 1973).

Data from our study generally agree with Forbes (1970); however, intake was not measured beyond 5-wk pre-partum. Stanley et al. (1993) reported that forage intake among pregnant cows of the same genotype used in our study continued to increase until calving, even though ruminal capacity decreased. The cattle in their study appeared to compensate for reduced ruminal capacity via increased rate of passage.

Trial 2. Cow ADG and BCS change did not differ ($P \geq 0.13$) among treatments during the pre-partum period (Table 6). In other circumstances, pre-partum RUP supplementation increased cow BCS (Van Saun et al., 1993) and weight gain (Dhuyvetter et al., 1993; Lalman et al., 1993). A possible explanation for discrepancies in response to RUP supplementation is variation in MP balance. A positive response to RUP supplementation is more likely when MP supply is inadequate to support the level of performance allowed by the dietary energy provided. In our trial, it appeared that MP supply was sufficient to maximize performance within the constraints of energy supply.

Subsequent Julian calving date, pregnancy rate and calving interval were not different ($P \geq 0.62$) among treatments. Pregnancy rate has not usually been influenced by RUP supplementation (Wiley et al., 1991; Dhuyvetter et al., 1993; Triplett et al., 1993); however, post-partum interval has been reduced by supplemental RUP in some cases (Wiley et al., 1991; Triplett et al., 1995).

Pre-partum supplementation with RUP did not affect ($P \geq 0.55$) calf birth weight, ADG, or weaning weight. Conversely, Rusche et al. (1993) reported that post-partum RUP supplementation increased calf ADG, possibly through increased milk production. Other researchers reported that RUP had no effect on calf gain (Sletmoen-Olson et al., 2000).

Implications

Pregnant cows consuming low-quality tallgrass forage and supplemented with common feeds to provide RDP at 0.09% of body weight daily appeared to have been fed sufficient MP to maximize performance within the constraints of energy supply. Therefore, altering supplemental protein composition in order to provide additional RUP under such conditions is not warranted. Cows appeared to compensate for the nutritional demands of pregnancy by consuming more forage DM. Increased forage intake in response to advancing pregnancy was interpreted to suggest that the quantity of supplemental RDP required to maximize forage intake and digestion may increase prior to parturition.

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Table 4. Effects of RUP supplementation on intake and digestibility by pregnant beef cows fed low-quality, warm-season forage (Exp. 1)

	RUP Level[a]			
Item	LOW	MOD	HI	SEM
Total-tract DM digestibility, %	51.8	51.8	52.4	0.27
Total-tract NDF digestibility, %	58.4[b]	57.6[c]	58.4[b]	0.24
Forage DM intake, % BW	2.31[b]	2.14[c]	2.10[c]	0.02
Total DM intake, % BW	2.61[b]	2.45[c]	2.42[c]	0.02
Total digestible DM intake,% BW	1.36[b]	1.27[c]	1.26[c]	0.01

[a] Supplements contained similar amounts of RDP (0.09% BW) and increasing amounts of RUP: 0.05% BW (LOW), 0.07% BW (MOD), or 0.09% BW (HI).
[b, c] Means within rows having common superscripts do not differ ($P < 0.05$)

Table 5. Effects of decreasing time to parturition on intake and digestibility by beef cows fed low-quality, warm-season forage (Exp. 1)

	Week relative to average calving date											*P*-value		
Item	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	SEM	Lin	Quad	Cubic
Total tract digestibility														
DM, %	54.0	53.0	52.6	52.1	52.1	51.8	51.6	50.8	51.1	50.5	0.48	0.01	0.36	0.39
NDF, %	58.7	58.5	58.4	58.0	58.3	58.3	58.3	57.2	57.9	57.5	0.51	0.03	0.81	0.88
Intake														
Forage DM, % of BW	1.67	1.98	2.08	2.08	2.18	2.28	2.41	2.23	2.44	2.50	0.04	0.01	0.01	0.01
Total DM, % of BW	1.98	2.29	2.39	2.39	2.48	2.58	2.72	2.54	2.75	2.80	0.04	0.01	0.01	0.01
TDDMI, % of BW	1.06	1.20	1.25	1.24	1.29	1.34	1.40	1.29	1.43	1.45	0.02	0.01	0.01	0.03

Table 6. Effects of RUP supplementation on cow and calf performance (Exp. 2)

	RUP Level[a]			
Item	LOW	MOD	HI	SEM
Cow				
ADG, kg/d	0.10	0.07	0.02	0.033
BCS change	-0.19	-0.20	-0.39	0.094
Julian calving date	68	66	64	2.2
Pregnancy rate, %	95	95	92	4.4
Calving interval, d	364	368	366	3.6
Calf				
Birth weight, kg	41	39	39	0.9
Weaning weight, kg	244	245	243	5.7
ADG (birth to wean), kg/d	0.99	0.99	0.97	0.02

[a] Supplements contained similar amounts of RDP (0.09% BW) and increasing amounts of RUP: 0.05% BW (LOW), 0.07% BW (MOD), or 0.09% BW (HI).

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BOTANICAL COMPOSITION OF DIETS GRAZED BY MATURE, LACTATING COWS WITH CALVES AND MATURE, NON-LACTATING COWS MAINTAINED ON EITHER BURNED OR UNBURNED NATIVE TALLGRASS PRAIRIE

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ABSTRACT: Our objective was to compare diet selection preferences of 32 mature, lactating beef cows (**L**; initial BW = 566 ± 55.9 kg) suckling calves with 32 mature, non-pregnant, non-lactating beef cows (**NL**; initial BW = 551 ± 53.2 kg) grazing burned or unburned native Tallgrass prairie during summer. Our study was conducted on 8 pastures (97 ± 39.9 ha); 4 were burned in mid-April and 4 had no recent burning history. Grazing commenced May 15. Predominant forage species were Andropogon *geradii*, Schizachyrium *scoparium*, Bouteloua *curtipendula* (BC), Bouteloua *gracillis* (BG), Panicum *virgatum* (PV), Sorghastrum *nutans* (SN), Amorpha *canescensi* (AC), Symphyotrichum *ericoides* (SE), Liatris *punctata* (LP), and Dalea *purpurea* (DP). Four L and 4 NL cows were grouped randomly and assigned to graze a single burned or unburned pasture for 120 d. Fecal samples were collected from each animal on d 30, 60, 90, and 120 of the grazing period. Range-plant fragments in fecal samples were quantified using a modified microhistological technique; plant fragment prevalence in fecal material was assumed to equal % botanical composition of the diet. Selection of SN decreased ($P < 0.01$) over time while selection of PV, BG, AC, and SE increased ($P < 0.01$) over time. Cows selected more PV, AC, and DP ($P < 0.03$) in unburned pastures than in burned pastures. Conversely, cows selected more ($P < 0.01$) BC in burned pastures than in unburned pastures. Total graminoid selection was greater ($P < 0.01$; 74.2%) on burned than unburned pastures (71.8%). In contrast, selection of forbs was greater ($P < 0.01$; 28.2%) on unburned than burned pastures (25.8%). Cows tended to select more (burn × period; $P < 0.09$) PV and AC in unburned pastures and selected more (burn × period; $P < 0.01$) BC in burned pastures over time. There were no differences ($P > 0.05$) in diet selection patterns between **L** and **NL** cows. Under the conditions of our study, botanical composition of beef cow diets was influenced by spring burning of native Tallgrass pastures but was not influenced by lactation and pregnancy status.

Key Words: beef cows, botanical composition, diet selection, grazing

Introduction

The diet selection process is dynamic because of changes in animal and plant characteristics (Wallace et al., 1972; Volesky et al., 2007). Lactating animals have greater energy requirements than non-lactating animals. Farruggia et al. (2006) reported lactating beef cows grazed more selectively than non-lactating dry cows and suggested greater selectivity was the result of the animal searching to fulfill specific nutrient needs to support lactation. Lactating ewes (Penning et al., 1995) and lactating dairy cows (Gibb et al., 1999) grazed for longer periods of time and had greater intakes than their non-lactating counterparts. Conversely, Parsons et al. (1994) reported no differences in diet selection between lactating and non-lactating ewes.

Microhistological analysis of fecal material is a widely used method for quantifying the botanical composition of a grazing animal's diet since first described by Baumgartner and Martin in 1939 (Holechek, 1982; Alipayo et al., 1992). Little research has been conducted on how diet selection preferences of lactating beef cows with suckling calves and non-lactating beef cows are influenced by prescribed burning. Our objective was to characterize differences in diet selection between lactating beef cows suckling calves and non-pregnant, non-lactating beef cows grazing either burned or unburned native tallgrass prairie during summer.

Materials and Methods

All procedures used in the care and handling of animals in our study were approved by the Kansas State University Institutional Animal Care and Use Committee.

Our study was conducted at the Kansas State University Commercial Cow-Calf Unit located approximately 5 km northwest of Manhattan, KS. Pasture forage species composition was described by Towne and Owensby (1984) and included big bluestem (*Andropogon geradii*), little bluestem (*Schizachyrium scoparium*), sideoats gramma (*Bouteloua curtipendula*), blue gramma (*Bouteloua gracilli*), switchgrass (*Panicum virgatum*), indian grass (*Sorghastrum nutans*), lead plant (*Amorpha*

canescens), heath aster (*Symphyotrichum ericoides*), dotted gayfeather (*Liatris punctata*), and purple prairie clover (*Dalea purpurea*). Average chemical composition of the pasture forage during the study is shown in Table 1.

Table 1. Average chemical composition of native tallgrass-prairie forage grazed by beef cows and calves during summer

Item	June 15	July 15	August 15	September 15
DM %	92.8	92.6	93.3	93.9
OM %	8.5	9.5	8.4	8.8
CP, %DM	10.2	7.9	5.3	4.3
NDF, %DM	61.5	63.3	68.8	69.7
ADF, % DM	40.0	40.8	44.2	44.9

We compared botanical composition of diets selected by 32 mature, pregnant, lactating beef cows suckling calves (**L**; initial BW = 566 ± 55.9 kg) with 32 mature non-pregnant, non-lactating beef cows (**NL**; initial BW = 551 ± 53.2 kg). Our study was conducted on 8 native tallgrass pastures (97 ± 39.9 ha); 4 were burned in mid-April and 4 had no recent burning history. Grazing commenced May 15. Four L and 4 NL cows were grouped randomly by treatment and assigned to graze a single burned or unburned pasture for 120 d. The L and NL cows were allowed to comingle within pastures and remained in their assigned pasture throughout the study. Water, salt, and a granular, salt-based mineral supplement (17% NaCl, 16% Ca, 8% P, 0.2% Mg, 3,300 ppm Zn, 1,200 ppm Cu, and 0.22 ppm Se) were available to cattle continually.

Cows were gathered into a corral and fecal grab samples were collected from each animal on d 30, 60, 90, and 120 of the grazing period. Each grab sample was hand-mixed to ensure homogeneity and a 40-g subsample was retained for analysis.

Sample preparation was conducted as described by Eckerle et al. (2009). Wet fecal samples were soaked overnight in 50% EtOH (v/v). After soaking, samples, EtOH was decanted and samples were homogenized and washed with de-ionized H_2O through a No. 200 US-standard sieve to remove contaminants. Samples were then re-homogenized, strained, and dried at 55°C for 96 h. Dried samples were ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1-mm screen and stored for slide preparation (Bennett et al., 1999).

Slide preparation methods were described by Eckerle et al. (2009). Subsamples (0.5 g) of dried, ground, and wash fecal material were soaked in de-ionized H_2O for 1 h to soften them. Approximately 20 mL of NaOH (0.05M) was then added to each sample. Samples were incubated for 20 min at room temperature to destroy plant pigments. Samples were subsequently rinsed with de-ionized H_2O over a No. 200 US-standard sieve to remove NaOH and then homogenized in a blender with 20 mL of de-ionized H_2O for 1 min. Samples were rinsed a second time over a No. 200 US-standard sieve.

Samples were placed on slides using an eyedropper, 1 to 3 drops of Hertwig's solution was applied, and the slide was placed over a propane flame until dry. One to 2 drops of Hoyer's solution was added to mount a cover slip. Slides were dried for 96 h in a 55°C-oven before viewing.

Slides were viewed on a compound microscope at 10 × magnification. The microscope was equipped with a digital camera; each slide field was photographed for comparison with standard slides (Eckerle et al., 2009). Twenty fields per slide were selected randomly from the entire slide view and were used to measure the frequency with which plant fragments appeared (Holechek and Vavra, 1981). Individual plant species were identified according to their histological characteristics. Big bluestem and little bluestem were grouped together for the purposes of analysis because of histological similarities.

Plant fragment prevalence in slide fields was assumed to be equivalent to prevalence in fecal samples and equivalent to % botanical composition of the diets grazed by beef cows (Sparks and Malechek, 1968). Plant fragments that were not among the 10 predominant range plants for which standards were prepared were classified as either an unknown grass or an unknown forb; however, these were present in trace amounts only and were not reported.

Data were analyzed as a mixed-model, completely randomized design with a split-split plot using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC). Class variables included burn regime, pasture, treatment, animal, and period. The MODEL statement included terms for: burn regime, treatment, treatment x burn regime, period, period × burn regime, treatment × period, and treatment × period × burn regime. The RANDOM statement included terms for: pasture within burn; treatment × pasture within burn; animal within burn, pasture, and treatment; period × pasture within burn; period × treatment × pasture within burn; and period × animal within burn, pasture, and treatment. Type-3 F-tests were used to test for differences for all fixed effects. Main effects of treatment and period were reported when treatment × period effects ($P \geq 0.10$) were not detected. Means were separated using the method of Least Significant Difference and were reported with pooled standard errors. Means were considered different when $P < 0.10$.

Results and Discussion

The objective of our study was to characterize differences in diet selection between mature pregnant, lactating beef cows with suckling calves and mature non-pregnant, non-lactating beef cows grazing either burned or unburned native tallgrass prairie during summer. We speculated that during the summer grazing season, lactating cows with calves and non-lactating cows would display distinctive preferences for certain species. Furthermore, we anticipated that these diet selection preferences might be influenced by prescribed burning.

There were no treatment differences ($P \geq 0.11$) in the botanical diet composition between lactating and non-lactating cows (Table 2). Similar findings were reported

by Parsons et al. (1994) who found no differences in diet composition between lactating ewes and non-lactating ewes. Conversely, Farruggia et al. (2006) reported lactating cows grazed more selectively than non-lactating, non-pregnant cows. Rook et al. (2004) suggested that lesser maintenance requirements could result in less selective foraging behaviors by non-lactating compared to lactating ruminants.

Cows consumed more (P = 0.01; 74.2 vs. 71.8%, respectively) grasses and fewer (P = 0.01; 25.8 vs. 28.2%, respectively) forbs on burned pastures compared to unburned pastures (Table 3). McGinty et al. (1983) reported greater selection of forbs on unburned pastures compared to burned pastures because forb availability was reduced by burning. Cows ate more ($P < 0.01$) sideoats grama and less ($P \leq 0.02$) switchgrass, leadplant, and purple prairie clover on burned pastures than unburned pastures. Prescribed burning is widely practiced in the Kansas Flint Hills to remove standing, dead growth and litter, to improve weight gains of stocker cattle, to manipulate plant species composition, and to eradicate invasive woody plants (Launchbaugh and Owensby, 1978; Towne and Owensby, 1984; Gibson and Hulbert, 1987).

As the grazing season progressed, selection of switchgrass increased (burn × period effect, P = 0.09) sharply in both burned and unburned pastures, whereas selection of sideoats grama generally decreased (burn × period effect, $P < 0.01$; Table 4). Selection of leadplant doubled (burn × period effect, P = 0.04) on burned pastures month-by-month but selection was inconsistent in unburned pastures. Selection of dotted gayfeather ranged from 12.3 to 20.4% of the diet in June, July, and August and diminished to 8.5 to 8.9% in September (burn × period effect, P = 0.05).

Cows selected more ($P < 0.01$) switchgrass, sideoats grama, leadplant, and heath aster over time, whereas they selected less ($P < 0.01$) indiangrass over time (Table 5). Palabitility is a major factor driving selection preferences by grazing herbivores (Vallentine, 1990) and is reduced as plants approach reproductive maturity and dormancy (Holechek et al., 2001). Under unrestricted grazing conditions, herbivore preference for specific forage plants is known to change over time (Parsons et al., 1994; Hester et al., 1999; Rutter et al., 2004; Soder et al., 2007). The cows used in our study may have modified their diets over time to select greater proportions of plants that were slower to reach maturity. Alternatively, decreased consumption over time may have been related to diminishing availability or regrowth of certain forage plants.

Consumption of all grasses and all forbs changed slightly ($P < 0.01$, Table 5) from month to month during the grazing season; however, the relative proportions of grasses and forbs remained consistently within the range of 71 to 75% grasses and 25 to 29% forbs.

Implications

The botanical composition of diets grazed by beef cows during summer in the Kansas Flint Hills was influenced by prescribed spring burning but was not influenced by lactation status. We interpreted these data to suggest that forage selection preferences of beef cows can be altered with spring burning of native tallgrass pastures. Further research in this area appears warranted.

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Table 2. Effect of collection period on botanical composition of diets (%) selected by lactating cows with calves or non-lactating, non-pregnant cows grazing the Kansas Flint Hills during summer

						P-Values		
Item	June 15	July 15	August 15	September 15	SEM	Trt	Period	Trt × Period
Total grasses, %								
Lactating	74.1	70.8	72.3	72.7	1.03	0.18	< 0.01	0.45
Non-lactating	76.7	71.3	73.6	72.4				
Big bluestem + little bluestem, %								
Lactating	6.1	12.9	13.9	10.9	1.15	0.37	< 0.01	0.15
Non-lactating	8.2	11.9	13.2	11.8				
Indiangrass, %								
Lactating	50.4	37.7	35.2	29.4	1.36	0.12	< 0.01	0.60
Non-lactating	51.6	37.6	38.3	31.3				
Switchgrass, %								
Lactating	4.2	4.4	7.5	10.4	0.76	0.88	< 0.01	0.41
Non-lactating	3.7	5.0	7.1	10.8				
Blue grama, %								
Lactating	1.8	2.3	5.4	14.9	0.51	0.83	< 0.01	0.16
Non-lactating	2.5	2.5	4.5	10.8				
Sideoats grama, %								
Lactating	8.6	11.7	8.6	4.7	0.69	0.94	< 0.01	0.20
Non-lactating	7.5	11.9	8.67	5.5				
Total forbs, %								
Lactating	25.9	29.2	27.7	27.3	1.03	0.18	< 0.01	0.45
Non-lactating	23.3	28.7	26.4	27.6				
Purple prairie clover, %								
Lactating	7.4	6.9	6.9	7.9	0.80	0.92	0.25	0.88
Non-lactating	7.5	6.5	6.9	8.5				
Leadplant, %								
Lactating	0.6	0.8	1.7	3.4	0.43	0.25	< 0.01	0.41
Non-lactating	0.9	0.9	1.5	4.0				
Dotted gayfeather, %								
Lactating	15.5	19.8	15.9	8.8	1.02	0.11	< 0.01	0.35
Non-lactating	12.7	19.5	15.1	8.6				
Heath aster, %								
Lactating	0.6	0.9	2.5	6.2	0.69	0.63	< 0.01	0.07
Non-lactating	1.0	0.9	2.1	5.2				

Table 3. Main effects of pasture burning regime on botanical composition of diets selected by beef cows grazing the Kansas Flint Hills during summer

Item	Burned	Unburned	SEM	*P*-Value
Total grasses, %	74.2	71.8	0.52	0.01
Switchgrass, %	5.3	7.2	0.27	< 0.01
Sideoats grama, %	9.0	7.1	0.26	< 0.01
Total forbs, %	25.8	28.2	0.52	0.01
Leadplant, %	1.1	1.7	0.12	< 0.01
Purple prairie clover, %	6.4	8.3	0.50	0.02

Table 4. Burn regime × collection period effects on botanical composition of diets selected by beef cows grazing the Kansas Flint Hills during summer

Item	June 15	July 15	August 15	September 15	SEM	*P*-Value
Switchgrass, %						
Burned	2.9	4.2	6.3	9.9	0.80	0.09
Unburned	5.2	5.3	8.5	11.4		
Sideoats grama, %						
Burned	10.1	13.8	8.9	5.2	0.78	< 0.01
Unburned	6.4	10.0	8.4	5.0		
Leadplant, %						
Burned	0.4	0.8	1.5	3.2	0.37	0.04
Unburned	1.2	0.9	1.7	4.2		
Dotted gayfeather, %						
Burned	16.0	18.9	15.8	8.9	1.04	0.05
Unburned	12.3	20.4	15.2	8.5		

Table 5. Main effect of collection period on botanical composition of diets selected by beef cows grazing the Kansas Flint Hills during summer

Item	June 15	July 15	August 15	September 15	SEM	*P*-Value
Total grasses, %	75.5	71.1	73.0	72.5	0.71	< 0.01
Indiangrass, %	51.0	37.7	36.7	30.3	0.97	< 0.01
Switchgrass, %	3.9	4.7	7.3	10.6	0.51	< 0.01
Blue grama, %	2.1	2.4	5.0	12.7	1.23	< 0.01
Total forbs, %	24.5	28.9	27.0	27.5	0.7	< 0.01
Leadplant, %	0.7	0.8	1.6	3.7	0.30	< 0.01
Heath aster, %	0.8	0.9	2.3	5.7	0.61	< 0.01

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EFFECT OF CALVING PERIOD ON ADG, REPRODUCTION, AND FIRST CALF CHARACTERISTICS OF HEIFER PROGENY

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ABSTRACT: Records from 1997 through 2009 were used to determine the effect of calving date on ADG, reproduction, and first calf characteristics in spring born heifer calves (n = 1,019) at the Gudmundsen Sandhills Laboratory near Whitman, NE. Heifers were classified as being born in the first, second, or third 21-d period of the calving season within year. Continuous data were analyzed using MIXED procedure of SAS and binomial data with GLIMMIX. Calf birth BW was lower ($P < 0.01$) for calves born in the first period compared to the second or third. Calf ADG from birth to weaning tended ($P = 0.10$) to be least for heifers born in the first calving period. Calf weaning BW decreased ($P = 0.03$) with advancing calving period. Calf ADG from weaning to prebreeding tended ($P = 0.07$) to be least for heifers born in the first period; however, prebreeding BW was greatest ($P < 0.01$) for calves born in the first period. Heifer ADG from the beginning of the breeding season to pregnancy diagnosis was greater ($P = 0.03$) for heifers born in the third vs. first calving period. The percentage of heifers cycling at the beginning of the breeding season decreased ($P < 0.01$) with advancing calving date (70, 58, and 39%, respectively) and 45 d pregnancy rates were lowest ($P = 0.02$) for heifers born in the third calving period (90, 86, and 78%, respectively). Birth date of the heifer's first calf and birth BW decreased ($P < 0.01$) if the heifer was born in the first calving period. Also, more ($P < 0.01$) calves were born in the first 21 d of the calving season if the heifer herself was born in the first calving period. First calf progeny had the greatest ($P \leq 0.10$) weaning BW if born to a heifer born in the first calving period. Heifer calves born during the first 21 d of the spring calving season had greater weaning, prebreeding, and precalving BW; greater percent cycling before breeding, and greater pregnancy rates compared to heifers born in the third calving period. First calf progeny also had earlier birth date and greater weaning BW. Calving period of heifer progeny significantly impacts development and first calf characteristics.

Key Words: ADG, Beef Cattle, Reproduction

Introduction

Rate of postweaning growth has been determined to be an important factor affecting age of puberty in heifer calves, which in turn influence pregnancy rates (Arije and Wiltbank, 1971; Ferrell, 1982; Short and Bellows, 1971; Wiltbank et al., 1966). This and other research conducted during the late 1960s through the early 1980s indicated puberty occurs at a genetically predetermined size, and only when heifers reach their target weight can high pregnancy rates be obtained (reviewed by Patterson et al., 1992). Guidelines were established indicating replacement heifers should achieve 60 to 65% of their expected mature BW by breeding. Traditional approaches for postweaning development of replacement heifers used during the last several decades have primarily focused on feeding heifers to achieve or exceed an appropriate target weight, and thereby maximize heifer pregnancy rates. Substantial changes in the economy and cattle genetics have occurred over this time, indicating traditional approaches should be re-evaluated.

More recent research has demonstrated feeding replacement heifers to traditional target weights increases development costs relative to more extensive heifer development systems where heifers were developed to lower target weights ranging from 51 to 57% (Funston and Deutscher, 2004; Larson et al., 2009; Martin et al., 2007; Roberts et al., 2009).

The majority of heifer development research has focused on the postweaning phase. In a review by Patterson et al. (1992) numerous studies were cited suggesting the preweaning growth phase exerts a greater influence on puberty in beef heifers than postweaning growth rate.

Thus, data from 13 production years were summarized to determine the effect of time of calving on subsequent pre- and postweaning ADG and BW and impact on reproduction and first calf characteristics in beef heifers.

Materials and Methods

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved the procedures and facilities used in this experiment.

Data were collected from the University of Nebraska Gudmundsen Sandhills Laboratory beef cattle research herd near Whitman, NE. The data for the spring calving herd, collected between 1997 and 2009, were used for this analysis. As varying nutritional and breeding treatments were applied to the yearling heifers during breeding, two year-old cows were removed from this analysis to avoid confounding the results. The breeding season begins on approximately June 15. Heifers were classified as being born in the first, second, or third 21-d period of the calving season within year.

Continuous data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) and binomial data with the GLIMMIX procedure of SAS. The model included the fixed effect of period the calf was born. The model also included the random effect of year and any

treatments imposed on each particular herd within each year.

Results and Discussion

The data demonstrating the effect of calving period on subsequent pre- and postweaning ADG and BW and impact on reproduction and first calf characteristics is presented in Table 1.

Heifer calves classified in the first calving period were 16 d older than those in the second and 36 days older than those in the third period ($P < 0.001$). Calf birth BW was lower ($P < 0.001$) for heifers born in the first period.

As the time of calving became more advanced, calf ADG from birth to weaning tended ($P = 0.10$) to be lowest for heifers born in the first calving period. Regardless of greater birth BW and preweaning ADG, heifer calf weaning BW decreased ($P = 0.03$) with advancing calving period. Calf ADG from weaning to prebreeding tended ($P = 0.07$) to be least for heifers born in the first period; however, prebreeding BW was greatest ($P < 0.01$) for calves born in the first period. Heifer ADG from the beginning of the breeding season to pregnancy diagnosis was greater ($P = 0.03$) for heifers born in the third vs. first calving period. The percentage of heifers cycling at the beginning of the breeding season decreased ($P < 0.01$) with advancing calving date (70, 58, and 39%, respectively) and 45 d pregnancy rates were lowest ($P = 0.02$) for heifers born in the third calving period (90, 86, and 78%, respectively).

Heifers born later in the calving season appear to have greater pre- and postweaning ADG and lower fertility. This is in contrast to data cited by Patterson et al. (1992) indicating preweaning growth exerts a greater influence on puberty than postweaning growth. In the current data set it appears neither pre- nor postweaning growth influenced percent cycling before the breeding season or pregnancy rates. Studies cited by Patterson et al. (1992) dated back to the 1950s. Considerable change in beef cattle genetics has likely occurred since these observations were made and perhaps age rather than rate of gain is more important in determining when an animal reaches puberty and conceives. Research from our group would certainly support the theory rate of gain prior to breeding has minimal impact on heifer pregnancy rate (Funston and Deutscher, 2004; Larson et al., 2009; Martin et al., 2007).

Birth date of the heifer's first calf and birth BW decreased ($P < 0.01$) if the heifer was born in the first calving period. Also, more ($P < 0.01$) calves were born in the first 21 d of the calving season if the heifer herself was born in the first calving period. Regardless of greater dam weight at calving and lower birth BW for heifers calving that were born in the first period, calving assistance and dystocia score were similar ($P \geq 0.18$). First calf progeny had the greatest ($P \leq 0.10$) weaning BW if born to a heifer born in the first calving period. Cow BW at weaning her first calf and pregnancy rate after the first calf were similar ($P \geq 0.20$).

Implications

Heifer calves born during the first 21 d of the spring calving season had greater weaning, prebreeding, and precalving BW; greater percent cycling before breeding, and greater pregnancy rates compared to heifers born in the third calving period. First calf progeny also had an earlier birth date and greater weaning BW. Calving period of heifer progeny significantly impacts development and first calf characteristics.

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Table 1. Effect of calving period on ADG, reproduction, and first calf characteristics of heifer progeny

	Calving period[1]				
Item	1	2	3	SEM	*P*
n	651	304	64		
Birth Date, julian d	77[a]	93[b]	113[c]	2.02	<0.001
Calf birth BW, kg	36[a]	37[b]	38[b]	0.70	<0.001
Calf weaning BW, kg	219[a]	213[b]	197[c]	4.90	0.03
Preweaning ADG, kg/d	0.83	0.83	0.86	0.04	0.10
Prebreeding ADG, kg/d	0.39	0.41	0.41	0.03	0.07
Prebreeding BW, kg	296[a]	292[b]	276[c]	4.18	<0.001
Cycling beginning of breeding, %	70[a]	58[b]	39[c]	9.35	<0.001
Breeding ADG, kg/d	0.72[a]	0.74[ab]	0.77[b]	0.04	0.03
Pregnancy diagnosis BW, kg	373[a]	371[a]	358[b]	5.33	<0.001
Pregnancy rate, %	90[a]	86[a]	78[b]	5.62	0.02
Precalving BW, kg	429	430	418	6.65	0.06
First calf birth date, julian d	68[a]	73[b]	75[b]	2.03	<0.001
Calved in first 21 d, %	81[a]	69[b]	65[b]	8.41	<0.01
First calf birth BW, kg	36[a]	37[b]	38[b]	0.69	<0.001
Assisted births, %	23	29	33	8.37	0.26
Dystocia score[2]	1.29	1.40	1.34	0.11	0.18
Cow weaning BW, kg	419	422	422	7.71	0.68
Calf weaning BW, kg	193	189	186	5.17	0.10
Pregnancy rate after first calf, %	93	90	84	6.61	0.20

[1] 1 = calved in the 1st 21 d, 2 = calved in the 2nd 21 d, 3 = calved in the 3rd 21 d of the spring calving period.

[2] Scoring system 1 to 5: 1 = no assistance; 2 = easy pull; 3 = mechanical pull; 4 = hard mechanical pull; and 5 = Caesarean section.

[abc] Means without a common superscript differ ($P \leq 0.05$).

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EVALUATING CONVENTIONAL AND SEXED SEMEN IN A COMMERCIAL BEEF HEIFER DEVELOPMENT PROGRAM

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ABSTRACT: Objectives of this study were to evaluate the use of sexed semen in a commercial heifer development program. Heifers (n = 500) were fed 0.5 mg/d of melengestrol acetate per animal for 14 d and 19 d later, administered $PGF_{2\alpha}$ and Estrotect heat detection patches were placed on their tail heads. Following $PGF_{2\alpha}$ injection, heifers were detected for standing estrus and AI approximately 18-24 hr following detection of standing estrus. Three days following $PGF_{2\alpha}$ injection, heifers with activated Estrotect patches and observed in standing estrus prior to 0800 h were sorted for breeding late the same day. Heifers detected the previous morning and early afternoon were AI early morning on d 3. Heifers detected in heat late on d 2 were inseminated early afternoon of day 3. Following the early afternoon AI, heifers not detected in estrus were given a GnRH injection and AI. Following timed AI, heifers detected the morning of d 3 were inseminated as late as possible with consideration given to the number of heifers to inseminate and remaining daylight. Heifers were AI with one of two sires, either conventional or sexed semen, creating four possibilities for AI sire. At each AI session, heifers were divided evenly to receive either sexed or conventional semen from the same sires. Pregnancy was determined via ultrasonography 55-58 d after AI. Heifers were identified as pregnant by AI, bull, or non-pregnant and sorted accordingly. Pregnancy rate was greater ($P < 0.01$) for heifers inseminated with conventional than sexed semen (58 vs. 41%). In addition, more ($P < 0.01$) heifers detected in standing estrus were pregnant (56% or greater) than heifers time AI (24%). Non-pregnant heifers (n = 124) and heifers pregnant by bull (n = 247) were returned with bulls and checked for pregnancy via ultrasound approximately 60 d later. Overall pregnancy rate was 93%.

Keywords: Artificial Insemination, Beef Heifer, Sexed Semen

Introduction

Sex-sorting sperm relies on the fact the bovine X chromosome has 3.8% more DNA than the Y chromosome. This principle enables sperm to be sorted using a flow cytometer (Garner and Seidel, 2008). However, the process does damage sperm and reduces fertility when compared to conventional semen (Tubman et al., 2004).

As the process is refined, the use of sexed semen becomes more applicable to the beef producer. Calves resulting from sexed semen do not exhibit more genetic abnormalities nor does it affect calf characteristics (Tubman et al., 2004).

Protocols for AI with sexed semen have been similar to those utilized with conventional semen without modification. Objectives of this study were to evaluate the use of sexed semen compared to conventional semen in a commercial heifer development program with a slightly modified, commonly used synchronization system for beef heifers.

Materials and Methods

Yearling heifers (n = 500) were managed together at the Kelly Ranch (**KR**), Sutherland, NE. Approximately one week prior to initiation of synchronization, a subset (n = 100) of heifers was randomly sorted and transported to the University of Nebraska West Central Research and Extension Center, North Platte, NE (**WCREC**); the balance of heifers (n = 400) remained at the KR.

Heifers at the KR grazed dormant upland Sandhills range receiving 1.3 kg/d (DM) dried distillers grains. Sixty-six d before initiation of synchronization, each heifer also began receiving 2.9 kg/d (DM) alfalfa. Alfalfa was fed *ad libitum* beginning the end of March through early April due to decreasing winter range.

Heifers at WCREC were placed in a dry lot and fed 11.6 kg/d (DM) of a diet consisting of 10% corn, 71% prairie hay, 16% wet corn gluten feed, and 3% heifer supplement. Heifer BW was measured (294 kg) upon arrival to WCREC.

Beginning April 8, heifers at both locations were fed 0.5 mg/d melengestrol acetate (**MGA**; Pfizer Animal Health, New York, NY) per animal for 14 d. At WCREC, MGA pellet was added as part of the complete diet; at KR, MGA pellet was mixed with 2.1 kg/d ground hay and 0.8 kg/d wet distillers grains (DM). Prostaglandin $F_{2\alpha}$ (**PGF**; Lutalyse, Pfizer Animal Health, New York, NY) was administered intramuscularly (**i.m.**) 19 d later and heat detection patches (Estrotect, Spring Valley, WI) were placed on tail heads. In addition, BW was measured (326 kg) for heifers at WCREC.

Following PGF injection, heifers were detected for estrus by one of two methods; visual observation of standing estrus or activated heat detection patches. Three people detected estrus at KR, while two detected estrus at WCREC during daylight hours. Heifers were AI approximately 18-24 hr following detection of standing estrus to place insemination closer to ovulation, due to sperm damage in the sex-sorting process (Tubman et al., 2004). Heifers detected in estrus before 0800 h were AI late the same day. Heifers detected between 0800 and 1400 h were AI early the next morning.

Heifers detected between 1400 h and the end of the day were AI early afternoon the next day.

Three days following PGF injection, heifers with activated Estrotect patches and observed in standing estrus prior to 0800 h were sorted for breeding late the same day. Heifers detected the previous morning and early afternoon were AI early morning on d 3. Heifers detected in estrus late on d 2 were inseminated early afternoon of day 3. Following the early afternoon AI, heifers not detected in estrus were given a GnRH (Fertagyl, Intervet/Schering-Plough Animal Health, Summit, NJ) injection i.m. and AI (mass bred, **MB**). Following timed AI, heifers detected the morning of d 3 were inseminated as late as possible with consideration given to the number of heifers to inseminate and remaining daylight.

Heifers were AI with one of two sires, either conventional or sexed semen, creating four possibilities for AI sire. At each AI session, heifers were divided evenly to receive either sexed or conventional semen from the same sires. Six AI technicians were used at the KR and two at WCREC.

The sexed semen was sorted at 90% purity for heifer calf pregnancies. Each sexed semen straw contained 2×10^6 sperm.

The day after MB, heifers at WCREC were transported back to KR. Heifers were managed as one group, grazing upland Sandhills range. Clean-up sires (n = 12) were turned in with heifers 12 d after MB, at a ratio of 1 bull to 42 heifers.

Fifty-five d after MB, BW was measured (365 kg), and pregnancy was detected via transrectal ultrasonography. Heifers were identified as pregnant by AI, bull, or open and sorted accordingly. Non-pregnant heifers (n = 124) and heifers pregnant by bull (n = 247) were returned with bulls for an additional 18 d and checked for pregnancy via ultrasound approximately 60 d later.

Data was analyzed using PROC GLIMMIX of SAS (SAS Inst. Inc., Cary, NC).

Results and Discussion

The subset of heifers at WCREC had an ADG of 0.77 kg/d during the 45 d period at WCREC. This same group of heifers weighed 370 kg at the time of ultrasound, for an ADG of 0.75 kg/d from AI to first pregnancy detection. Location did not affect ($P = 0.28$) pregnancy rates.

There was no ($P > 0.10$) sire × type of semen (conventional or sexed) interaction; therefore, sires were combined for analysis. Pregnancy rate was greater ($P < 0.01$) for heifers inseminated with conventional than sexed semen (58 vs. 41%, Table 1). These results agree with previous research indicating pregnancy rates using sexed semen are generally 70-90% of conventional semen (Seidel, 2010; Rhinehart and Parish, 2009; Doyle et al., 1999) with quality of herd management playing a key role (Garner and Seidel, 2008).

More ($P < 0.01$) heifers detected in standing estrus were pregnant (56% or greater, Table 2) than heifers MB (24%). A review by Seidel (2003) indicated most inseminations with sexed semen have been conducted at 12 or 24 h after observed standing estrus, and fertility with timed AI was markedly lower. Doyle et al. (1999) found pregnancy rates in lactating cows from insemination 6 to 14 h after estrus detection were similar to inseminations 21 to 26 h after estrus detection, recommending detection of estrus and once a day breeding . Pregnancy rates using sexed semen were not statistically ($P = 0.22$) different between sires; however, there was a 10% numerical difference. Doyle et al. (1999) reported a difference in fertility rates among bulls when using sexed semen. Overall pregnancy rate (including natural service) was 93%.

Breeding costs based on breeding system were highest numerically for AI with sexed semen (Table 3), due to lower pregnancy rates and greater semen costs ($14 for conventional vs. $45 for sexed). A portion of the pregnant heifers (n = 417) were marketed following the breeding season. Heifers pregnant by AI were sold at $1,344/animal and heifers pregnant by natural service sold at an average of $1,238/animal. Gender difference for replacement heifers AI with sexed semen was not considered as all AI pregnant heifers sold for the same price.

Further research is needed to establish the optimum estrus synchronization program with sexed semen.

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Table 1. Pregnancy rates by sire for conventional and sexed semen

	Conventional semen	Sexed semen	SE	P value
Both sires, % pregnant	58.4[a]	41.0[b]	4.2	<0.01
Sire 1, % pregnant	59.4[a]	36.1[b]	5.4	<0.01
Sire 2, % pregnant	57.5[a]	46.2[b]	5.6	<0.01

[a,b]Row means without a common superscript differ ($P < 0.05$).

Table 2. Pregnancy rates by insemination time for conventional and sexed semen

	AM[1]	EPM[2]	LPM[3]	MB[4]	SE	P value
Overall	64.2[a]	55.9[a]	57.0[a]	24.0[b]	6.8	<0.01
Conventional	69.6[a]	59.9[a]	68.0[a]	34.9[b]	7.0	<0.01
Sexed	58.4[a]	51.9[a]	45.3[a]	15.8[b]	9.0	<0.01

[1] Heifers detected in estrus between 0800 and 1400 h were AI early the next morning.
[2] Heifers detected in estrus between 1400 h and the end of the day were AI early afternoon the next day.
[3] Heifers detected in estrus before 0800 h were AI late the same day.
[4] Heifers not detected in estrus were given a GnRH injection and mass AI.
[a,b]Row means without a common superscript differ ($P < 0.05$).

Table 3. Various costs for AI with conventional semen, sexed semen, and natural service in a commercial beef heifer development program

	Conventional semen	Sexed semen	Natural Service
Semen cost/straw, $	14.00	45.00	--
Semen cost/AI pregnancy, $	24.39	109.22	--
Breeding system cost per pregnant heifer, $	68.66	111.47	63.39
Pregnant Heifer Net Cost, $	1,264.00	1,308.00	1,259.00

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EFFECT OF RESIDUAL FEED INTAKE ON TEMPORAL PATTERNS OF GLUCOSE, INSULIN, AND NEFA CONCENTRATIONS AFTER A GLUCOSE CHALLENGE IN TARGHEE EWES [1]

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ABSTRACT: The objective of this study was to evaluate the effect of a glucose challenge on glucose, insulin, and NEFA concentrations in high and low residual feed intake (**RFI**) ewes. The null hypotheses were that temporal concentrations of glucose (**Gluc**), insulin (**I**), and NEFA in response to a glucose challenge do not differ between high and low RFI ewes. Residual feed intake was determined for 49, 9-mo-old Targhee ewe lambs fed a commercially available pelleted-diet formulated for growing lambs during a 49-d trial using the GrowSafe system. Ewes were ranked by RFI score and the 6 highest and 6 lowest RFI scoring ewes were selected for the glucose challenge. The glucose challenge began 10 d after the GrowSafe trial. Ewes were fasted and fitted with a jugular catheter 24 h before the start of the glucose challenge. Blood samples were collected at -30, -15, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after infusion of glucose (59 mM/kg BW) at time = 0 h (1015h). Gluc, I, and NEFA assayed using colorimetric assays and insulin was assayed by RIA. Temporal patterns of Gluc, I, and NEFA were analyzed by repeated measures of SAS. Concentrations of Gluc, I, and NEFA over time did not differ ($P > 0.10$) between ewes of the RFI classes. However, time to 50% disappearance of Gluc from circulation, estimated from quadratic equation fitting of concentrations after the maximum concentration tended ($P = 0.07$) to be greater in high RFI ewes than in low RFI ewes (110 and 86 min, respectively), and Gluc to I ratios tended ($P = 0.08$) to be greater in high RFI ewes than low RFI ewes (273 and 204, respectively). Thus, Targhee ewes selected for high RFI appeared to be more insulin-resistant than ewes selected for low RFI.

Key words: RFI, ewe, energy metabolism

Introduction

Feed is a major cost in sheep production and improved conversion of feed to product is one approach for increasing profitability (Cammack et al, 2004). A measure of feed efficiency is residual feed intake (**RFI**), defined as the difference between actual feed intake of an animal and its predicted intake based on body size and level of performance (Koch et al., 1963). However, there is little or no knowledge of the biological mechanisms controlling RFI in sheep. Understanding changes in metabolites and metabolic hormones that regulate manifestation of RFI could be useful to evaluate the overall metabolic status of sheep that diverge in RFI. Since RFI is independent of the level of production it represents inherent variations in basic metabolic processes related to efficiency (Herd and Bishop, 2000). Thus, there is the possibility that differences in energy homoeostatic mechanisms underlie variation in RFI scores.

It is well known that glucose utilization is primarily controlled by insulin in mammals (Hadley and Levine, 2006). A primary tool for assessing glucose utilization in mammals is a glucose tolerance or challenge test. This test was used to address the primary question of this study as to whether differences in RFI scores of sheep are related to energy homeostasis, in particular, glucose utilization.

The objective of this study is to evaluate the effect of a glucose challenge on glucose, insulin, and NEFA concentrations in ewes selected for high (inefficient) and low (efficient) RFI scores. The null hypotheses were that temporal concentrations of glucose, insulin, NEFA and glucose to insulin ratios in response to a glucose challenge do not differ between high and low RFI ewes.

Materials and Methods

Animals

Forty-nine, 9-mo-old Targhee ewe lambs were selected from the Montana State University, Red Bluff Research Ranch flock for determining RFI scores. All experimental procedures were approved by the Montana State University Agricultural Animal Care and Use Committee.

Feeding Trial

A 49-day trial was employed to estimate RFI scores during the active growth phase using the GrowSafe feed intake system (GrowSafe Systems Ltd., Airdrie, AB, Canada). Ewes weighed 44 ± 1 kg (mean ± SD) at the start of the trial. Details of the feeding trial for determining RFI were given in Redden et al. (2011). In brief, ewe-lambs were given ad libitum access to a pelleted diet (Table 1) and water. Ewe lambs were allowed a 2-wk adaptation period. Lambs were weighed at 7-d intervals and 2-consecutive-day BW measurements were obtained at the beginning and end of the GrowSafe trial.

[1] This study was supported by the Montana Agric. Exp. Sta. and USDA, NIFA Special Grants Program.

Table 1. Ingredient composition and chemical composition of pelleted diet[1]

Item	Pelleted diet
Ingredient[2]	
Alfalfa sun-cured	43.6
Corn	29.9
Wheat middlings	15.0
Soybean hulls	10.0
Molasses cane	4.0
Calcium carbonate	2.0
Ammonium chloride	0.6
Pelleting agent	0.5
Premixes[3]	0.4
Nutrient analysis[4]	
DM	88.5
CP	15.8
NDF	36.0
ADF	22.2

[1] Ewe-lambs had free access to the pelleted diet.
[2] Dietary components are on an as fed basis.
[3] Contained 2,000 mg/kg Mo; 44,000IU/kg vitamin E; 9% Fe; 10% Zn; 6% Mn; 3.4% I; 0.03% Co; 0.02% Se; 23,700 IU/mg vitamin A; and, 2,250 IU/mg vitamin D.
[4] Values for CP, NDF, and ADF based on percent of DM.

Residual Feed Intake Calculations

A detailed description of the methods used for estimating RFI was given in Redden et al. (2011). Briefly, daily feed intakes and feeding behavior traits of each ewe were computed by using the Process Intakes and Export Behavior Data routine of the GrowSafe Data Acquisition software. No daily assigned feed disappearance values were less than 95% for any ewe. Growth rates of individual ewes were modeled by linear regression of ewe BW by day using PROC GLM procedures of SAS (SAS Inst. Inc., Cary, NC), and regression coefficients were used to compute average daily gain (ADG), initial and final BW, and metabolic BW (MBW; mid-test $BW^{0.75}$) as described by Lancaster et al. (2009). Residual feed intake was calculated for each individual as the difference between actual feed intakes and expected feed intake. Expected feed intake was modeled by linear regression of dry matter intake against MBW and ADG during the trial using PROC REGRESS of SAS. Residual feed intake for each ewe was then determined by subtraction of expected feed intake minus actual feed intake. Ewes were then classified into low, medium, and high RFI groups that were < 0.5, within ± 0.5, and > than 0.5 SD, respectively, from the mean RFI.

Glucose Challenge

All ewes were ranked by RFI score and the 6 highest and 6 lowest RFI scoring ewes were selected for a glucose challenge that began 10 d after the GrowSafe trial. Body weights of these ewes were 57.7 ± 3.4 kg and 57.8 ± 5.6 kg for high and low RFI ewes, respectively. Ewes were fitted with an indwelling jugular catheter and fasted for 24 h before the start of the challenge. Each ewe was infused with glucose at a rate of 59 mM/kg BW in 10 mL of sterile physiological saline (0.9%) over a 2-min interval at time = 0 h (1015 h). Each catheter was immediately flushed with 10 mL of sterile saline.

Blood Sampling and Processing

Blood samples (10 mL) were collected at -30, -15, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after infusion of glucose infusion. The first 1 to 2 mL of blood at each sampling time was discarded. Catheters were flushed with 10 mL sterile saline after collection of each blood sample. Samples were stored on ice, allowed to clot overnight and centrifuged at 1,850 x *g* for 30 min. Serum was decanted into 12 mm x 75 mm plastic culture tubes, capped and stored at -22° C, until assayed for glucose, insulin, and NEFA.

Glucose, Insulin, and NEFA Assays

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). Sensitivity of the assay using ewe serum was 12.5 mg/dL. Intra- and inter-assay CV were < 10% ewe serum pools that contained 180 and 60 mg/dL of glucose.

Insulin was assayed in duplicate using solid-phase RIA kits (SRI-13k; Millipore, Billerica, MA, USA) validated for sheep serum in our laboratory. The sensitivity of the assay was 0.02 ng/mL. Percent recoveries for serial dilutions of 0.75 ng/mL insulin standard in pooled ewe serum ranged from 96 to 108%. Intra- and inter-assay CV were 9.2 and 12%, respectively, for a ewe serum containing 0.2 ng/mL of insulin.

Concentrations of NEFA were quantified with a commercially available enzymatic-colorimetric assay (HR Series NEFA – HR [2]; Wako Diagnostics, Richmond, VA). Sensitivity of the assay using ewe serum was 0.062 mmol/L. Inter- and intra-assay CV were < 10% for a ewe serum pool that contained 0.415 mmol/L; and, 14 and 5.6%, respectively, for a serum pool that contained 0.100 mmol/L.

Statistical Analyses

Glucose, insulin, NEFA, and glucose to insulin ratios were analyzed initially using the PROC MIXED for repeated measures analysis with compound symmetry of SAS (SAS Inst., Inc., Cary, NC). The model included RFI class, time, and the interaction between these variables as fixed effects. Animal was the experimental unit (random effect) with time after glucose infusion as the repeated measure. Means were separated using Bonferroni's multiple comparison tests.

Areas under the glucose and insulin concentrations by time curves for each ewe were calculated using Sigma Plot (Systat Software, Inc., Chicago, IL, USA). Time to 50%

disappearance of glucose from circulation for each ewe was estimated from quadratic equation fitting of concentrations after the maximum concentration. Areas under the concentration by time curves and time to 50% disappearance of glucose were analyzed by separate one-way ANOVA using SAS. The model included RFI class and means were separated with Bonferroni's mean comparison tests.

Results

Patterns of glucose, insulin, and NEFA concentrations over the 270-min sampling period did not differ ($P > 0.10$) between high and low RFI ewes. As expected, there was a time ($P < 0.01$) effect for each of these variables. Glucose reached maximum and minimum concentrations at 5.4 ± 1.4 min and 120 ± 10 min after infusion. Insulin concentrations reached a maximum at 16.4 ± 11.3 min and a minimum at 142.1 ± 30.6 min. Concentrations of NEFA decreased ($P < 0.01$) from 1.431 ± 0.103 mmol/L before time 0 min to a nadir of 0.831 ± 0.106 mmol/L at 90 min, then rose ($P < 0.01$) to pre-infusion concentrations by 180 min.

Time between peak glucose and insulin concentrations did not differ ($P > 0.10$) between high and low RFI ewes (Table 2). However, time to 50% disappearance of glucose, from maximum concentration to 240 min after infusion, tended ($P = 0.07$; Table 2) to be greater in high RFI ewes than in low RFI ewes. Also, ratios of glucose to insulin concentrations tended ($P = 0.08$; Table 2) to be greater in high RFI ewes than in low RFI ewes. Area under concentration by time curves of glucose and insulin did not differ ($P > 0.10$) between high and low RFI ewes (Table 2).

Table 2. Least squares means of characteristics of temporal concentrations patterns of glucose and insulin in ewe lambs classified by high and low residual feed intake (RFI) score

	RFI class[1]			
Item	high	low	SEM	$P =$
n	6	6		
Time from peak glucose to peak insulin, min	15.0	7.5	11.0	0.29
50% disappearance	110.1[a]	86.3[b]	21.0	0.07
G:I ratio	273.2[a]	204.7[b]	61.3	0.08
AUC, glucose[2]	2150.0	2003.2	520.1	0.64
AUC, insulin[2]	8.5	11.1	2.6	0.10

[1] RFI class defined as less than 0.5, within ± 0.5, and greater than 0.5 SD, respectively, from the mean RFI.

[2] AUC = area under concentration by time curves in arbitrary units.

[a,b] Values within rows differ.

Discussion

Residual feed intake is a measure of feed efficiency (Koch et al., 1963). This measure is independent of level of production and is thought to represent inherent variations of basic metabolic processes underlying efficiency of feed conversion (Herd and Bishop, 200). Understanding changes in metabolites and metabolic hormones that regulate or are intimately associated with RFI could be useful to evaluate the overall metabolic status of sheep with the possibility that selection for RFI could reduce feeding cost in sheep. Kelly et al. (2010) provided evidence to suggest that genetic selection for low (efficient) RFI in beef heifers appeared to be associated with altered glucose utilization or metabolism. However, there is little or no knowledge of an association between energy homeostasis or glucose utilization and RFI in sheep.

The premise of present study was that energy homeostasis varies among sheep of differing RFI scores. As such, selecting ewes that had the very highest and lowest RFI scores from a large population of sheep would allow us to evaluate whether differences in energy homoeostasis is a physiologically important variable that is the underlying basis of variation in RFI scores in sheep. Thus, the objective of this study is to evaluate the effect of a glucose challenge on glucose, insulin, and NEFA concentrations in ewe lambs selected for high (inefficient) and low (efficient) RFI scores.

We found that temporal patterns of glucose, insulin, and NEFA concentrations did not differ between ewes lambs classified by low (efficient) or high (inefficient) RFI score in response to a glucose challenge. Furthermore, most variables used to characterize utilization of glucose, such as, time from peak glucose concentration to peak insulin concentration, and area under concentration by time curves, did not differ between high and low RFI ewe lambs in response to a glucose challenge. These results are in line with those reported by Kolath et al. (2006) and Kelly et al. (2010) in growing steers and beef heifers. These authors concluded that changes in glucose metabolism may not be directly related to selection for RFI.

We did find that the time to clear 50% of the maximum glucose concentration from the systemic circulation tended to be shorter in low RFI ewe lambs than in high RFI ewe lambs. This is an interesting observation, although the statistical power of this test is low with such few ewes per class. The interpretations of this result are equivocal. This could mean that either efficient ewe lambs are more sensitive to their own insulin or that high RFI ewe lambs more efficiently take up glucose across cell membranes of their livers and muscles with the same insulin milieu as inefficient or low RFI ewe lambs. On the other hand, this may mean that inefficient or high RFI ewe lambs are less sensitive to insulin than efficient or low RFI ewe lambs. However, in support of either of these is the tendency of the glucose to insulin ratio to be greater in high or inefficient ewe lambs than in low or efficient ewe lambs. The interpretation of this result may be that at the same insulin concentration low RFI ewe lambs tend to utilize glucose more efficiently that high RFI ewes. These results differ somewhat with those in growing heifers

reported by Kelly et al. (2010). They found no association between glucose to insulin ratios in high and low RFI beef heifers; however, they did find a positive correlation between glucose: insulin ratios and gain to feed ratios in growing beef heifers. Nevertheless, results of the present study appear to support the hypothesis provided by Richardson et al. (2004) and Browne (2005) that "energetically inefficient animals may have developed a decrease in insulin sensitivity in muscle tissue" resulting in greater glucose to insulin ratios similar to what we observed in the present study with ewe lambs.

In conclusion, the response of inefficient or high RFI and efficient or low RFI ewe lambs during the growing phase to a glucose challenge, in terms of temporal concentrations of glucose, insulin and NEFA, indicates that energy homeostasis or glucose utilization is not directly related to RFI and may not be the underlying physiological basis for efficiency as measured by RFI score in sheep. However, there is an indication that low RFI ewe lambs may be more efficient at utilizing glucose than high RFI ewes.

Implications

The underlying physiological mechanism of selecting for RFI may not be involved directly with energy homeostasis in growing ewe lambs. This means that an efficient ewe lamb is no more likely to utilize glucose for growth than an inefficient ewe lamb. Nevertheless, results of this study merit further research to evaluate the possibility that energy homeostatic mechanisms at the cell-molecular level may be altered by selecting for efficient RFI in growing lambs

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SUPERIMPOSING MELENGESTROL ACETATE PRE-FEEDING AND(OR) CONTROLLED INTRAVAGINAL DRUG RELEASE ON THE SELECT SYNCH ESTROUS SYNCHRONIZATION PROTOCOL IN BEEF COWS

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ABSTRACT: The combined effect of melengestrol acetate (**MGA**) and controlled intravaginal drug release (**CIDR**) on estrous and pregnancy response in beef cows (n = 507) was compared with 3 Select Synch-based estrous synchronization protocols over 2 years. Angus-based cows (BCS = 5.1 ± 0.52) were randomized by BCS and assigned to 1 of 4 treatments. All cows received Select Synch (100 ug GnRH im followed by 25 mg $PGF_{2\alpha}$ im 7 d later); progestin treatments included: 1) MGA fed for 14 d beginning 26 d before Select Synch (**MGA**), 2) CIDR for 7 d concurrent with Select Synch (**CIDR**), 3) MGA fed for 14 d beginning 26 d before Select Synch and CIDR for 7 d concurrent with Select Synch (**MGA+CIDR**), and 4) no progestin with Select Synch (**Control**). Range cubes with or without MGA (carrier) were fed daily in 2 separate pastures. Cows were observed for behavioral signs of estrus for at least 60 min twice daily (morning and evening) for 72 h following $PGF_{2\alpha}$. Cows observed in estrus within 72 h of $PGF_{2\alpha}$ were inseminated half a day later. Remaining cows were inseminated 77.4 ± 1.68 h after $PGF_{2\alpha}$ and given GnRH. There were no ($P > 0.10$) yr × MGA or yr × CIDR interactions for estrous or pregnancy response. Hence, data were pooled across yr. Estrous response was greater ($P < 0.001$) in CIDR (46.4%) and Control (41.3%) cows than MGA+CIDR (20.2%) or MGA (17.8%) cows. Overall, 62.1% of cows became pregnant to AI, but pregnancy rate did not differ ($P > 0.10$) among treatments: MGA = 61.8%, CIDR = 63.6%, MGA+CIDR = 63.0%, and Control = 56.8%. When data were evaluated for a progestin effect, there was no effect ($P > 0.10$) of either MGA or CIDR presence on pregnancy rate. In contrast, estrous response rate within 72 h of $PGF_{2\alpha}$ was markedly less ($P < 0.001$) for cows that received MGA (19.0%) vs. no MGA (43.7%), but was not different ($P > 0.10$) among cows that received CIDR (33.2%) compared with cows not receiving CIDR (29.9%). Results indicate that combining both MGA feeding and CIDR with Select Synch does not increase pregnancy rate to AI. However, estrous response within 72 h of $PGF_{2\alpha}$ was decreased in MGA-treated cows.

Key words: Beef cows, Estrous synchronization, Progestins

Introduction

The Select Synch estrous synchronization protocol (GnRH-PG) is an effective method to synchronize estrus in a large percentage of the cowherd (Geary et al., 2000). However, detection of estrus in advance of the $PGF_{2\alpha}$ injection is necessary to maximize its effectiveness (Kojima et al., 2000). Geary et al. (2000) reported that cows in d 15 to 17 of their estrous cycle at the time of GnRH injection consistently expressed estrus prior to the $PGF_{2\alpha}$ injection in the Select Synch protocol. To avoid early estrus [as documented by Geary et al. (2000) and Kojima et al. (2000)] in these cows, progestins have been incorporated into the GnRH-PG protocol to "pre-synchronize" cows and reduce the length of time necessary to observe estrus, and ultimately improve the protocol's synchronization efficiency (Patterson et al., 2003).

Commonly used progestins include melengestrol acetate (**MGA**) and controlled intravaginal drug release (**CIDR**). The inclusion of MGA into the Select Synch protocol resulted in a greater pregnancy response than Select Synch alone (Perry et al., 2002). Similarly, inclusion of CIDR into a GnRH-PG protocol resulted in a greater pregnancy rate than a CIDR insert alone (Lamb et al., 2001).

Researchers have compared MGA- and CIDR-based protocols, although the main effects of MGA and CIDR have not been evaluated in the same trial, and the combined effects of MGA and CIDR on reproductive performance have not been reported. Therefore, the objectives of this experiment were to: 1) evaluate the effectiveness of a "Double Progestin Select Synch" (14 d MGA and 7 d CIDR in the same protocol) compared to 3 commonly-used Select Synch-based protocols, and 2) determine the main effects of MGA and CIDR in a Select Synch-based protocol on estrous and pregnancy response in suckled beef cows.

Materials and Methods

In 2 consecutive years, estrous and pregnancy response of crossbred (Angus-based) beef cows (n = 507 combined; n = 260, yr 1; n = 247, yr 2) was evaluated on a northern Colorado ranch. Cows had an average BCS of 5.1 ± 0.52. In each yr, cows were randomized by BCS into 4 treatment groups. All cows were at least 30 d postpartum before initiation of estrous synchronization protocols.

All cows received the Select Synch estrous synchronization protocol, which included an injection of 100 ug GnRH (Fertagyl®, Intervet Inc., Millsboro, DE) im followed 7 d later with an injection of 25 mg of PG (Lutalyse®, Pfizer Animal Health, Kalamazoo, MI) im. As illustrated in Figure 1, the 4 treatments included: 1) MGA (Pfizer Animal Health) fed for 14 d beginning 26 d prior to Select Synch (**MGA**; Long-Term MGA Select Synch; n = 126), 2) insertion of CIDR (EAZI-BREED™ CIDR® Cattle Insert, Pfizer Animal Health) for 7 d concurrent with Select Synch (**CIDR**; CIDR Select Synch; n = 121), 3) MGA fed for 14 d beginning 26 d prior to insertion of CIDR for 7 d concurrent with Select Synch (**MGA+CIDR**; Double

Progestin Select Synch; n = 123), and 4) Select Synch without a progestin (**Control**; Select Synch; n = 137).

Both MGA and MGA+CIDR cows received MGA for 14 d prior to Select Synch initiation. Conversely, cows in the CIDR and MGA+CIDR treatments were given a CIDR insert at the same time Select Synch was initiated. The CIDR was removed 7 d later at the time of PG administration.

During the estrous synchronization period, cows were fed range cubes (RanchWay Feeds, Fort Collins, CO; approximately 1.8 $kg \cdot hd^{-1} \cdot d^{-1}$) daily with or without MGA in 2 separate pastures beginning 26 d prior to the start of the Select Synch protocol. The MGA-containing cubes provided MGA at a rate of 0.5 $mg \cdot hd^{-1} \cdot d^{-1}$ for 14 d to cows in the MGA and MGA+CIDR treatments.

Cows were observed for behavioral signs of estrus for at least 60 min twice daily for 72 h following PG. Cows observed in estrus (**EAI**) during this 72-h period were inseminated 13.0 ± 5.28 h after estrus was first observed. At approximately 72 h after PG, any cows that had not yet been observed in estrus were sorted and mass inseminated (**TAI**) 77.4 ± 1.68 h after PG, and administered 100 ug GnRH im. Clean-up bulls were turned out 10 d following TAI, and pregnancy rates to AI were determined by transrectal ultrasonography (Aloka 500V equipped with a 5.0-MHz linear array transducer; Corometrics Medical Systems, Wallingford, CT) 40 d after TAI.

Reproductive performance (including estrous and pregnancy response) was analyzed as a 2 × 2 factorial (factors were MGA and CIDR) using logistic regression (PROC GENMOD, SAS Inst., Inc. Cary, NC) with animal as the experimental unit. Initial models for reproductive response contained the fixed effects of treatment, BCS, yr, sire, and technician, in addition to relevant 2- and 3-way interactions. When an interaction was not significant, it was removed from the model. If the yr × treatment interaction was not significant, data were pooled across yr. Significance was identified as $P < 0.05$. Main effects were determined using contrast statements; comparisons made were: 1) with MGA vs. without MGA and 2) with CIDR vs. without CIDR.

Results and Discussion

There was no yr × treatment interaction for either estrous ($P = 0.74$) or pregnancy response ($P = 0.28$); therefore data were combined across yr. Overall estrous response within 72 h of PG was 31.5%, and pregnancy rate to AI was 61.2%. A comparison of estrous and pregnancy response among treatments is reported in Table 1. Estrous response was not different ($P > 0.10$) between MGA+CIDR and MGA or between CIDR and control. However, CIDR and Control cows had greater ($P < 0.0001$) estrous response than MGA+CIDR and MGA cows. Overall estrous response (to all 4 treatments) within 72 h of PG was lower than has been reported for similar studies (Geary et al., 2000; Larson et al., 2006). Conversely, previous research involving Select Synch-based estrous synchronization protocols in the same cowherd as the current study has resulted in similar 72 h estrous response rates of 31.4 to 37.5% (Ahola et al., 2009). Interestingly, only 7.2% of cows were observed in estrus within the first 48-h period after PG administration, even with the presence of a no progestin treatment (Control). The mean periods of time from PG administration to observed estrus by treatment (mean ± SD) were: 48.5 ± 5.94 h (MGA), 48.3 ± 6.88 h (CIDR), 49.2 ± 3.48 h (MGA+CIDR), and 47.3 ± 9.87 h (Control).

Pregnancy rates by treatment have been reported separately for EAI vs. TAI cows (Table 1). Among cows inseminated by either EAI or TAI, pregnancy rate to AI did not differ ($P > 0.10$) among the 4 treatments. Pregnancy rate to AI in Control cows was numerically lower, yet a statistical difference was absent due in part to the binomial nature of pregnancy data. There were no differences ($P > 0.10$) among treatments for EAI cows; however, within TAI cows the MGA+CIDR treatment tended ($P = 0.06$) to have a higher pregnancy rate to AI compared to the Control treatment. Perry et al. (2002) reported greater pregnancy rates in cows receiving MGA Select Synch (62%, n = 313 cows) compared to cows receiving Select Synch alone (45%, n = 528). Comparing the effectiveness of MGA vs. CIDR as a progestin, Kojima et al. (2004) replaced 14-d MGA feeding with 14-d CIDR insertion and improved the synchrony of estrus and pregnancy rate in beef heifers.

The pregnancy rate to AI of 61.2% across all treatments in the current experiment was higher than previously published trials in beef cows using Select Synch-based estrous synchronization protocols (Larson et al., 2006; Ahola et al., 2009). Pregnancy response to estrous synchronization in the current trial was comparable with previous research while estrous response within 72 h of PG was low; suggesting that estrous response following PG may have been delayed in some cows.

In a large study involving over 2,500 beef cows at 14 locations, Larson et al. (2006) evaluated the efficacy of adding a CIDR to GnRH-PG protocols. Two of the treatment protocols compared by the authors were identical to those evaluated in the current study (CIDR Select Synch and Select Synch), which included EAI for at least 72 h following PG administration, and TAI at approximately 82 to 92 h post PG. The estrous response rates within 72 h of PG administration reported by Larson et al. (2006) at 61 and 69% in the Select Synch and CIDR Select Synch treatments, respectively, were much higher than the current study. Additionally, the authors reported EAI conception rates of 70 and 67% and TAI conception rates of 39 and 26% for Select Synch and CIDR Select Synch treatments, respectively. Compared to Larson et al. (2006), EAI conception rates for Control and CIDR cows in the current study were similar while TAI conception rates in the current study were much higher at 49.3 and 55.9% for the Control and CIDR treatments. The large difference in TAI conception rate between the 2 studies may be associated with a higher percentage of cows being in estrus after 72 h post PG in the current study vs. Larson et al. (2006), although this was not evaluated in either study.

In a multi-location study that involved the comparison of MGA pre-feeding and CIDR inclusion within the Select Synch estrous synchronization protocol with TAI only, Schafer et al. (2007) reported no difference in pregnancy rate among treatments. The authors reported very acceptable TAI pregnancy rates of 61% (MGA) and 66% (CIDR), which are comparable to the current study.

Both MGA and the CIDR insert were combined in the MGA+CIDR treatment to evaluate if exposing cows to an additional progestin could improve synchrony of estrus. The authors are not aware of published literature for comparing pregnancy rate to AI of cows administered both MGA and CIDR (MGA+CIDR) within the Select Synch protocol. However, within a group of beef cows given the Long Term MGA Select Synch protocol, DeJarnette et al. (2004) similarly evaluated the addition of a progestin (MGA) from 1 d after GnRH administration until 1 d prior to PG administration. In primiparous cows, the authors reported a delay in the interval from PG to estrus due to the additional exposure to MGA, but no effect on estrous response and an apparent improvement in conception rate. However, DeJarnette et al. (2004) also reported that in multiparous cows the addition of MGA fed during the Select Synch protocol negatively affected reproductive performance and resulted in a lower pregnancy rate to AI. Since the progesterone source provided concurrently with Select Synch in the current trial was via a CIDR and not MGA, results from DeJarnette et al. (2005) cannot be directly compared. Regardless, since the combination of 2 progestins in the current trial did not improve pregnancy rate to AI over other Select Synch protocols that have included a progestin, it is evident that there is no additional benefit of incorporating both MGA and CIDR.

The main effects of progestin (MGA and CIDR) are also presented (Table 2). There was no yr × MGA interaction or yr × CIDR interaction for estrous ($P > 0.71$) or pregnancy response ($P > 0.51$), thus data were pooled across yr. Pregnancy rate to AI was not affected by the presence of either MGA ($P = 0.46$) or CIDR ($P = 0.43$). In contrast, estrous response was lower ($P < 0.001$) for cows that received MGA compared to cows that did not receive MGA. Estrous response was not affected ($P = 0.24$) by CIDR. Based on these data, the proportion of cows pre-synchronized with MGA that were observed in estrus within 72 h of PG was somewhat inconsistent with the proportion of cows that became pregnant to AI, suggesting that some cows either had been in estrus within 72 h of PG or displayed estrus sometime after 72 h after PG, but were never observed in estrus. Since the overall pregnancy rate was comparable to previously published research even though estrous response was poor, it is more likely that TAI cows were in estrus after 72 h post PG.

Implications

Several proven Select Synch-based estrous synchronization protocols that incorporate progestins are available for beef cattle producers, and can result in acceptable estrous and pregnancy rates. However, combining 2 progestin sources (melengestrol acetate and controlled intravaginal drug release) with Select Synch does not result in a higher pregnancy rate to AI in cows with good body condition. Furthermore, estrous response was decreased in cows treated with melengestrol acetate.

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Table 1. Comparison of beef cow reproductive performance among 4 Select Synch-based estrous synchronization protocols over 2 years[1,2]

	Treatment[3]			
Item	MGA	CIDR	MGA+CIDR	Control
n =	126	121	123	137
Estrous response within 72 h of PG, %	17.8[a]	46.4[b]	20.2[a]	41.3[b]
Pregnancy rate to AI, %				
Overall (all cows)	61.8	63.6	63.0	56.8
EAI cows[4]	66.7	74.0	61.9	66.0
TAI cows[5]	59.6	55.9	64.0	49.3

[1]All cows were administered the Select Synch protocol (100 ug GnRH followed by 25 mg PG 7 d later).
[2]Due to no yr × treatment for estrous ($P = 0.74$) or pregnancy response ($P = 0.28$), data were pooled across yr
[3]MGA = MGA fed for 14 d beginning 26 d prior to Select Synch; CIDR = CIDR for 7 d concurrent with Select Synch; MGA+CIDR = MGA fed for 14 d beginning 26 d prior to CIDR for 7 d concurrent with Select Synch; Control = Select Synch without a progestin.
[4]Cows inseminated based on an observed estrus (EAI) from 0 to 72 h following PG administration.
[5]Cows fixed-time inseminated (TAI) with GnRH at 77.4 h following PG administration.
[a,b]Within each row, means without common superscripts are different ($P < 0.001$).

Table 2. Main effect of progestin type and presence on beef cow reproductive performance within the Select Synch estrous synchronization protocol over 2 years[1,2]

	MGA effect[3]		CIDR effect[4]	
Item	with MGA	without MGA	with CIDR	without CIDR
n =	249	258	244	263
Estrous response within 72 h of PG, %	19.0[a]	43.7[b]	33.2	29.9
Pregnancy rate to AI, %	62.4	60.0	63.3	59.2

[1]All cows were administered the Select Synch protocol (100 ug GnRH followed by 25 mg PG 7 d later).
[2]Due to no ($P > 0.51$) yr × MGA or yr × CIDR for estrous or pregnancy response, data were pooled across yr.
[3]With MGA = includes cows in both MGA (MGA fed for 14 d beginning 26 d prior to Select Synch) and MGA+CIDR (MGA fed for 14 d beginning 26 d prior to CIDR for 7 d concurrent with Select Synch) treatments. Without MGA = includes cows in both CIDR (CIDR for 7 d concurrent with Select Synch) and Control (Select Synch without a progestin) treatments.
[4]With CIDR = includes cows in both CIDR (insertion of CIDR for 7 d concurrent with Select Synch) and MGA+CIDR (MGA fed for 14 d beginning 26 d prior to insertion of CIDR for 7 d concurrent with Select Synch) treatments. Without CIDR = includes cows in both the MGA (MGA fed for 14 d beginning 26 d prior to Select Synch) and Control (Select Synch without a progestin) treatments.
[a,b]For the MGA effect, means without a common superscript are different ($P < 0.001$).

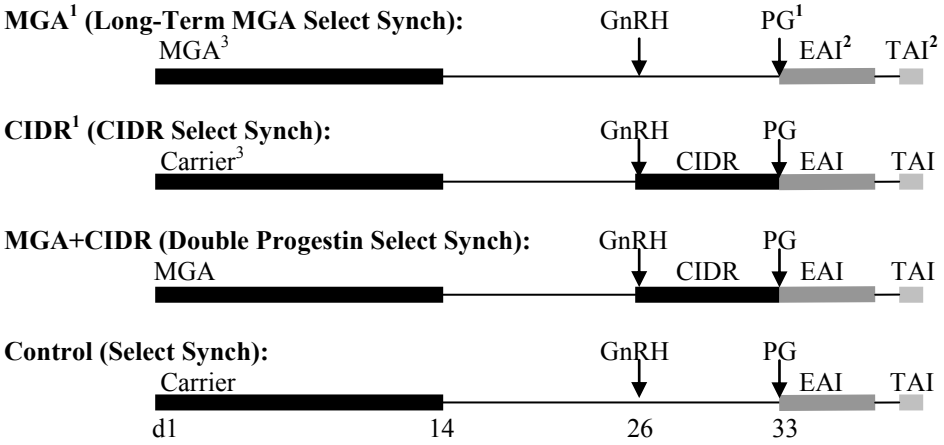

Figure 1. Treatment timelines for 4 Select Synch-based estrous synchronization protocols in beef cows over 2 years

[1]MGA = melengestrol acetate; CIDR = controlled internal drug release device; PG = prostaglandin $F_{2\alpha}$.
[2]All cows were observed for signs of behavioral estrus for a period of 72-h post PG. Cows observed in estrus were inseminated 13.0 ± 5.28 h later (EAI) while cows not observed in estrus were mass inseminated 77.4 ± 1.68 h post PG and given 100 ug GnRH im (TAI).
[3]Range cubes (approximately 1.8 $kg \cdot hd^{-1} \cdot d^{-1}$) with or without MGA (carrier) were fed daily in 2 separate pastures for 14 d beginning 26 d prior to the initiation of the Select Synch protocol.

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EFFECTS OF DIGESTED AND UNDIGESTED SNAKEWEED INGESTION ON BLOOD COMPONENTS OF FEMALE SPRAGUE-DAWLEY RATS

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Abstract: Snakeweed (*Gutierizia sarothrea*) is a noxious plant infesting rangelands in the western U.S., northern Mexico, and southern Canada. An experiment was conducted and replicated over time (Exp. 1 and 2) to examine effects of snakeweed (SW) ingestion on serum components in female Sprague-Dawley rats. In both experiments, 36 rats were offered either *In Vitro* ruminally digested (DSW) or undigested (USW) snakeweed. Treatments were 15% digested SW (15% DSW; n = 6), 15% undigested SW (15% USW; n = 6), or 20% digested SW (20% DSW; n = 6) of 5001 Rat Chow® diet. Additionally, each of these rats was assigned a pair-fed, non-snakeweed control 5001 Rat Chow ®diet (control; n=6/treatment). Pair-fed control rats were fed based on the intake of their treated pair to eliminate any nutritional variation due to feed intake. The pair- fed control diet was adjusted using corn meal and the 5001 Rat Chow® diet to make the diet iso-caloric and iso-nitrogenic. Data were analyzed as a completely randomized design and feed intake as a repeated measures. Rats were fed for 10 d, after which blood samples were collected via heart venipuncture. In Exp. 1, daily feed intake and BW were similar ($P > 0.05$) among treatments compared to controls. In Exp. 2, rats consumed 15% DSW and 15% USW had greater ($P < 0.05$) feed intake than rats consumed 20% DSW. Additionally, rats consumed 15% DSW had greater ($P < 0.05$) feed intake compared to rats fed 15% USW on d 3, 5, 6 and 9. In Exp. 2, BW decreased ($P < 0.05$) in all treatments compared to controls. In Exp 1 and 2, serum constituents were similar ($P > 0.05$) among treatments except for triglycerides which decreased ($P < 0.05$) in Exp. 2 in rats consuming SW treatments compared to pair-fed controls. This may be an effect of reduced BW in treated rats compared to pair-fed controls, as body fat is most likely being mobilized. These results indicate that SW ingestion causes mild toxic effects in rats.

KEYWORDS: Snakeweed, Sprague- Dawley rat, Enzymes

Introduction

Rangelands in the Western United States contain a wide range of plant species, some of them are of great benefit to animals while others are very toxic. Toxic plant species can cause large economical losses in livestock industry, both directly and indirectly (James et al., 1992). Direct losses include reduce growth rates, low birth rates, weakened newborns, abortion, and increased death loss. Indirect losses include preventative measures, such as feeding supplements and managing livestock to avoid poisoning, as well as decreased forage availability and lower stocking rates.

Snakeweed (*Gutierizia sarothrea*) is a noxious plant the western United States, northern Mexico and southern Canada (Florez-Rodreguez et al., 1989; Smith et al., 1991). Approximately 60% of New Mexico and 22% of Western Texas have been invaded by snakeweed (Torell et al., 1988). Many methods have been developed to control snakeweed. The most common is herbicidal treatment, however, burning, mowing and biological control are also used (McDaniel, 1991).

Snakeweed poisoning occurs during periods in which forage is scarce. Snakeweed is unpalatable to animals, but under severe conditions like drought when more palatable forage is in short supply, animals are more likely to graze this toxic plant (Gardner et al., 1999). Clinical signs of snakeweed poisoning vary according to the degree of poisoning, but may include loss of appetite and weight loss, reduction in growth rate, low birth weight, weakened newborns, abortion, and increased death loss (James et al., 1980; Dollahit et al., 1957; James et al., 1992; Smith et al., 1994).

Poisoning effect of snakeweed is the result of the presence of compounds such as saponins, alkaloids, terpenes, flavanols and other resinous substances (Smith et al., 1991). A diet containing 20% or more snakeweed will usually lead to poisoning (Smith et al., 1994). Many researchers have found that saponins are the primary toxicant in snakeweed (Dollahite et al., 1962; Kinsbury, 1965; Smith et al., 1994), and multiple studies have shown that rats are sensitive to SW toxicity (Florez-Rodrequez et al., 1989, 1990; Edrington et al., 1990, 1993a, 1993b). Using rats as a model in ruminants may not be appropriate because of the potential changes snakeweed may undergo in the rumen so, SW has been digested before offering it to rats. Rumen and abomasum compartments of ruminant animals have potential to alter compounds found in various feedstuffs due to the presence of microflora in the rumen and low pH in the abomasum (McDonald et al., 2002). This possibility leads to a potential for toxic compounds in SW to be altered in a manner that could render them more or less toxic to the animal. The objective of this study was to examine effects of snakeweed ingestion on certain blood

serum components of female rats fed either ruminally digested or undigested snakeweed.

Materials and Methods

All procedures and protocols described below were approved by the New Mexico State University Institutional Animal Care and Use Committee (IACUC). All snakeweed samples were harvested from the Chihuahuan Desert Range Research Center (CDRRC), located 37 km north of Las Cruces, New Mexico. Snakeweed was harvested during the pre-bloom stage via hand clipping. Approximately 5 to 10 cm of the distal portion of the plants were harvested. Snakeweed samples were stored frozen at -20°C and were ground to pass through a 2 mm screen at the time of use.

***In vitro ruminal digestion*:** Two salt solutions were used in the *in vitro* digestion. Salt solution A consisted of 7.3 g K2HPO4*3H20/L de-ionized (DI) water. Salt solution B consisted of 6.9 g KH2PO4, 12.0 g (NH4)2SO4, 12.0 g NaCl, 2.5 g MgSO4*7H2O, and 25 g CaCl2*2H2O. Forty mL of solution A and 40 mL of solution B were mixed together, along with 0.6 g of cestein hydrochloride, 1 mL resazorine (1.0%), and 875 mL DI water to create the complete buffer. Buffer was then adjusted to a pH of 7.0, autoclaved and cooled. Finally, 8% Na2CO3 was added, and the buffer was bubbled with CO_2 for 3 min (Russel and Martin, 1984). Rumen fluid was obtained from a canulated Angus cow fed *ad libitum* sorghum hay. The buffer and rumen fluid were mixed at a ratio of 50:50. Ground snakeweed was added at 0.5g per 50 mL of the mixed buffer and rumen fluid. After incubation in a water bath at 39ºC for 24h, the content of each flask was filtered and snakeweed was collected, air dried, and kept frozen until use. The mixture was shaken every 2 h during incubation. and an estimated 40% recovery rate was obtained from filtration. Nutrient composition of snakeweed was analyzed by SDK Laboratories (Hutchinson, Kansas; Table 1).

Table 1. Nutrient analysis (% dry matter) for digested and undigested snakeweed used to create diets.

Item	Treatment	
	DSW[1]	USW[2]
Dry Matter, %	23.9	61.2
Crude protein, %	10.7	11.3
Acid detergent fiber, %	37.5	26.5
Ether extract, %	11.3	11.7

[1]Ruminally digested snakeweed
[2]Undigested snakeweed

Rats were housed at the Small Animal Care Facility at New Mexico State University. Mature female rats were weighed and placed individually in plastic box-type cages. Rats were randomly assigned to 1 of 3 treatments: 15% digested snakeweed (15% DSW), 20% digested snakeweed (20% DSW), and 15% undigested snakeweed (15% USW) with 5001 Rat Chow® as the remainder of the diet. Each treatment contained 6 rats each treated rat was assigned a pair-fed control. Pair-fed rats received rat chow mixed with corn to make it isoenergic and isonitrogenic. The experiment was replicated over time (36 rats per replicate: 6 per treatment, 18 controls). Treatment diets were offered for 10 d. Initial weight, final weight, and daily feed intake were recorded. After d 10, a blood sample was collected via heart venipuncture for blood serum component analysis and rats were euthanized by decapitation.

Statistical Analysis

Data were analyzed using the mixed procedure of SAS (SAS Inst., Inc., Cary, NC). Model effects were treatment, time, and treatment x time, and the animal was considered the experimental unit. Intake was analyzed as a split plot using the mixed procedure of SAS with the repeated measures function. Treatment was in the main plot and day and the interaction were in the sub-plot. For each experiment, intake was compared between snakeweed conditions for each of the 10 treatment days. Where *F* values were significant ($P < 0.05$), means were separated by contrast statements.

Results and Discussion

In Exp 1, BW was similar ($P > 0.05$) among all groups over the 10-d treatment period. However, in Exp 2, rats fed 15% DSW and 20% DSW lost more weight ($P < 0.05$) compared to controls and USW (Table 2). Feed intake was similar ($P > 0.05$) among all treatments in Exp 1 (Figure 1). However, in Exp 2, 20% DSW reduced ($P < 0.05$) feed intake compared to other diets except on d 3, 5, 6, and 9, where rats fed 15% USW consumed the least ($P < 0.05$; Figure 2). Loss in BW associated with similar or reduced feed intake among treatments indicates adverse effects of snakeweed on nutrient metabolism and absorption, as saponins in snakeweed have been reported to decrease growth rate and absorption of nutrients (Cheeke, 1971). Decreased BW and feed intake could also be due to the fact that snakeweed is unpalatable.

In Exp 2, blood serum triglyceride concentrations decreased ($P < 0.05$) in all treatments compared to pair-fed controls (15% DSW: 78.7 ± 9.9 vs. 163.0 ± 9.9; 15% USW: 90.0 ± 16.7 vs. 158.3 ± 16.7; and 20% DSW: 79.3 ± 21.2 vs. 200.3 ± 21.2, respectively). Reduced triglycerides may be an effect of reduced BW, as body fat metabolism occurs when glycogen is depleted after fasting or losing BW. Rates of body fat synthesis and mobilization are closely related to energy intake (Parmley and McNamara, 1996). Florez-Rodriguez et al. (1989), Edrington et al. (1991), and Edrington (1993) reported similar effects of snakeweed on glucose and triglycerides related to decrease BW after snakeweed consumption. Decreased triglycerides in Exp. 2 may be related to under-nutrition and reduced BW in rats consuming snakeweed.

Implications

Findings from this study indicate that snakeweed ingestion reduces feed intake and BW and causes mild toxicity as indicated by blood serum components. Digested snakeweed elicited the most dramatic changes, and thus further testing of additional serum components is needed to determine if snakeweed causes changes in metabolism and absorption of nutrients.

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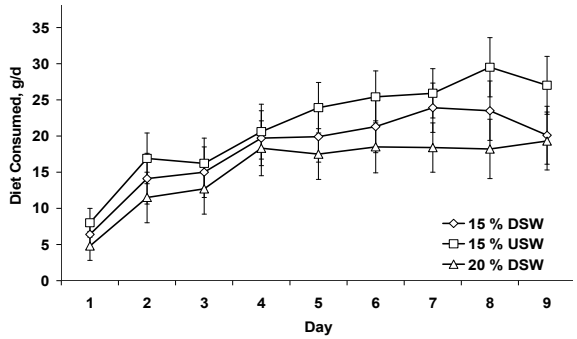

Figure 1. Daily feed intake of rats (n = 6) offered ruminally digested snakeweed (DSW) or undigested snakeweed (USW) over a 10-d period in Exp 1. A treatment x day interaction was observed ($P < 0.05$).

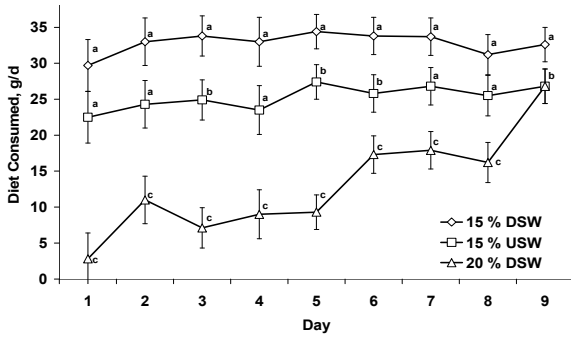

Figure 2. Daily feed intake of rats (n = 6) offered ruminally digested snakeweed (DSW) or undigested snakeweed (USW) over a 10-d period in Exp 2. A treatment x day interaction was observed ($P < 0.05$). Differing superscripts indicate differing means ($P < 0.05$).

Table 2. Body Weight (g) of rats fed ruminally digested snakeweed (DSW) and undigested snakeweed (USW) over a 10-d feeding period.

	Control[1]	15%DSW[1]	Control	15%USW	Control	20%DSW	SE[2]
Exp 1							
BW change	-45.3	-34.8	13.0	-8.0	-9.8	-18.9	18.5
Exp 2							
BW change	-2.6[a]	-25.6[b]	27.6[a]	-9.7[b]	13.9[a]	-20.4[b]	8.3

[a, b]Means with different superscripts differ (P < 0.05) from controls.
[1]Pair-fed rats.
[2]Standard error (n

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FIRST PARITY EVALUATION OF PEAK MILK YIELD FOR RANGE COWS DEVELOPED IN THE SAME ECOPHYSIOLOGICAL SYSTEM BUT RECEIVING DIFFERENT CONCENTRATIONS OF HARVESTED FEED INPUTS[1]

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ABSTRACT: Can range livestock producers reduce harvested feed inputs, during heifer development, and maintain production goals? To address this, we conducted a 2-yr study measuring milk production (kg/d) and milk constituent concentrations (g/d) in primiparous beef heifers (n = 32; 16/yr reared under two different feed inputs). Heifers were born from dams receiving 1.8 or 1.2 kg/d winter supplementation for approximately 80 d and then randomly assigned to heifer development treatments that provided ad-libitum or 20% less feed post weaning. Heifers developed on the ad-libitum treatment also received 1.8 kg/d winter supplementation for life, whereas heifers developed on the 20% less treatment received 1.2 kg/d winter supplementation for life. Milk production was measured with a portable milking machine every other week from d 28 to126 post partum. Milk yield for the 126-d lactation period was calculated from area under the lactation curve approximated by trapezoidal summation. The analysis of variance model included dam winter nutrition, heifer development treatment, and their interaction. Total milk yield, day of peak yield, and peak yield did not differ between dam winter nutrition ($P \geq 0.57$) or heifer development treatment ($P \geq 0.09$). Milk urea N, butterfat, lactose, and solids non-fat did not differ due to dam winter nutrition ($P \geq 0.09$) and milk urea N, protein, lactose and solids non-fat did not differ between heifer development treatment ($P \geq 0.09$). Milk butterfat was greater ($P = 0.04$) in heifers receiving ad-libitum feeding during heifer development (212 vs. 183 ± 9.7 g/d, respectively). Heifers born from dams receiving 1.2 kg/d winter supplementation had greater ($P = 0.03$) milk protein than heifers born from dams receiving 1.8 kg/d of winter supplementation (211 vs. 184 ± 8.3 g/d, respectively). In summary a heifer's dam (*in utero*) and development/lifetime winter plane of nutrition influenced first parity milk composition but not first parity milk yield.

Key words: Feed intake**,** milk constituents and yield, primiparous beef cow, rangeland

Introduction

Previous research has demonstrated that BW, BCS, and productivity of heifers reared on reduced harvested feed input during postweaning development and subsequent winters may be dependent on amount of winter supplemental feed fed to the heifers' dam, thus indicating the possibility of a uterine programming effect (Roberts et al., 2009b). Research indicates that cows with similar genetic potential for mature weight may have different production efficiencies depending on milk yield (Montano-Bermudez and Nielsen, 1990). Our objective for the present research was to evaluate effects of amount of supplement fed to cows during late gestation and level of feed provided to their daughters during postweaning development on first parity milk yield and constituents.

Materials and Methods

Study Location and Environment

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 April 2009 through August 2010. The LARRL encompasses 22,500 ha and has an average elevation of 730 m. 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 340 mm with the majority of precipitation occurring from April through September from convectional thunderstorms. Predominant grass genera at study sites include grama (*Bouteloua*), needlegrass (*Hesperostipa*), and wheatgrass (*Pascopyron*)

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within a mixed-grass dominated rangeland (Küchler, 1964). The average annual forage standing crop at the study site is 870 ± 14 kg/ha (Grings et al., 2005). Quantity of forage availability was in excess of cattle needs (low stocking rate) in both years of the study.

Herd Management

All research protocols used in this study were approved by the Fort Keogh Institutional Animal Care and Use Committee. Heifers used in this study were from a stable composite population (**CGC**; ½ Red Angus, ¼ Charolais, ¼ Tarentaise).

Heifers were born from dams receiving adequate (**ADEQ**; 1.8 kg/d) or marginal (**MARG**; 1.2 kg/d) of good quality alfalfa hay as winter supplementation for approximately 80 d prior to parturition. At weaning, heifers were stratified into groups based on weaning weight and were randomly assigned to 1 of 22 to 24 pens. Each pen contained 6 individual feed bunks equipped with electronic Calan gates (American Calan, Northwood, NH) to allow heifers to be fed individually. Within each pen, heifers were randomly assigned to a 140-d postweaning development treatment that provided either ad-libitum (**AL**) or 80% of ad-libitum (**LAL**). Heifers received their experimental diets while being trained to open their assigned Calan gate for approximately 1 mo and then a 140-d development period was initiated. Level of feed provided for LAL was adjusted at 4-wk intervals using the following formula: [0.80 x (mean BW of restricted/mean BW control) x mean daily feed intake (as fed basis) of controls over the 28-d period]. Heifers that were developed in AL treatment received 1.8 kg/d winter supplementation for life, whereas heifers that were developed on the LAL treatment received 1.2 kg/d winter supplementation for life.

After the 140-d treatment period heifers were combined and managed together until the following winter when they were again separated by treatment. Bred heifer that received the AL development treatment were then supplemented at the ADEQ level (1.8 kg/d) and heifers developed in the LAL treatment received the MARG (1.2 kg/d) level of winter supplementation. Further details of experimental design and heifer management have previously been reported (Roberts et al., 2007; Roberts et al., 2009a; Roberts et al., 2010).

Experimental Animals

Sixteen heifers with steer calves were selected each year for two years from the CGC population. Heifers were selected to provide equal numbers for dam winter treatment, by heifer development classification and for having a similar birth date. Selected heifers grazed native rangeland in a 76 ha pasture for the duration of the study.

Measurements

Milk yield was measured every 14 d starting approximately 28 ± 0.81 d after parturition and continuing for 16 wk using a modified weigh-suckle-weigh technique (Wiley et al., 1991; Triplett et al., 1995; Waterman et al., 2006). Beal et al. (1990) indicated that use of a milking machine provides a more repeatable method than multiple weights on a calf before and after a suckling event. On the day of each milking, heifers were gathered from pasture, calves were removed from their dam, and cows were administered an i.m. injection of oxytocin (40 USP) 5 min before milking to facilitate milk letdown. The time interval from oxytocin administration to milk collection was recorded (Beal et al., 1990). Cows were then milked dry using a portable milking machine (SuperKart, Coburn Company, Inc., Whitewater, WI) until machine pressure could not extract any additional fluid, at which time individual teats were hand stripped by a technician. Milk collected from the initial milking was discarded. Heifers were kept separate from calves for 4.5 h and then milked a second time using the same procedures as previously mentioned. Milk weight was recorded after the final milking and an aliquot was retained for analysis of milk protein, lactose, butterfat, solids non-fat, and milk urea-N (**MUN**) by Rocky Mountain DHIA (Logan, UT). Final milk weight collected 4.5 h after initial milking was then multiplied by an appropriate factor to provide an estimate of 24-h milk yield. Daily (24-h) milk constituent secretion (g/d) was calculated by multiplying constituent concentration by daily milk production (Appeddu et al., 1997; Waterman et al., 2006). Body weight of steer claves was recorded within 24 h of calving and again on d 21, 35, 70, 98, and 126 postpartum.

Statistical Analysis

Milk production data and steer calf BW were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), with a model that included fixed effects of dam winter supplementation, heifer development treatment, collection date, and their interaction. The RANDOM statement included year with the subject heifer within (dam × heifer treatment). The REPEATED statement included collection date with the subject heifer within (dam and heifer treatment). Statistical significance was set at $P \leq 0.05$. No interactions ($P < 0.10$) were observed in the present analyses; therefore only main effects due to dam winter supplementation, heifer development treatment, and collection date are reported. The Tukey-Kramer method was used to adjust *P*-values to correct for multiple comparisons in the current study (SAS Institute, 2004). Means separations were conducted using PDMIX800 (Saxton, 1998). Failure to detect differences in measurements when $P < 0.30$ and > 0.05 may be due to the marginal number of animals used in the experiment and suggests the need for additional animals.

Results and Discussion

In the present study, milk yield and constituents were measured every 2-wk beginning on d 26 and continuing through d 126 postpartum. Milk peak yield (kg), d to peak milk, milk AUC, average milk yield (kg/d), milk urea N, and BW of steer claves did not differ due to dam winter nutrition ($P \geq 0.17$) or heifer development treatment ($P \geq 0.21$; Table 1). Milk butterfat, lactose, and solids non-fat did not differ due to dam winter nutrition ($P \geq 0.09$) and milk protein, lactose and solids non-fat did not differ

between heifer development treatment ($P \geq 0.09$; Table 1). Milk butterfat was greater ($P = 0.04$) in heifers receiving ad-libitum feeding during heifer development (Table 1). Heifers born from dams receiving MARG winter supplementation had greater ($P = 0.03$) milk protein than heifers born from dams receiving ADEQ of winter supplementation (Table 1). Body weight and BCS of heifers used in this study increased as d postpartum increased (Kelly et al., 2011).

Steer calf BW increased as days postpartum advanced ($P > 0.0001$; data not presented). Similarly, changes in milk yield and constituents occurred as weeks postpartum increased (Figure 1). Interestingly, no interactions between dam winter nutrition or heifer development treatment and week postpartum occurred indicating that this population of heifers responded similarly in regards to milk yield and constituents as lactation progressed postpartum (Figure 1). In summary, a heifer's dam (*in utero*) and subsequent development/lifetime winter plane of nutrition influenced first parity milk constituents but not first parity milk yield.

Implications

Cows managed on 2 levels of winter harvested feed inputs during late pregnancy may have lifetime influences on their female offspring. Furthermore, a reduction in harvested feed input during heifer development had no detrimental impact on milk yield, milk constituents, or calf BW. Continued research may indicate that reduction in harvested feed during late pregnancy and subsequent heifer development may result in improved economic and production efficiency.

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Table 1. Least square means ± SEM for milk production measurements and steer calf weight on first parity heifers over an 18 week period.

Item	Dam Winter Treatment*			Heifer Development Treatment†			*P* - value	
	ADEQ	MARG	SEM	AL	LAL	SEM	Dam Treatment	Heifer Treatment
Heifers, n =	16	16		16	16			
Milk Production								
Milk peak, kg	6.7	7.0	0.32	7.1	6.6	0.32	0.57	0.26
Milk Peak, d	66	63	6.6	70	60	6.6	0.74	0.28
Milk AUC, kg×126 d	644	657	37.4	683	618	37.4	0.81	0.22
Milk yield average, kg/d	5.6	6.1	0.28	6.1	5.6	0.28	0.17	0.25
Protein, g/d	184	211	8.3	206	189	8.3	0.03	0.15
Lactose, g/d	263	278	14.6	288	253	14.6	0.46	0.09
Butterfat, g/d	186	209	9.7	212	183	9.7	0.09	0.04
Solids-not-fat, g/d	495	543	23.5	548	490	23.5	0.16	0.09
Milk urea-N, mg/ 100mL	8.8	9.0	0.30	9.1	8.6	0.30	0.68	0.21
Calf Performance								
Calf BW, kg	79.9	77.9	2.50	79.6	78.2	2.50	0.56	0.69

* An evaluation of heifers born from dam receiving adequate (ADEQ; 1.8 kg/d) or marginal (MARG; 1.2 kg/d) winter nutritional treatments.

†Comparison of heifers fed ad-libitum (AL) or 80% of ad-libitum fed (LAL; adjusted to a common BW) for 140-d postweaning.

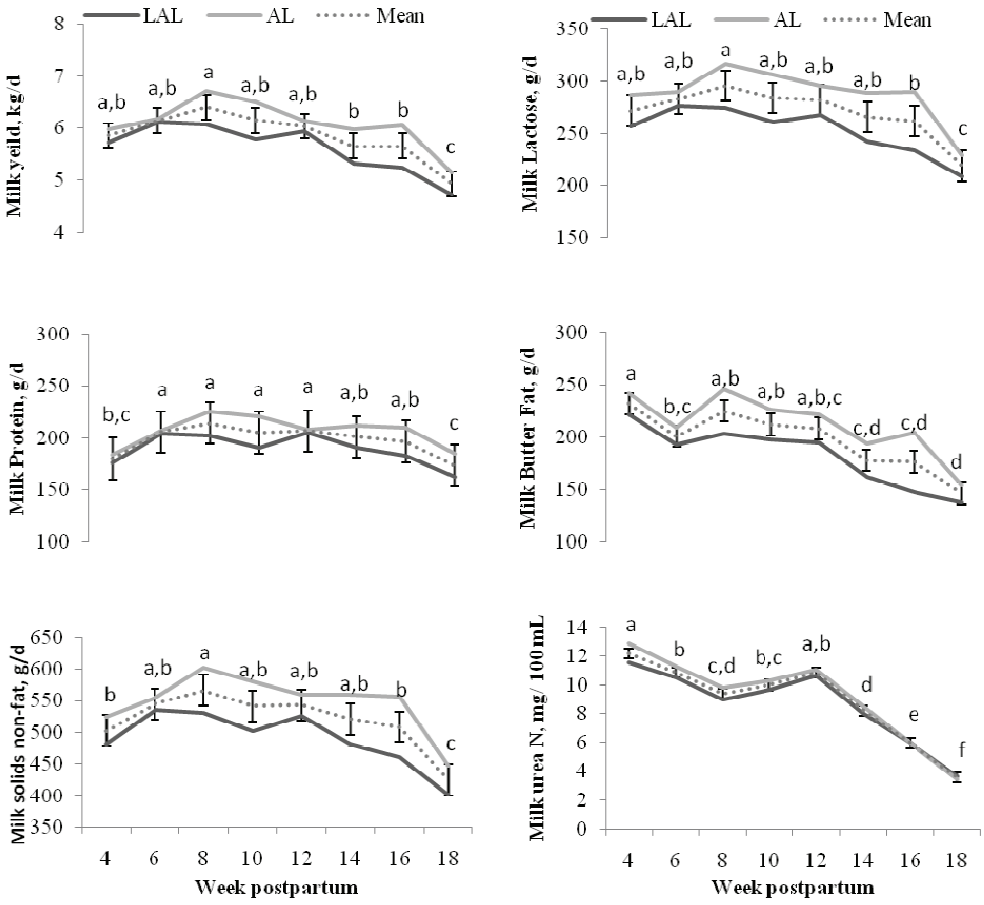

Figure 1. Mean response profiles for milk yield, lactose, protein, butter fat, solids not fat, and urea N for heifers fed either ad-libitum (AL) or 80% of ad-libitum (LAL; on a common body weight bases) during a 140-d postweaning period. Data are least square means ± SEM. No differences ($P > 1.0$) were observed between heifer developed on an ad-libitum (AL) or 80% of ad-libitum (LAL) diet. Superscripts indicate differences between weeks postpartum ($P > 0.0001$).

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FIRST PARITY EVALUATION OF BODY CONDITION, WEIGHT, AND BLOOD BETA-HYDROXYBUTYRATE DURING LACTATION OF RANGE COWS DEVELOPED IN THE SAME ECOPHYSIOLOGICAL SYSTEM BUT RECEIVING DIFFERENT HARVESTED FEED INPUTS

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ABSTRACT: Reduction of harvested feed inputs during heifer development could optimize range livestock production and improve economic feasibility for producers. The objective of this study was to measure body condition and weight as well as blood beta-hydroxybutyrate (**BHB**) concentrations for primiparous beef heifers born from dams receiving 1.8 or 1.2 kg/d of winter supplementation during late gestation and then developed within an ad-libitum or 20% less feed post weaning. Heifer (BW) and **BHB** concentrations were measured (n = 32; 16/yr reared under two different feed inputs) every 7 days from d 21 to 126 post partum and body condition was measured every 14 days from d 21 to 119 post partum. The analysis of variance model included dam nutritional plane, heifer development feed intake, day of collection, and their interaction. Body condition tended to be greater (P = 0.08) during the 126-d trial in first parity heifers that were developed in the ad-libitum treatment group. Body condition was not influenced by dam winter nutrition (P = 0.21). Weekly body weights were greatest (P = 0.0002) in first parity heifers that were developed with ad-libitum feed (433 vs. 379 ± 8.90 kg, respectively for ad-libitum or 20% less feed treatment). Weekly body weights were not influenced by dam winter nutrition (P = 0.63). BHB concentrations did not differ (P = 0.33) between heifers developed differently, but tended (P = 0.09) to be greater in heifers born from dams that received the 1.2 kg/d winter supplementation. These results indicate that by reducing feed during heifer development results in lower first parity body weight and condition, and that dam plane of winter nutrition may influence the metabolism of their offspring.

Key words: Feed intake, primiparous beef cow, rangeland

Introduction

Winter feeding programs and heifer development are major financial inputs in extensive western United States production systems. To maintain a sustainable beef cow/calf enterprise in these environments, the use of harvested feeds are necessary. Adequate nutrition is crucial for reproductive success especially if the goal is to have heifers conceive by 14 months of age and to have their first calf by 24 months of age. Therefore, identification of individuals that more efficiently utilize dietary feedstuffs and still reproduce equate to a more optimal production system. Roberts and coworkers (2009) indicate that heifers consuming 27% less harvested feed for 140-d during their yearling development had similar pregnancy rates to their unrestricted counterparts. In addition, heifers that received reduced levels of harvested feed had compensatory gains following the development period and out-gained ad libitum fed heifers. Furthermore, reduced fed heifers had a $21 per pregnant heifer advantage when compared to ad libitum fed heifers (Roberts et al., 2009). Funston and Deutscher (2004) report similar savings that equated to a $22 per heifer advantage when lower quality and thus lower-cost diets were consumed.

Economically, it may be beneficial to develop heifers on a lower plane of nutrition and accept a slightly lower pregnancy rate; however, effects if any does it have on lifelong efficiency and metabolism should be considered. Further economic benefits could be absorbed by lowering winter feed inputs during late gestation but how is the resulting heifer calf affected in utero? It has been suggested that heifers born to dams who received marginal winter supplementation, and were subsequently developed on a slightly lower plane of nutrition may have economical advantages through altered metabolism and (or) efficiency (Roberts et al., 2010). The current study evaluated heifers who were developed on a lowered plane of nutrition and were assigned to winter supplementation regimens consisting of either a traditional program or a program slightly lower than traditional. Heifer's dams were also under a reduced or normal winter feeding program. To assess the effect of treatments on these

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heifers during their first parity BW, beta-hydroxybutyrate **(BHB)**, and BCS were evaluated.

Materials and Methods

Procedures were approved by the Fort Keogh Livestock and Range Research Laboratory Animal Care and Use Committee. Heifers used in this study were a stable composite population (**CGC**; ½ Red Angus, ¼ Charolais, ¼ Tarentaise). Heifers were born during a 2-yr period (2007 & 2008) to dams receiving either 1.8 kg/d (**ADEQ**) or 1.2 kg/d (**MARG**) of winter supplement prior to calving as previously described by Roberts et al. (2010). After weaning, heifers were stratified into groups of 6 based on weaning weight and groups were randomly assigned to 1 of 24 pens. Each pen contained 6 individual feed bunks equipped with electronic Calan gates (American Calan, Northwood, NH). Heifers were randomly assigned within pen to receive either an ad-libitum (**AL**) or 20% less of the ad-libitum (**LAL**) growth ration for 140-d as previously described (Roberts et al., 2007, 2009). Upon completion of the 140-d trial, heifers were managed as one herd from breeding to December when they were again separated into treatment groups to allow differential winter supplementation. Heifers fed **AL** during postweaning development were fed the **ADEQ** level of winter supplement and heifers fed **LAL** during postweaning development were fed **MARG** winter supplement for life.

During the period of April 15, 2009 through July 29, 2009 and again from April 7, 2010 through July 21, 2010, 32 primiparous CGC 2-year-old cows (16 each year) were selected from the herd to provide 8 animals from each dam winter supplementation program by individual heifer development treatment classification. Body weight and blood BHB concentrations were measured on d 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112, 119, and 126 postpartum. Body condition was measured on d 21, 35, 49, 63, 77, 91, 105, and 119 postpartum. All measurements were taken in the morning before other cattle work was performed. Body condition scores (1= emaciated to 9 = extremely obese) were assigned by the same two experienced technicians, as described by Herd and Sprott (1986) and Wagner et al. (1988). Blood BHB concentrations were measured utilizing a commercially available diabetic monitor system (MediSence®; Precision Xtra™; Abbott laboratories, Abbott Park, IL 60064, USA). For each measurement taken, a new blood β-ketone test strip (MediSence®; Precision Xtra™; Abbott laboratories, Abbott Park, IL 60064, USA) was inserted into the monitor and calibration was verified. A clean, fresh drop of whole blood was recovered via tail or jugular vein and placed on the collection tip of the test strip. Data were recorded immediately at chute side. If the monitor recorded an error, a fresh strip was inserted into the monitor, a new blood sample was tested, and this continued until a valid reading could be recorded.

Statistical Analysis

Data for BW, BHB and BCS were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), with a model that included fixed effects of dam winter supplementation, heifer development treatment, collection date, and their interaction. The RANDOM statement included year with the subject heifer within dam and heifer treatment classification. The REPEATED statement included collection date with the subject heifer within dam and heifer treatment. Statistical significance was set at $P \leq 0.05$. No interactions ($P < 0.10$) were observed in the present analyses; therefore only main effects due to dam winter supplementation, heifer development treatment, and collection date are reported. The Tukey-Kramer method was used to adjust *P*-values to correct for multiple comparisons in the current study (SAS Institute, 2004). Means separations were conducted using PDMIX800 (Saxton, 1998).

Results and Discussion

Weekly BW were greatest ($P < 0.001$) in first parity heifers that were developed with ad-libitum feed (433 vs. 379 ± 8.90 kg, respectively for **AL** or **LAL**; Table 1). Body condition tended to be greater ($P = 0.08$) during the 126-d sampling period in heifers that were developed in the **AL** treatment group (Table 1). These data are further supported by similar results found by Roberts et al., (2007). Heifer BW and BCS were similar for dam winter nutrition ($P = 0.63$ and $P = 0.21$ respectively; Table 1). Body condition increased from d 21 to 126 regardless of heifer development treatment (Figure 1).

Hepatic ketogenesis occurs at similar rates with no discrimination in fed, nonpregnant, nonlactating goats, sheep, and dairy cows and hepatic tissue release of BHB increases in late gestation and early lactation (Heitmann et al., 1987). When the lipogenic pathway (synthesis from acetate into long-chain fatty acids in adipose tissue) and tri-carboxylic acid cycle (incorporation of acetate to convert oxaloacetate to citrate) are operating less efficient excess acetate is either oxidized as substrate in futile cycles or directed towards the synthesis of ketones (MacRae and Lobley, 1982; Armentano, 1992)

In the present study, BHB was evaluated every wk from d 14 through 126 to evaluate if dam winter nutrition or heifer development treatment influenced the production of BHB. Level of feed provided during heifer development and winter supplementation did not influence ($P = 0.33$) weekly BHB concentrations after first calving. However, there was a tendency for the heifers born from dams that received the MARG winter supplementation to have greater overall BHB concentrations ($P = 0.09$). As discussed above, increases in circulating levels of BHB suggest that the animal may be experiencing some level of negative energy balance. This might indicate gluconeogenic precursors are limiting, thus not allowing for efficient utilization of acetyl-CoA in the Krebs Cycle, resulting in the

conversion of acetyl-CoA to ketone bodies. Earlier research has suggested that an individual cow's metabolism could be programmed as early as in utero or yearling development (Roberts et al., 2010). Waterman et al. (2011) in the same population of cows identified a trend (P = 0.08) for glucose half-life to be shorter in heifers born from dams receiving the MARG in utero winter treatments. These findings disagree with findings observed with BHB in the present study. Although, the statistical analysis allowed for the detection of a tendency in BHB to be different, numerically they are fairly close (Table 1), and below levels previously observed in animals experiencing negative energy balance at approximately 3mM. Concentrations of BHB were different depending on week postpartum but the changes were consistent regardless of heifer development treatment (Figure 1).

Implications

The present research indicates that lighter BW and lower BCS observed during the first postpartum period in heifers provided less feed during postweaning development and for winter supplementation were not accompanied by differences in circulating levels of BHB that would be indicative of differences in energy balance. This observation provides additional evidence that reductions in harvested feed inputs may provide for improved profitability without major decreases in productivity, as previous research on this herd of cattle at Fort Keogh has revealed. Observed differences in BHB due to dam treatment extend the list of factors observed in this herd that appear to be altered by nutritional influences mediated through uterine/fetal programming. Continued long term research will be of value as this herd continues to further our understanding of the mechanisms at work and how to better utilize resources.

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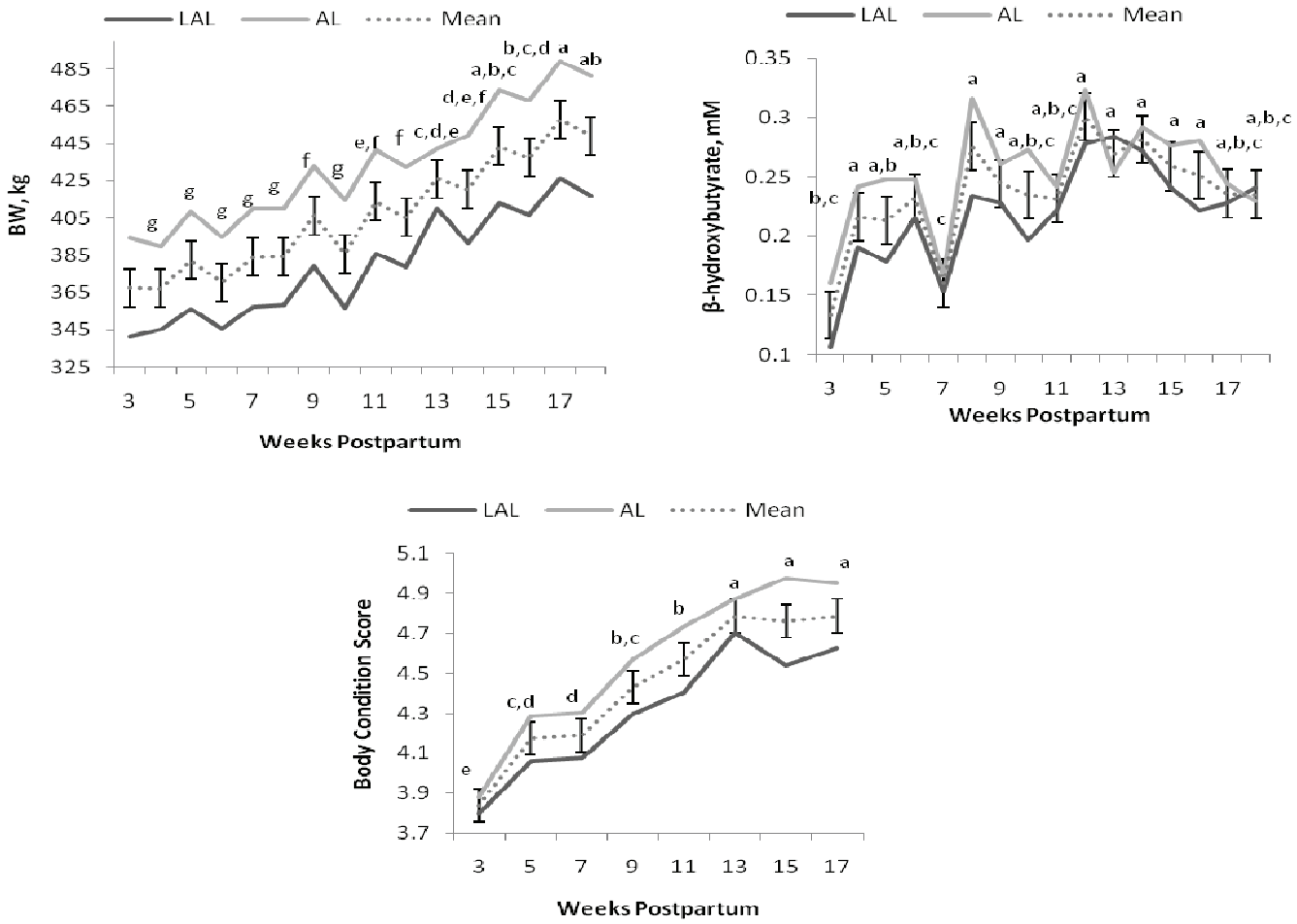

Figure 1. Body weight, β-hydroxybutyrate, and BCS means of postpartum heifers whose dams received either 1.8 kg/d or 1.2 kg/d of winter supplemental feed and then were developed for 140-d post-weaning at either ad-libitum (AL) or 20% less of ad-libitum (LAL). Data are given as least squares means ±SEM. Body weights differed between LAL and AL groups ($P < .001$).

Table 1. Least square means ± SEM for BW, β-hydroxybuterate (BHB), and BCS conducted on first parity heifers over a 16-week period.

	Dam Winter Treatment*			Heifer Development Treatment†			*P* - value	
Item	ADEQ	MARG	SEM	AL	LAL	SEM	Dam Treatment	Heifer Treatment
Heifers, n =	16	16		16	16			
Body Weight, kg	409.4	403.3	8.9	433.4	379.4	8.9	0.63	0.001
BHB, mM	0.218	0.263	0.17	0.253	0.228	0.017	0.09	0.33
BCS	4.61	4.5	0.06	4.63	4.47	0.06	0.81	0.22

*An evaluation of heifers born from dam receiving adequate (ADEQ) or marginal (MARG) winter nutritional treatments.
†A comparison of heifers developed on a ad-libitum (100%) or reduced (80%; fed at 80% of that consumed by controls adjusted to a common BW) 140-d heifer development diet.

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DOES PURE DINOPROST TROMETHAMINE (PROSTAGLANDIN $F_{2\alpha}$) INHIBIT GROWTH *IN VITRO* OF *STAPHYLOCOCCUS AUREUS* ASSOCIATED WITH BOVINE MASTITIS?

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ABSTRACT: Certain fatty acids have antimicrobial properties on the mastitis-causing pathogen, *Staphylococcus aureus (S. aureus)*. The objective of this study was to determine the effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), in a pure and solid form of dinoprost tromethamine, on growth of *S. aureus*. Glass tubes containing tryptic soy broth were inoculated with a strain of *S. aureus* Novel, and subsequently treated with $PGF_{2\alpha}$ at concentrations of 0, 2.4, 4.8 and 9.6 mg/mL. Cultures were incubated at 37°C and sampled every 6 h for 24 h. Bacterial growth was assessed by measuring turbidity using optical density at 600 nm (OD_{600}) and counting colony forming units (log CFU). Data were analyzed by analysis of variance (repeated measures), where the model included treatment, repeated factor (time), and their interaction. There was an effect of treatment and treatment by time interaction ($P < 0.05$) on mean OD_{600} and log CFU, indicating that bacterial growth over 24 h was different across treatments. Pre-planned contrasts were conducted to compare the mean OD_{600} and log CFU values between treatment concentrations at 24 h. At time of inoculation (0 h), mean OD_{600} and log CFU values were not different ($P > 0.5$) between treatments and averaged 0.13 ± 0.18 OD_{600} units and 7.1 ± 0.05 log CFU. However, at 24 h, mean OD_{600} units for each $PGF_{2\alpha}$ treatment was different ($P < 0.05$) from control (16.67 ± 0.17). Mean OD_{600} value of 2.4 mg/mL (7.45 ± 0.17) was different ($P < 0.05$) from 4.8 mg/mL (5.92 ± 0.17) and 9.6 mg/mL treatments (5.81 ± 0.17) at 24 h. In contrast, mean OD_{600} values of 4.8 mg/mL and 9.6 mg/mL treatments were not different ($P > 0.2$) from each other at 24 h. Log CFU values (number of live cells) at 24 h, for each $PGF_{2\alpha}$ treatment, were different ($P < 0.05$) from control (10.77 ± 0.06), in a dose dependent manner. Mean log CFU values at 24 h were 9.85 ± 0.06, 8.66 ± 0.06, and 8.42 ± 0.06, for $PGF_{2\alpha}$ treatments 2.4, 4.8, and 9.6 mg/mL, respectively. These results provide evidence for the first time that $PGF_{2\alpha}$, in the form of pure dinoprost tromethamine, has inhibitory effects on growth of *S. aureus in vitro*.

Key words: mastitis, prostaglandin $F_{2\alpha}$, *Staphylococcus aureus*

Introduction

Infec†tions caused by *Staphylococcus aureus* bacteria result in a wide variety of diseases that affect both domesticated animals and humans (Amir et al., 1999). In the bovine species, *S. aureus* is involved in uterine and intramammary infections, such as metritis (Smith and Risco, 2002) and mastitis (Bannerman et al. 2004), respectively.

S. aureus is a contagious pathogen (Blowey and Edmondson, 1995) that has adapted to survive within the mammary gland. Lombard et. al. (2008) reported 43% of dairies in 17 states in the U.S. identified *S. aureus* as the most contagious pathogen present in the herd.

The spread of contagious mastitis pathogens cause large economic losses to the dairy industry. These losses include increased involuntary culling rate, reduced milk production, increase in somatic cell count, and discarded milk (Philpot, 1984; Zepeda, 1998). Costs associated with mastitis infections for the U.S. dairy industry have been estimated at about $2 billion per year (Degraves and Fetrow, 1993).

Cure rates for subclinical *S.aureus* mastitis treated with antibiotics range widely, most likely due to the type of antibiotic used, as well as various cow factors (Barkema et al., 2004). The age of the cow, the number of infected quarters, and the duration of the infection are factors that may alter the response to antibiotic treatments (Ruegg, 2004). A variety of treatments and immunizations against *S. aureus* mastitis have been researched for years (Anderson, 1998); however, the intracellular survival of *S. aureus* is a large factor contributing to the difficulty in clearing *S. aureus* infections following antibiotic therapy (Sears et al., 2001). If cure rates are low, it is generally not considered cost-effective to treat cows with chronic *S. aureus* infections (Ruegg, 2004).

The bactericidal activities of various fatty acids on bacteria have been studied as an alternative to antibiotics (Walker, 1926; Bayliss, 1936; Kabara et al., 1977; Kanai and Kondo, 1979; Kelsey et al., 2006). The fatty acid arachidonic acid is a precursor to the synthesis of several prostaglandins in response to various stimuli. One of these prostaglandins, $PGF_{2\alpha}$, is readily available as dinoprost tromethamine for use in estrous synchronization. Because $PGF_{2\alpha}$ is a fatty acid, we hypothesized that pure dinoprost tromethamine has antimicrobial properties that resemble those of fatty acids, and inhibits the growth of *S. aureus in vitro*. The objective of this study was to determine the effects of $PGF_{2\alpha}$ on *S.*

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aureus in vitro by characterizing the growth response of *S. aureus* to pure dinoprost tromethamine.

Materials and Methods

Experimental design and treatment. A strain of *S. aureus* Novel was prepared by inoculating a single colony into 3 mL of tryptic soy broth (TSB) followed by overnight incubation, at 37°C with shaking at 250 rpm.

A total of 8 tubes (2 tubes/treatment) were inoculated at a concentration of 1:100, with the overnight culture of *S. aureus* and subsequently treated with $PGF_{2\alpha}$, in the form of dinoprost tromethamine (Pfizer Animal Health, New York, NY), at concentrations of 0, 2.4, 4.8 and 9.6 mg/mL. Tubes were incubated at 37°C and shaken at 250 rpm for 24 h. At time of bacterial inoculation (0 h), and every 6 h, for 24 h, 1 mL samples were taken from each tube, and the experiment was repeated twice. Bacterial growth was assessed using a spectrophotometer to visually determine the optical density at 600 nm (OD_{600}). Optical density measures turbidity, with the assumption that when turbidity of the broth increases, so does bacterial growth.

To determine colony forming units (CFU), samples of 100 uL were taken at each time point to perform serial dilutions. The 100 uL samples were added to 900 uL of fresh TSB, and this process was repeated until dilutions appropriately coincided with the turbidity of the broth. Samples were plated on agar plates (EMD Chemicals Inc., Darmstadt, NJ) and after 24 h incubation plate counts were performed in duplicate per sample from each tube.

Statistical Analysis. At each 6 h time point, the OD_{600} was recorded three times, and results for duplicate concentrations were averaged. OD_{600} was determined by taking the mean of two 24-h growth curves for each concentration at each time point (modified from Kelsey et al., 2006). An analysis of variance (repeated measures) was carried out using Mixed procedure of SAS® (SAS Institute, Cary, NC), where the model included treatment, repeated factor (time), and their interaction.

The number of live cells, as measured by CFU, was determined by averaging the number of cells for the duplicate concentrations of two plates at each 6 h time point. Results for two growth curves were also pooled for each concentration, at each time point. The analysis of variance procedures carried out for the CFU responses were similar to those carried out for the OD_{600} responses.

Results and Discussion

Based on bacterial growth curves, $PGF_{2\alpha}$, in the form of dinoprost tromethamine, has inhibitory effects on growth of *S. aureus* (Novel). Overall growth of *S. aureus* decreased with increasing concentrations of $PGF_{2\alpha}$, with the treatment 9.6 mg/mL of $PGF_{2\alpha}$ determined to be the most inhibitory concentration.

There was a significant effect of treatment and treatment by time interaction, on mean OD_{600} and log CFU ($P < 0.05$), indicating that bacterial growth over 24 h was different across treatments. Pre-planned contrasts were conducted to compare mean OD_{600} and log CFU values

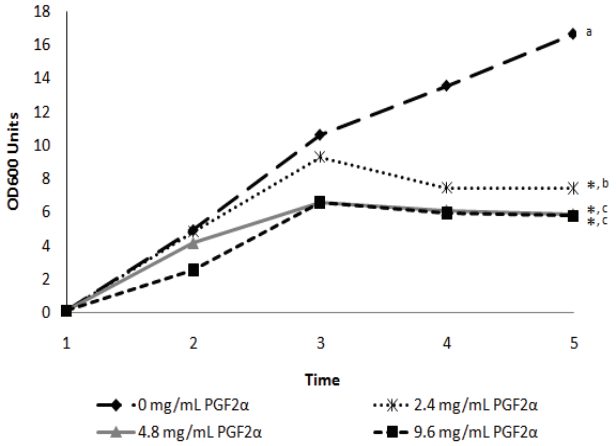

Figure 1. Growth of *S. aureus* as measured by optical density at 600 nm (OD 600 units), treated with $PGF_{2\alpha}$, at concentrations of 2.4 mg/mL (dotted star), 4.8 mg/mL (gray triangle), and 9.6 mg/mL (dashed triangle). Control (0 mg/mL $PGF_{2\alpha}$) consisted of tryptic soy broth (TSB) (long dashed diamond). * Means of each treatment at 24 h differ from control ($P < 0.05$). [a, b, c,] Means of treatments with different letters differ at 24 h ($P < 0.05$)

between concentrations at 24 h. At 0 h mean OD_{600} and log CFU values were not significantly different between treatments and averaged 0.13 ± 0.18 OD_{600} units (Fig. 1) and 7.1 ± 0.05 log CFU (Fig. 2). However, at 24 h of growth, the mean OD_{600} value for each dinoprost tromethamine treatment was different ($P < 0.05$) from the control (Fig. 1). At 24 h, mean OD_{600} value of 2.4 mg/mL (7.45 ± 0.17) was different ($P < 0.05$) from 4.8 mg/mL (5.92 ± 0.17) and also from 9.6 mg/ml treatments (5.81 ± 0.17) (Fig. 1). Mean OD_{600} values of 4.8 mg/mL and 9.6 mg/mL treatments were not different ($P > 0.2$) from each other (Fig. 1).

At 24 h of growth, the log CFU value for each dinoprost tromethamine treatment was different from one another ($P < 0.05$) and from the control (10.77 ± 0.06) (Fig

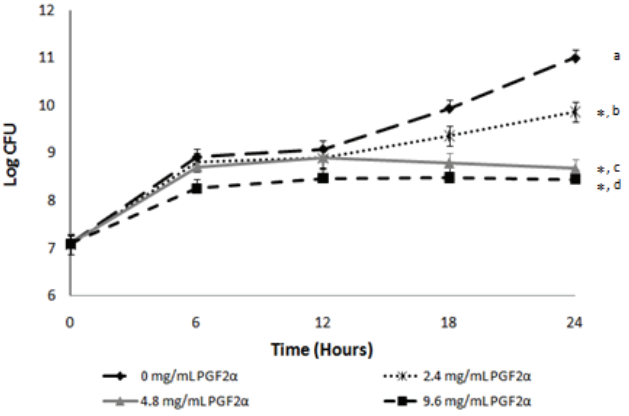

Figure 2. Growth of *S. aureus* as measured by colony forming units (log CFU), treated with $PGF_{2\alpha}$ at concentrations of 2.4 mg/mL (dotted star), 4.8 mg/mL (gray triangle), and 9.6 mg/mL (dashed triangle). Control (0 mg/mL $PGF_{2\alpha}$) consisted of tryptic soy broth (TSB) (long dashed diamond). * Means of each treatment at 24 h differ from control ($P < 0.05$). [a, b, c, d] Means of treatments with different letters differ at 24 h ($P < 0.05$)

2). Bacterial growth at 24 h responded in a dose dependent manner, where mean log CFU values (number of live cells) were 9.85 ± 0.06, 8.66 ± 0.06, and 8.42 ± 0.06, for $PGF_{2\alpha}$ treatments 2.4, 4.8, and 9.6 mg/mL, respectively (Fig. 2, Table 1). These results provide evidence for the first time that $PGF_{2\alpha}$ in the form of dinoprost tromethamine has inhibitory effects on growth of *S. aureus in vitro*.

Table 1. Mean values of Log CFU of *S. aureus*, at 0 and 24 h, treated with different concentrations of $PGF_{2\alpha}$ over 24 h.

Concentration $PGF_{2\alpha}$	0 h	24 h
0 mg/mL	7.1 ± 0.05^{a}	10.77 ± 0.06^{b}
2.4 mg/mL	7.07 ± 0.05^{a}	9.86 ± 0.06^{c}
4.8 mg/mL	7.1 ± 0.05^{a}	8.66 ± 0.06^{d}
9.6 mg/mL	7.1 ± 0.05^{a}	8.42 ± 0.06^{e}

[a, b, c, d, e] Means of treatments within columns and rows with different letters differ ($P < 0.05$)

The antimicrobial properties of fatty acids on bacteria have been studied for years. Walker (1926) showed that fatty acid soaps inhibit growth of *Streptococci*, and later Bayliss (1936) studied *S. aureus, E. coli-communis, Diplococcus pneumoniae* and *Streptococcus lacti*s, and their susceptibility to 27 different soaps. The effectiveness of fatty acids in inhibiting growth of gram-positive bacteria has also been demonstrated (Kabara et al., 1977; Kanai and Kondo, 1979), and concurs with the results from our present study.

Research by Kelsey et al. (2006) indicated that certain fatty acids (laureic acid, capric acid, myristic acid) and monoglycerols were more potent than others (linoleic acid, *cis*-9, *trans*-11 conjugated linoleic acid, and *trans*-10, *cis*-12 conjugated linoleic acid) in inhibiting two different strains of *S. aureus*. More importantly, the strain used for this study (*S. aureus* Novel) was the same as used in previous studies (Smith et al., 1998; Kelsey et al., 2006). The fatty acid used for this study was prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Prostaglandins are involved in regulating numerous processes in the body, including reproductive function (Bardin, 1970), neurotransmitter release, platelet aggregation, kidney function, and modulation of immune function (Bergström et al.,1968; Goetzl et al., 1995; Phipps et al., 1991). $PGF_{2\alpha}$ is synthesized from arachidonic acid (Bergström et al., 1968), a fatty acid originally derived from linoleic acid (Marcel et al., 1968). Kodicek and Worden (1945) characterized the actions of linoleic acid on pathogens as bacteriostatic. Kelsey et al. (2006) noted that linoleic acid inhibited growth of *S. aureus* Novel. Linoleic acid also inhibited bacterial growth of *Lactobacillus helveticus, Bacillus subtilis* and *Clostridium sporogenes* in a similar fashion (Laser, 1952; Nieman, 1954). Gram-positive bacteria such as *Streptococcus faecalis, Staphylococcus epidermidis, Lactobacillus acidophilus, Bacillus megaterium* and *Corynebacterium equi* were inhibited by arachidonic acid (Knapp and Melly, 1986). Since linoleic acid and arachidonic acid have been shown to affect bacterial growth, we therefore hypothesized that $PGF_{2\alpha}$, synthesized from these fatty acids, may perhaps have similar antibacterial properties. Our results presented here indicate that $PGF_{2\alpha}$, in the form of dinoprost tromethamine, inhibits the growth of *S. aureus in vitro*. Growth was inhibited in a dose dependent manner, resembling the actions of fatty acids in previously described literature (Bayliss, 1936; Kodicek, 1949; Knapp and Melly, 1986; Petschow et al., 1996; Kelsey et al., 2006).

The mechanism by which $PGF_{2\alpha}$ inhibited *S. aureus* growth cannot be determined in the current study. Dinoprost tromethamine is composed of 24 carbons, making it a long chain fatty acid, as well as two double bonds. Kabara et al. (1977) revealed that the position and presence of a double or triple bond, was an important factor in inhibition of bacteria by long chain fatty acids ($> C_{14}$). The inhibitory properties of fatty acids were also noted to be more distinct as the degree of unsaturation and chain length both increased (Nieman, 1954). The strong affinity to fatty acids may be due to their hydrophobic surface and the cell wall's lipid rich interior, enabling the ease of penetration of fatty acids into the plasma membrane. The membrane may also be disrupted as the hydrocarbon chain of the fatty acids are inserted in the phospholipid bilayer and increase the negative charge of the membrane surface (Kondo and Kanai, 1972; Camien and Dunn, 1957). The proposed mechanism may occur via an interaction of the lipid at the cell membrane, resulting in a change in membrane permeability (Nieman, 1954). Other studies showed that some lipids such as lauric acid may disrupt the signal transduction pathways of *S. aureus* that are involved in the production of exoprotein virulence factors (Projan et al., 1994). Although the mechanism by which $PGF_{2\alpha,}$ in the form of dinoprost tromethamine, inhibits the growth of *S. aureus in vitro* is not known, it is likely via similar membrane interactions and multi-step mechanisms. Nonetheless, further research will be necessary to elucidate how $PGF_{2\alpha}$ inhibits *S. aureus* growth.

Implications

The difficulty in curing chronic intramammary infections, particularly those caused by *S. aureus,* has pressed for a reassessment of current and potential treatment strategies. Numerous studies have demonstrated various effects of fatty acids on bacteria. The current study determined the antimicrobial effects of $PGF_{2\alpha}$, a fatty acid hormone readily available for use in estrous synchronization. This study established for the first time, that $PGF_{2\alpha,}$ in the form of dinoprost tromethamine, inhibits growth of *S. aureus in vitro*. With further research $PGF_{2\alpha}$ may potentially aid in curing mastitis infections caused by the *S. aureus* pathogen.

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CAMELINA MEAL SUPPLEMENTATION TO BEEF CATTLE: III. EFECTS ON ACUTE-PHASE AND THYROID RESPONSES

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ABSTRACT: Fourteen halter-trained Angus steers were ranked by initial BW (average 191 ± 2.1 kg), and assigned (d 0) to receive supplements containing (as-fed basis): 1) 84 % corn, 14 % soybean meal, and 2 % mineral mix (CO); and 2) 70 % corn, 28 % camelina meal, and 2 % mineral mix (CAM). Treatments were offered individually, at a daily rate of 1.65 and 1.52 kg of DM/steer for CO and CAM, respectively. Alfalfa-grass hay was offered ad libitum during the study (d 0 to 36). On d 24, steers were fitted with a jugular catheter and were infused (i.v.) on d 25 with 0.5 µg of bovine corticotropin-releasing hormone (CRH)/kg of BW. Blood samples were collected hourly from –2 to 0 h and 4 to 8 h, and every 30 min from 0 to 4 h relative to treatment infusion (0 h). Blood samples were also collected via jugular venipuncture every 6 h from 12 to 72 h, and every 24 h from 96 to 168 h. All samples were analyzed for plasma concentrations of cortisol, ceruloplasmin, and haptoglobin. No treatment effects were detected (P = 0.28) for cortisol concentrations, which peaked for both treatments at 0.5 h relative to CRH infusion (time effect; P < 0.01). Ceruloplasmin concentrations were greater for CO vs. CAM steers at 6, 18, 42, 120, 144, and 168 h relative to CRH infusion (treatment × time interaction, P < 0.01). Mean haptoglobin concentrations tended to be greater (P = 0.10) for CO vs. CAM steers (1.73 vs. 1.54 absorbance @ 450 nm × 100, respectively). On d 34, steers were again fitted with a jugular catheter and were infused (i.v.) on d 35 with 0.33 µg of bovine thyrotropin-releasing hormone (TRH)/kg of BW. Blood samples were collected hourly from –2 to 0 h and 4 to 8 h, every 30 min from 0 to 4 h, and every 4 h from 8 to 24 h relative to treatment infusion (h 0) for determination of serum T_3 and T_4. No treatment effects were detected for T_3 (P = 0.58) and T_4 (P = 0.54) concentrations, which peaked, respectively, at 3 and 5 h relative to TRH infusion in both treatments. In conclusion, camelina meal supplementation did not affect thyroid gland function following a TRH challenge, but alleviated the acute-phase protein response following a CRH challenge in beef steers.

Key Words: Acute-phase, camelina meal, thyroid

Introduction

The acute-phase response is an important component of the innate immune system, but it can be detrimental to cattle performance, particularly when stimulated by stressors such as weaning, transport and feedlot entry (Duff and Galyean, 2007; Araujo et al., 2010, Cooke et al., 2010). Alternatives to prevent this reaction, including supplementation of polyunsaturated fatty acids (PUFA), are thus beneficial to cattle productivity (Cooke et al., 2010). Moreover, Cooke and Bohnert (2011) reported that corticotropin-releasing hormone (CRH) challenge stimulates an acute-phase response in cattle, and this research model can be used to investigate the physiological components and develop alternatives to modulate the stress-induced acute-phase response.

Camelina meal results from the mechanical processing of the seeds for oil extraction, and may contain up to 20% oil with the majority of the fatty acid content as PUFA (Moriel et al., 2010). Therefore, camelina meal may serve as a nutritional alternative to modulate the acute-phase response in cattle subjected to stress of management. However, camelina meal contains elevated concentrations of glucosinolates, which may impair thyroid gland activity in cattle (Lardy and Kerley, 1994) resulting in impaired growth rates (Burel et al., 2001). However, Moriel et al. (2010) reported that camelina meal supplementation did not impair thyroid function in beef heifers. Therefore, we hypothesized that camelina meal supplementation alleviates stress-induced acute-phase responses without impairing thyroid function in beef cattle. The objectives of this study were to evaluate the effects of camelina meal supplementation on concentrations of acute-phase proteins and thyroid hormones in beef steers following a CRH and thyrotropin-releasing hormone (TRH) challenges, respectively.

Materials and Methods

The experiment was conducted in accordance with an approved Oregon State University Animal Care and Use Protocol. Fourteen weaned Angus steers were utilized in these studies. All steers were exposed daily (d -60 to d 0) to halter-training techniques to become acclimated to human interaction; thus preventing confounding effects between human handling, weaning and hormone challenges measured herein (Cooke et al., 2009). Steers were ranked by initial BW (average 191 ± 2.1 kg), and assigned on d 0 to receive 1 of 2 treatments: 1) supplement containing (as-fed basis) 84 % corn, 14 % soybean meal, and 2 % mineral mix (CO); and 2) supplement containing (as-fed basis) 70 % corn, 28 % camelina meal, and 2 % mineral mix (CAM). Treatment intakes were formulated to be iso-caloric and iso-nitrogenous, and offered individually at a daily rate of 1.65 and 1.52 kg of DM/steer for CO and CAM,

respectively. Alfalfa-grass hay was offered ad libitum during the study (d 0 to 36).

On d 24 and 34 of the study, steers were fitted with a jugular catheter according to procedures described by Merrill et al. (2007), and were infused (i.v.) on d 25 and 35 with 0.5 µg of bovine CRH/kg of BW (Exp. 1) and 0.33 µg of bovine TRH/kg of BW (Exp. 2), respectively. In Exp. 1, blood samples were collected hourly from –2 to 0 h and 4 to 8 h, and every 30 min from 0 to 4 h relative to treatment infusion (0 h) via jugular catheters. Blood samples were also collected via jugular venipuncture every 6 h from 12 to 72 h, and every 24 h from 96 to 168 h. All samples were analyzed for plasma concentrations of cortisol, ceruloplasmin, and haptoglobin. In Exp. 2, blood samples were collected via jugular catheters hourly from –2 to 0 h and 4 to 8 h, every 30 min from 0 to 4 h, and every 4 h from 8 to 24 h relative to treatment infusion (h 0) for determination of serum T_3 and T_4. Blood samples were harvested for plasma and serum, and stored at −80°C until assayed for plasma concentrations of cortisol Endocrine Technologies Inc., Newark, CA), ceruloplasmin (Demetriou et al., 1974) and haptoglobin (Makimura and Suzuki, 1982), and serum concentrations of T_3 and T_4 (Endocrine Technologies Inc.).

Data from Exp. 1 and 2 were analyzed using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement contained the effects of treatment, time, and the interaction. Data were analyzed using steer(treatment) as the random variable. The specified term for the repeated statement was time and the covariance structure utilized was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. Results are reported as least square means and separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effects, or according to the highest-order interaction detected.

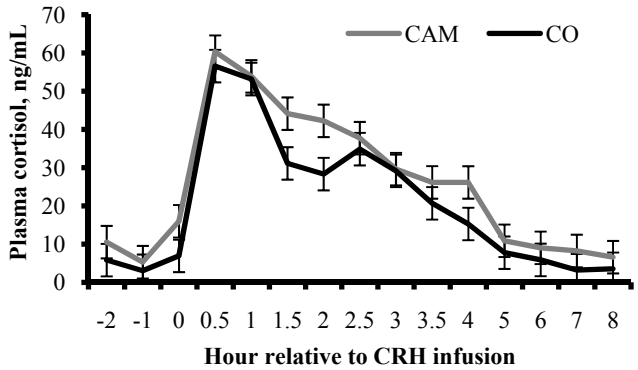

Figure 1. Plasma cortisol concentrations of steers supplemented (CAM) or not (CO) with camelina meal and receiving 0.5 µg of bovine corticotropin-releasing hormone (CRH)/kg of BW at h 0. No treatment effect (P = 0.28) or treatment × time interaction (P = 0.09) were detected.

Results and Discussion

In Exp. 1, no treatment (P = 0.28) effects were observed for cortisol concentrations (Figure 1). Steers receiving CAM tended (P = 0.10) to have reduced mean haptoglobin concentrations compared to CO steers (1.54 vs. 1.73 absorbance @ 450 nm × 100, respectively; Figure 2). A treatment x time interaction (P < 0.001) was detected for ceruloplasmin concentrations, because CAM steers had reduced ceruloplasmin concentrations compared with CO steers at 6, 18, 42, 120, 144, and 168 h relative to CRH infusion (Figure 2). These results suggest that CAM and CO steers experienced a similar increase in plasma cortisol concentrations (Cooke and Bohnert, 2011), but camelina meal supplementation reduced the acute-phase protein response stimulated by the CRH challenge. Similarly, previous research from our group reported that PUFA supplementation alleviated the acute-phase response in beef steers following transport and feedlot entry (Cooke et al., 2010).

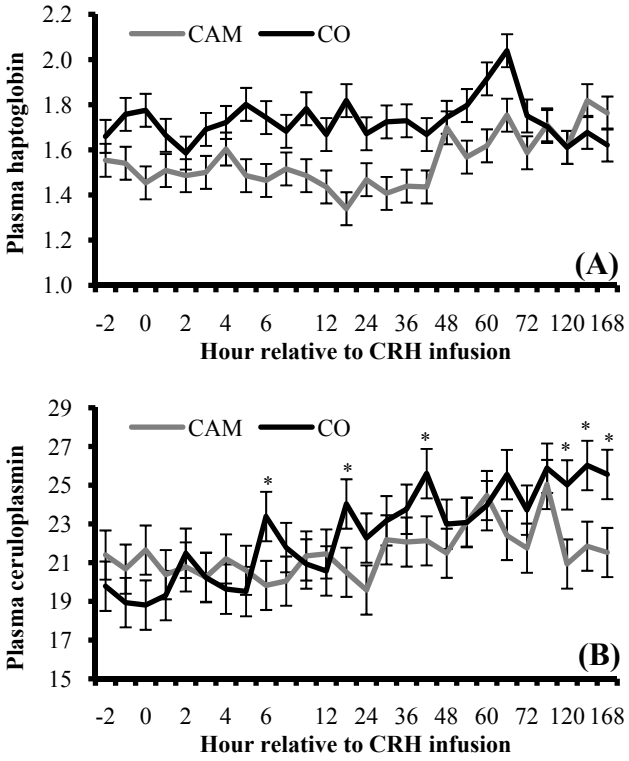

Figure 2. Plasma haptoglobin (panel A; absorbance at 450 nm × 100) and ceruloplasmin (panel B; mg/dL) concentrations of steers supplemented (CAM) or not (CO) with camelina meal and receiving 0.5 µg of bovine corticotropin-releasing hormone (CRH)/kg of BW at h 0. Steers receiving CAM tended (P = 0.10) to have reduced mean haptoglobin concentrations compared to CO steers. A treatment × time interaction was detected for ceruloplasmin concentrations (treatment comparison within time: * P < 0.05).

In Exp. 2, no treatment effects were detected for serum T_3 (P = 0.58) and T_4 (P = 0.55) concentrations, which peaked, respectively, at 3 and 5 h relative to TRH infusion in both treatments (Figure 3). Moriel et al. (2010) reported that heifers fed camelina meal had greater T_3 concentrations compared to cohorts fed a corn-soybean meal diet, whereas no differences were detected for serum T_4 concentrations. Therefore, camelina meal does not impair thyroid gland function in beef cattle when supplemented at the rates utilized herein and by Moriel et al. (2010)

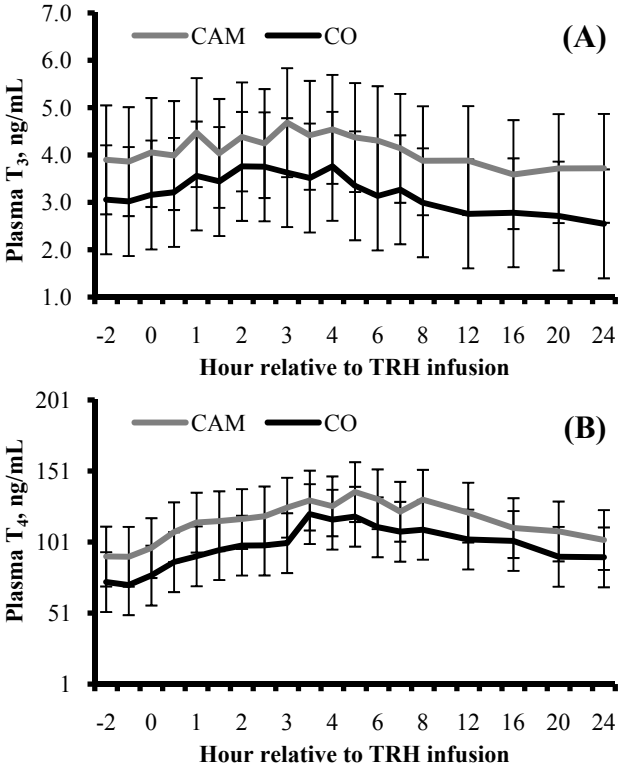

Figure 3. Plasma concentrations of T_3 (panel A) and T_4 (panel B) of steers supplemented (CAM) or not (CO) with camelina meal and receiving 0.33 µg of bovine thyrotropin-releasing hormone (TRH)/kg of BW at h 0. No treatment effects were detected ($P > 0.55$).

Implications

Camelina meal supplementation did not impair thyroid gland function following a TRH challenge, but alleviated the acute-phase protein response stimulated by CRH challenge in beef steers.

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Estrous response following the PG 6-d CIDR protocol for heifers that do and do not exhibit estrus prior to CIDR insertion and its usefulness as a fixed-time AI protocol.[1]

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ABSTRACT: Inducing luteal regression 3 d prior to an injection of GnRH and CIDR insertion has increased control of follicular development in beef heifers and pregnancy success in beef cows. However, a proportion of animals exhibit estrus during this 3 d period of time. Therefore, the objectives of these studies were to determine if estrous response following CIDR removal differed between heifers that did or did not exhibit estrus prior to CIDR insertion and to determine whether the PG 6-d CIDR protocol could be used for fixed-time AI in beef heifers. In experiment 1, heifers at one location (n=159) were synchronized with the PG 6-d CIDR protocol [$PGF_{2\alpha}$ (25mg; i.m.) on d -9, GnRH (100μg; i.m.) and insertion of a CIDR on d -6, $PGF_{2\alpha}$ (25mg; i.m.) and CIDR removal on d 0]. Estrotect estrous detection patches were placed on each heifer on d -9. At time of CIDR insertion, activated patches were recorded. At time of CIDR removal, all activated patches were replaced and estrus was observed visually. In experiment 2, heifers at one location (n=517) were randomly assigned to one of two treatments: 1) PG 6-d CIDR or 2) GnRH and insertion of a CIDR on d -5 and CIDR removal with 2x $PGF_{2\alpha}$ (6 h interval) at CIDR removal (5-d CIDR). Heifers were time-inseminated at 66 h (PG 6-d CIDR) or at 72 h (5-d CIDR) after CIDR removal. In experiment 1, interval to estrus following CIDR removal did not differ (P=0.18) between heifers that did (n=72) and did not (n=87) exhibit estrus before CIDR insertion (51.8 ± 1.0 and 53.6 ± 1.0 h, respectively). Variance for the interval to estrus tended to differ (P=0.07) between heifers that did (47.5) and did not (76.0) exhibit estrus before CIDR insertion. In experiment 2, pregnancy rates were greater (P<0.01) for heifers receiving PG 6-d CIDR (64%) compared to 5-d CIDR (42%). In summary, interval to estrus did not differ between heifers that did or did not exhibit estrus prior to CIDR insertion, and timed AI pregnancy rates were improved by using the PG 6-d CIDR protocol compared to the 5-d CIDR protocol.

Key Words: Estrous synchronization, Fixed-time AI, Heifers

Introduction

Several estrous synchronization protocols have been developed that utilize GnRH at the time of Controlled Internal Drug Releasing (CIDR) device insertion to better control follicular development (Lamb et al., 2006; Larson et al., 2006). However, the ability of GnRH to induce ovulation and initiate a new follicular wave is dependent on the stage of the estrous cycle (Atkins et al., 2008). Only 45 to 50% of heifers (Pursley et al., 1995; Atkins et al., 2008) at various stages of the estrous cycle ovulated in response to an injection of GnRH. An injection of $PGF_{2\alpha}$ administered three days before the injection of GnRH increased the percentage of heifers that responded to the injection of GnRH at time of CIDR insertion (Grant et al., Submitted).

When luteal regression was induced prior to an injection of GnRH, serum concentrations of estradiol increased, and serum concentrations of progesterone decreased (Grant et al., Submitted). Furthermore, administration of $PGF_{2\alpha}$ to cows between d 6 and 16 of the estrous cycle, resulted in the majority of cows regressing their CL and exhibiting estrus within 5 d, and the peak in estrus activity occurred between 60 and 80 h (See review by, Lauderdale, 2009). Therefore, administering an injection of $PGF_{2\alpha}$ three days before an injection of GnRH should decrease progesterone, increase estradiol, and allow for the majority of cycling animals to be in estrus around the time of GnRH administration. The objectives of these studies were to determine if estrous response following CIDR removal differed between heifers that did or did not exhibit estrus prior to CIDR insertion and to determine whether the PG 6-d CIDR protocol could be used for fixed-time AI in beef heifers.

Materials and Methods

Experiment 1

Experimental Design: All procedures were approved by the South Dakota State University (SDSU) Institutional Animal Care and Use Committee. One hundred fifty-nine beef heifers at one location received an injection of $PGF_{2\alpha}$ (25 mg as 5 mL of Lutalyse i.m.; Pfizer Animal Health, New York, NY) on d -9, an injection of GnRH (100 μg as 2 mL of Cystorelin i.m.; Merial, Athens, GA) and insertion of a CIDR (Pfizer Animal Health, New York, NY) on d -6, and a $PGF_{2\alpha}$ injection and CIDR removed on d 0 (**PG 6-d CIDR**).

Blood Sampling, and Radioimmunoassays: Blood samples were collected on d -9 (day of $PGF_{2\alpha}$) via venipuncture of the jugular vein into 10-mL evacuated

[1] Mention of a proprietary product does not constitute a guarantee or warranty of the product by South Dakota State University, USDA or the authors and does not imply its approval to the exclusion of other products that may also be suitable. South Dakota State University and USDA, ARS, Northern Plains Area, are equal opportunity/ affirmative action employers. All agency services are available without discrimination.

tubes (Fisher Scientific, Pittsburgh, PA) for determination of serum concentrations of progesterone. Blood samples were placed on ice, stored at 4°C overnight, and centrifuged at 1,200 x g for 30 min. Serum was harvested and stored at -20°C until analysis was performed by RIA. Circulating concentrations of progesterone were analyzed using methodology described by Engel et al., (2008). Intra- and interassay coefficients of variation for progesterone assays were 6.9 and 4.2%, respectively. Assay sensitivity was 0.4 ng/mL.

Estrous Detection and Artificial Insemination: Estrotect (Western Point, Inc., Apple Valley, MN) estrous detection aids were placed on the tailhead of each heifer on d -9. At time of CIDR insertion (d -6), activated patches were recorded. Animals were classified in standing estrus when the patch had been completely activated (Figure 1a), or partially activated (Figure 1b). Animals were classified as not in estrus when the patch had no signs of activation (Figure 1c). At time of CIDR removal, all activated and partially activated patches were replaced and standing estrus was detected by continuous visual observation during daylight hours with the aid of Estrotect estrous detection aids.

Animals were artificially inseminated approximately 12 hr after detection in standing estrus according to the AM/PM rule by a single technician to one of ten sires. Pregnancy success was determined 45 days after AI using an Aloka 500V ultrasound with a 7.5 MHz transrectal linear probe (Aloka, Wallingford, CT). Presence of a viable fetus was determined by the presence of a heartbeat.

Experiment 2.

Experimental Design: Five hundred seventeen Angus-cross beef heifers at one location were utilized in accordance with the South Dakota State University Institutional Animal Care and Use committee. Heifers were randomly assigned to receive either: 1) the PG 6-d CIDR (as described in exp. 1), or 2) GnRH (100 μg as 2 mL of factrel i.m.) on d -5 and insertion of a CIDR, and on d 0, $PGF_{2\alpha}$ (25mg as 5 mL of Lutalyse i.m.) and CIDR removal and 6 h after CIDR removal **(5-d CIDR).** Heifers were time-inseminated at 66 h (PG 6-d CIDR) or at 72 h (5-d CIDR) after CIDR removal (Figure 2). All heifers were inseminated by one of four technicians to one of five sires that were allotted equally to both treatments. Bulls were withheld from heifers for a minimum of seven days following fixed-time insemination and pregnancy success was determined 67 d after fixed time AI by transrectal ultrasonography using an Aloka SSD-500V.

Statistical Analysis

Differences between heifers that exhibited estrus prior to CIDR insertion and heifers that did not in the interval to estrus were determined by analysis of variance in SAS by PROC GLM. When a significant ($P < 0.05$) effect of treatment was detected, LS means were separated by the PDiff option of SAS. Differences between heifers that exhibited estrus prior to CIDR insertion and heifers that did not in the variance associated with the interval to estrus were determined by an F-test of the variance. Effects of treatment (PG 6-d CIDR and 5-d CIDR) on pregnancy success were determined by chi square analysis in SAS PROC FREQ.

Results and Discussion

Administering an injection of $PGF_{2\alpha}$ three days before an injection of GnRH decreased progesterone, increased estradiol, and allowed for the majority of cycling animals to be in estrus around the time of GnRH administration (Grant et al., submitted). In the present study, 45% (72/159) of all heifers exhibited standing estrus between the injection of $PGF_{2\alpha}$ and insertion of the CIDR. However, there was no difference ($P = 0.77$) between heifers with concentrations of progesterone > 1 ng/mL on d -9 (d of $PGF_{2\alpha}$; 44%, 47/107) and heifers with concentrations of progesterone < 1 ng/mL on the d-9 (47%, 21/45) in the percentage of heifers that exhibited estrus between $PGF_{2\alpha}$ and CIDR insertion. Furthermore, there was no difference ($P = 0.78$) in BCS between heifers that did and did not exhibit estrus (5.9 ± 0.1 and 5.9 ± 0.1, respectively). When an injection of $PGF_{2\alpha}$ is administered to cows between d 6 and 16 of the estrous cycle, the majority of cows regressed their CL and exhibited estrus within 5 d, and the peak in estrus activity occurred between 60 and 80 h (See review by, Lauderdale, 2009).

Interval to estrus following CIDR removal did not differ ($P = 0.18$) between heifers that did (n = 72) and did not (n = 87) exhibit estrus before CIDR insertion (51.8 ± 1.0 and 53.6 ± 1.0 h, respectively). However, variance for the interval to estrus tended to differ ($P = 0.07$) between heifers that did (47.5) and did not (76.0) exhibit estrus before CIDR insertion. However, among both groups peak estrus activity occurred between 44 and 56 hr after CIDR removal (Figure 3). Tight synchrony of estrus is essential for the implementation of a fixed-time AI program. Therefore, it is important to determine if exhibiting estrus prior to an injection of GnRH at CIDR insertion influenced the distribution of estrus following CIDR removal.

Previous studies have reported differences in response to synchronization between pubertal and prepubertal heifers (Wood-Follis et al. 2004, Leitman et al. 2008). In the present study, interval to estrus following CIDR removal did not differ ($P = 0.26$) between heifers with elevated (> 1 ng/mL) or low (< 1 ng/mL) concentrations of progesterone on d -9 (52.2 ± 0.8 and 53.9 ± 1.3 h, respectively), and peak estrus occurred between 44 and 56 hr after CIDR removal (Figure 4). However, the variance for the interval to estrus was decreased ($P = 0.04$) in heifers with elevated progesterone on d 0 (50.9) compared to heifers with low progesterone (88.2).

Decreased pregnancy rates to fixed-time AI among beef heifers has been attributed to the inability to synchronize follicular waves with an injection of GnRH at the initiation of the synchronization protocol (Lamb et al., 2006). Even with low ovulatory response in heifers (Pursley et al., 1995; Atkins et al., 2008), an injection of GnRH at the initiation of a 7-d CIDR fixed-time AI

protocol decreased the standard deviation of pregnancy rates among 12 groups of heifers compared to heifers not receiving the injection of GnRH (Lamb et al., 2006). The 5-d CIDR protocol uses an injection of GnRH at time of CIDR insertion to try to control follicular development (Bridges et al., 2008). However, ovulatory response to an injection of GnRH is dependent on day of the estrous cycle when GnRH is administered (Atkins et al., 2008).

Two short duration CIDR protocols have been developed for fixed-time AI in beef heifers. Both the Select Synch + CIDR protocol [GnRH (100 μg i.m.) on d -7 and insertion of a CIDR, and on d 0 CIDR removal with $PGF_{2\alpha}$ (25 mg i.m.) at CIDR removal; Lamb et al., 2006] and the 5-d CIDR protocol utilize an injection of GnRH at random stages of the estrous cycle to control follicular development. However, Wilson and co-workers (2007) reported an increased fixed-time AI pregnancy success among heifers treated with the 5-d CIDR protocol compared to the Select Synch + CIDR protocol.

In experiment 2, pregnancy rates were greater ($P < 0.01$) for heifers receiving the PG 6-d CIDR (64%) compared to the 5-d CIDR (42%). When a new follicular wave was initiated at the start of a fixed-time AI protocol the percent of grade 1 and 2 embryos, total number of blastomeres, and proportion of live blastomeres were increased compared to cows that did not initiate a new follicular wave (Cerri et al., 2005). An injection of $PGF_{2\alpha}$ administered three days before the injection of GnRH increased the percentage of heifers that responded to the injection of GnRH at time of CIDR insertion and decreased the variation in follicle size at time of CIDR removal (Grant et al., Submitted). Presynchronization with an injection of $PGF_{2\alpha}$ increased pregnancy success to fixed-time AI in dairy cows (Moreira et al, 2001, El-Zarkouny et al., 2004, Nananukraw et al., 2004), and presynchronization with a progestin prior to an injection of GnRH increased the proportion of beef heifers that ovulated in response to GnRH (Lietman et al., 2008) and increased pregnancy success to fixed-time AI (Busch et al., 2007).

In summary, interval to estrus did not differ between heifers that did or did not exhibit estrus prior to CIDR insertion, and timed AI pregnancy rates were improved by using the PG 6-d CIDR protocol compared to the 5-d CIDR protocol.

Implications

Inducing luteal regression three days prior to the start of a 6-day CIDR protocol resulted in 45% of heifers exhibiting estrus prior to CIDR insertion. However, there was no difference in interval to estrus following CIDR removal for heifers that did or did not exhibit estrus prior to CIDR insertion or between heifers with concentrations of progesterone > 1 or < 1 ng/mL on d -9. The similar distribution of estrus and tightness of synchrony leads to the ability to use this protocol for fixed-time AI, and in comparison the PG 6-d CIDR protocol resulted in greater pregnancy success compared to the 5-d CIDR protocol in beef heifers.

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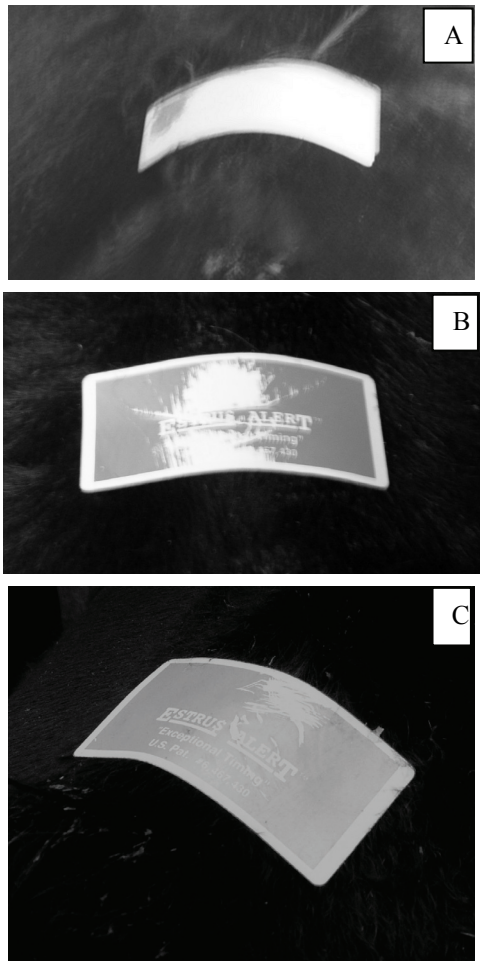

Figure 1. Examples of an Estrotect patches on an animal that was considered in standing estrus (A- activated and B- partially activated), or not in standing estrus (C).

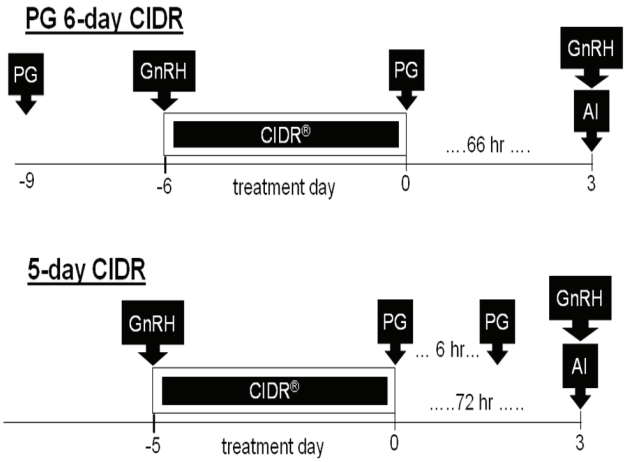

Figure 2. Treatment schedule for heifers assigned to the PG 6-day CIDR and the 5-day CIDR treatments. Heifers assigned to the PG-CIDR treatment received an injection of $PGF_{2\alpha}$ on d -9, an injection of GnRH and insertion of a CIDR on d -6, and a $PGF_{2\alpha}$ injection and CIDR removed on d 0. Fixed-time AI occurred 66 hr after CIDR removal. Heifers assigned to the 5-day CIDR treatment received an injection of GnRH and insertion of a CIDR on d -5 and on d 0 an injection of $PGF_{2\alpha}$ at CIDR removed and 6 hrs after CIDR removal. Fixed-time AI occurred at 72 hr after CIDR removal.

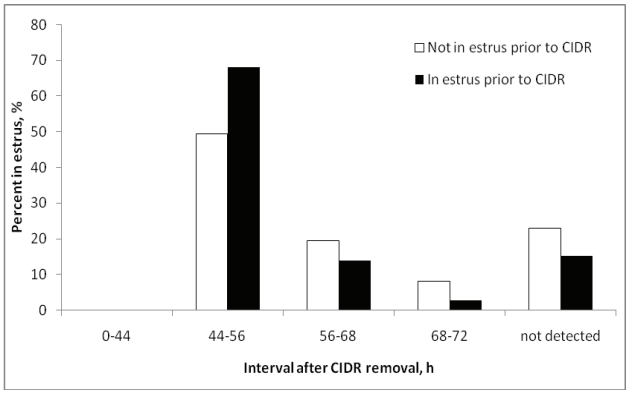

Figure 3. Percentage of heifers in estrus that did or did not exhibit estrus prior to CIDR insertion at specific times following CIDR removal.

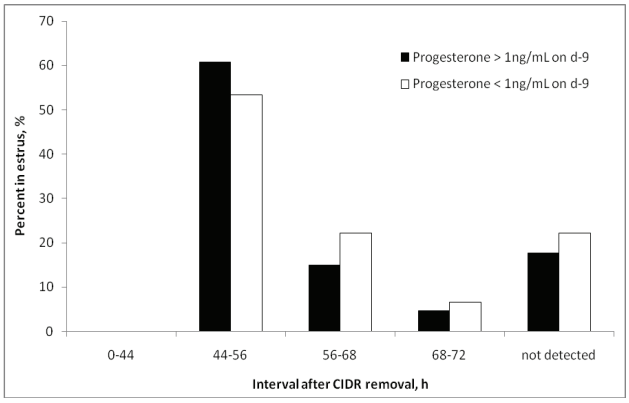

Figure 4. Percentage of heifers in estrus that had concentrations of progesterone > 1 or < 1 ng/mL on d -9 at specific times following CIDR removal.

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GROWTH AND REPRODUCTIVE PERFORMANCE OF BEEF REPLACEMENT HEIFERS FED WINTER DEVELOPMENT DIETS CONTAINING SOYBEAN MEAL OR WET DISTILLERS GRAINS

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ABSTRACT: Our objective was to determine the effects of replacing soybean meal with wet distillers grains plus solubles (**WDG**) in replacement heifer development diets on growth and reproductive performance. Spring-born Crossbred heifers (n =172; initial BW 319 ± 2 kg; age 282 ± 1 d), previously preconditioned, weaned, and fed a grower diet for 60 d, were stratified by BW and age. Heifers were then assigned randomly to be fed development diets containing either soybean meal (**CON**) or WDG as a protein supplement. Within treatment, heifers were allotted equally to 4 pens and adapted to diets for 14 d. Diets were formulated to be isonitrogenous and isocaloric and were fed ad libitum for 94 d. Heifer BW was measured every 28 d during the feeding period; paired serum samples were also collected at these times to define puberty status. Following the 94 d feeding period, heifers were removed from treatment pens and combined in a native-range pasture. Equal proportions of heifers from each treatment were exposed to ovulation synchronization and bred by fixed-time AI either 23 or 51 d after development diets ended and 10 d later were exposed to bulls for 35 d. Total DM delivered was 4695 kg lower ($P < 0.01$) for WDG than for CON. Likewise, daily DMI by WDG was 0.58 kg less ($P < 0.1$) than that by CON, which resulted in greater ($P < 0.01$) ADG throughout the development period for CON heifers (0.47 kg/d) than for WDG heifers (0.32 kg/d). Proportion of pubertal heifers was greater ($P < 0.01$) for CON compared to WDG after 28 and 56 d on feed but was not different ($P > 0.10$) after d 84 on feed or at ovulation synchronization for each breeding-group of heifers. Conception to fixed-time AI (46%) and pregnancy rate (86%) was not different ($P > 0.50$) between treatments. Under the conditions of our study, developing replacement heifers with WDG-containing diets may have some negative impacts on growth performance and age at puberty. Further research is needed to determine the effects of WDG inclusion rate, duration of feeding and diet composition on the reproductive performance of replacement heifers.

Key Words: beef heifers, distillers grains, puberty

Introduction

Proper development of replacement heifers is vital to ensure maximum lifetime productivity by reducing age at first parturition (Lesmeister et al., 1973). Development of heifers must also be achieved at low cost without sacrificing performance. Wet distillers grains plus solubles (**WDG**) are commonly used in beef cattle diets, especially in finishing diets. Due to increasing availability and relatively lower unit cost of CP on a DM basis, WDG is increasingly used as a winter protein supplement for beef heifers and cows. During ethanol production, starch is fermented and fiber, protein and ether extract are concentrated 3-fold (Klopfenstein et al., 2008); therefore, WDG typically replaces portions of the grain and most or all of the supplemental protein when used in feedlot diets. Currently, little information is available regarding the effects of replacing grain or supplemental protein in heifer development diets with WDG. Our objective was to determine the effects of replacing soybean meal with WDG in replacement heifer development diets on growth and reproductive performance.

Materials and Methods

All procedures involving the handling and care of animals used in our experiment were approved by the Kansas State University Institutional Animal Care and Use Committee (Protocol # 2799.3).

Animals and Experimental Design. Spring-born crossbred beef heifers (n = 172; initial body weight 319 ± 2 kg, age 282 ± 1 d) that were preconditioned, weaned, and fed a grower diet for 60 d were stratified by body weight and age in January. Heifers were then assigned randomly to development diets containing soybean meal (**CON**) or wet distillers grains (**WDG**) as protein supplements (Table 1). Heifers were allotted randomly to 8 pens (4 pens per treatment) and allowed to adapt to treatment diets for 14 d. Diets were formulated to be isonitrogenous and isocaloric (Table 1) and fed *ad libitum* for 94 d after the adaptation period. Following the development period, heifers were removed from treatment pens and combined in a native-range pasture. Equal proportions of heifers from each treatment were exposed to ovulation synchronization and bred via fixed-time artificial insemination (**AI**) either 23 or 51 d after development diets were terminated.

Data Collection. Heifer body weight was measured every 28 d during the feeding period and before ovulation synchronization. A BCS (scale 1 to 9; 1 = extremely thin, 9 = obese; Wagner et al., 1988) was assigned to each animal by two independent, trained evaluators on the d ovulation synchronization was initiated.

Weekly diet samples were collected from each pen, composited by treatment and immediately frozen. At the conclusion of the study, weekly composite samples

were combined to create diet treatment composites which were submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) and analyzed for DM, CP, NE_m, NE_g, NDF, ADF, TDN, Ca, P, and S.

Table 1. Ingredient and nutrient composition of control and wet distillers grains (WDG) diets fed to replacement heifers

	%, dry matter basis	
Item	Control diet	WDG diet
Ingredient composition		
Ground sorghum hay	82.5	65.3
Ground sorghum grain	9.42	7.91
Soybean meal	7.07	–
WDG	–	25.72
Vitamin/mineral premix	0.52	0.44
Calcium carbonate	0.26	0.44
Salt	0.26	0.22
Nutrient analysis[1]		
DM, %	79.4	67.5
CP, %	10.7	10.1
NE_m, Mcal/lb	0.67	0.67
NE_g, Mcal/lb	0.34	0.34
NDF, %	47.4	41.1
ADF, %	35.3	35.4
TDN, %	60.6	60.6
Calcium, %	0.86	0.99
Phosphorus, %	0.21	0.25
Sulfur, %	0.17	0.23

[1]Data represent the analysis from a composite of weekly samples for each diet collected from the bunk.

Puberty Evaluation. Paired blood samples were collected via jugular venipuncture monthly during the feeding period and again 10 d before and at ovulation synchronization. Samples were immediately placed on ice, allowed to clot for 24 h at 4°C and then centrifuged (1,500 × g) for 10 min. Serum was decanted into 12 × 75 mm plastic tubes and immediately frozen (-20°C). Concentration of progesterone (P4) in serum was subsequently quantified by RIA (Skaggs et al., 1986). Intra- and inter-assay CV were 7.0 and 7.9%, respectively, and assay sensitivity was 0.019 ng/mL. When samples contained concentrations of P4 ≥ 1 ng/mL heifers were considered to have attained puberty and be cycling.

Synchronization and Breeding. For each breeding group of heifers (inseminated 23 or 51 d after termination of treatment diets), ovulation was synchronized using the 7 d CoSynch+CIDR protocol and heifers were inseminated by fixed-time AI 54 to 56 h after CIDR removal. Heifers were exposed to bulls 10 d after fixed-time AI for the remainder of a 45-d breeding season. Conception to fixed-time AI was determined via ultrasound 35 d after insemination and final pregnancy rate was determined via rectal palpation 60 d after the end of the breeding season.

Statistical Analysis. Heifer performance data were subjected to ANOVA using GLM (SAS Inst. Inc., Cary, NC). The model included effects for treatment and pen. Treatment within pen was considered the experimental unit.

Pregnancy rates were analyzed using PROC CATMOD (SAS Inst. Inc., Cary, NC). The original model used to assess differences in fixed-time AI pregnancy rates and overall pregnancy rates included effects for treatment and breeding group. Breeding group effects and associated interactions were not significant and were removed from the model. Least Squares means for pregnancy rates were reported. Treatment differences in performance and pregnancy data were discussed when $P \leq 0.05$; trends and tendencies were discussed when $P > 0.05$ and ≤ 0.10.

Results

Chemical composition of diets was similar (Table 1). Crude protein was slightly greater in the CON diet compared to the WDG diet, but both supplied adequate MP. Although heifers were fed diets that were similar in chemical composition *ad libitum*, heifers fed the development diet containing WDG consumed less feed. Total DM delivered during the 94-d feeding period was 4,694 kg lower ($P < 0.01$) for heifers fed the WDG diet than for heifers fed the CON diet. Likewise, individual daily DM intake by heifers fed the WDG diet was 0.58 kg less ($P = 0.02$) than for heifers fed the CON diet. This resulted in greater ($P < 0.01$) BW change and ADG during each 28-d period and throughout the development period for CON-fed heifers than for WDG-fed heifers (Table 2).

Cattle consuming actively growing forages respond to ruminally undegradable protein (**RUP**) supplementation, because the protein in the forage is highly degraded in the rumen, causing a MP deficiency (Klopfenstein, 1996; Creighton et al., 2003). However, the WDG diet was likely deficient in ruminally degradable protein (**RDP**) (CP in WDG is 52% RUP compared to CP in soybean meal being 34% RUP; NRC 2000). Coupled with the low-quality forage sorghum hay used in the current study (< 8.0% CP), low RDP intake may have caused reduced intake and gain. Wickersham et al. (2008) found that supplemental RDP increased low-quality forage OM intake and digestible OM intake, and digestibility of total tract OM and NDF.

At the end of the development-diet feeding period, total BW change was greater ($P < 0.01$) for CON-fed compared to WDG-fed heifers; likewise, heifer BW also tended to be greater ($P < 0.10$) for CON compared to WDG (Table 2). Heifer BW and BCS were not different ($P > 0.70$) between treatments at the start of ovulation synchronization for breeding group 1. These heifers were bred 23 d after treatment diets were terminated. In contrast, BW and BCS was greater ($P < 0.01$) for CON heifers compared to WDG heifers at start of ovulation synchronization for breeding group 2. These heifers were bred 51 d after treatment diets were terminated. The reason for these differences is unclear.

Proportion of pubertal heifers was greater ($P < 0.01$) for CON compared to WDG after being fed treatment diets for 28 and 56 d, but was not different ($P > 0.10$) at 84 d or at initiation of ovulation synchronization for each breeding group of heifers (Figure 1). Conception to fixed-time AI (46%) and pregnancy rate (86%) were not different ($P > 0.50$) between treatments.

Diet cost for the WDG diet was less than for the CON diet ($80.44/metric ton vs. $115.45/metric ton, respectively). Therefore, the WDG diet may be considered a more cost-effective diet because WDG-fed heifers overcame their slower grow rate during the feeding period. Additionally, similar proportions of heifers were cycling before ovulation synchronization and conceived to fixed-time AI in both treatments. Clanton et al. (1983) found that pattern and timing of gain in replacement heifers did not affect subsequent performance, provided the necessary weight was reached by the beginning of the breeding season. These authors concluded there is flexibility in the procedures by which heifers can be developed and that achieving a targeted body weight should be accomplished as cost efficiently as possible. Similarly, Lynch et al. (1997) found that heifers accruing the majority of their BW late in the development period consumed less feed than heifers that were fed to achieve a consistent gain during development. The heifers that were initially restricted tended to display a greater first service conception rate in one of the two years examined. Funston and Deutscher (2004) reported that developing heifers to 53% of mature BW did not adversely affect reproduction or calf production traits compared to developing heifers to 58% of mature BW. Similar to our study, they also found that it decreased development costs.

Under the conditions of our study, developing replacement heifers with WDG-containing diets had negative effects on growth performance during the feeding period and age at puberty; however, the proportion of heifers that had attained puberty was similar between treatments at the time of fixed-time AI and did not affect first service conception rate.

Implications

Development cost was lower for WDG-fed heifers due to the lower cost of the WDG diet and less total feed consumed during the development period. In contrast, WDG-fed heifers attained puberty at an older age and conception rates could have been negatively affected if heifers had been exposed to breeding earlier in the year. Further research is warranted to determine the effects of WDG inclusion rate, duration of feeding, and diet composition on reproductive performance of replacement heifers.

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Table 2. Body weight (BW) and average daily gain (ADG) of heifers fed development rations containing soybean meal (CON) or wet distillers grains (WDG) as the protein supplement

	Treatment means			
Item	CON	WDG	SE	P-value
Initial BW, kg	323	327	3.3	0.31
ADG 1st 28 d (1/29 to 2/26), kg/d	0.62	0.37	0.03	< 0.001
ADG 2nd 28 d (2/26 to 3/26), kg/d	0.30	0.10	0.04	< 0.001
ADG 3rd 28 d (3/26 to 4/23), kg/d	0.83	0.40	0.03	< 0.001
BW change, 94-d feeding period (1/29 to 5/3), kg	44.0	30.4	1.8	< 0.001
BW at end of feeding, kg	367	357	3.9	0.09
ADG, 94-d feeding period (1/29 to 5/3), kg/d	0.47	0.32	0.02	< 0.001
BW at breeding (Group 1; 5/24/10), kg	361	362	5.0	0.92
Body condition score[1] at breeding (Group 1; 5/24/10)	5.78	5.82	0.07	0.73
BW at breeding (Group 2; 6/21/10), kg	386	368	4.54	0.004
Body condition score[1] at breeding (6/21/10)	6.14	5.88	0.06	0.003

[1] 1 = emaciated, 9 = obese

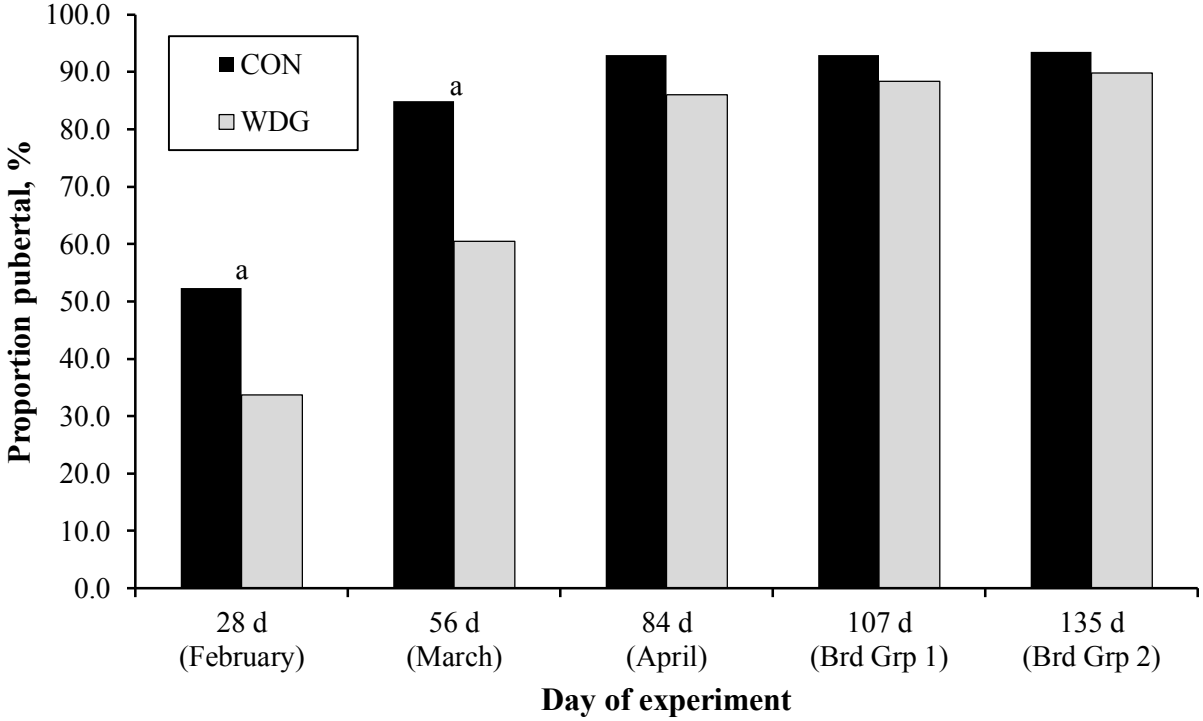

Figure 1. Proportion of heifers with serum progesterone concentrations ≥ 1.0 ng/mL in one or both blood samples collected 28, 56, or 84 d after feeding development rations containing soybean meal (CON) or wet distillers grains (WDG) as the protein supplement, and at breeding 23 d (Brd Grp 1) or 51 d (Brd Grp 2) after diets were terminated. [a] effect of development diet ($P \leq 0.01$).

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DEVELOPMENTAL POTENTIAL OF OOCYTES DERIVED FROM MATURE COWS AND FATTENED HEIFERS

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ABSTRACT: In exp 1, we compared developmental capacity of oocytes from ovaries from a slaughterhouse processing only feedlot steers and heifers and a second slaughterhouse specializing in culled cows; 2,248 oocytes were subjected to a factorial experimental design with 2 oocyte sources (cull cows and feedlot heifers); 3 sperm concentrations (0.25, 0.5, and 1.0 million sperm/mL), and semen from 2 bulls. Exp 2 was a retrospective evaluation of cleavage of oocytes from ovaries from heifers vs mature cows and subsequent embryonic development; we analyzed 72 *in vitro* production cycles (33,982 oocytes) in a factorial design with 2 ovary sources (heifers = 53 in vitro embryo production (IVP) cycles; cows = 19 IVP cycles) and semen from 4 bulls. In exp 1, oocytes from cows were superior to those from heifers in production of 8-cell embryos (74 ± 1.7 vs 62 ± 2.2%, $P < 0.01$), blastocyst rates (34 ± 1.4 vs 16 ± 1.3%, $P < 0.01$), and blastocyst cell number (138 ± 6.2 vs 116 ± 5.8 cells, $P < 0.01$). No difference was observed between 0.25, 0.5, and 1.0 x 10^6 sperm/mL in blastocysts/oocyte (24 ± 3.0 vs 26 ± 3.2 vs 25 ± 2.5%, respectively, $P > 0.10$). In exp 2, more grade-1 oocytes were obtained per ovary of heifers than those from cows (10.0 ± 0.3 vs 8.0 ± 0.5, $P < 0.01$); no differences in cleavage or 8-cell rates were found between heifer- and cow-derived embryos ($P > 0.1$). However, more blastocysts per oocyte (25.2 ± 1.9 vs 13.2 ± 1.2%) and per 8-cell embryo (45.5 ± 2.7 vs 25.5 ± 1.6%) were obtained from cow- than feedlot heifer-derived ovaries ($P < 0.01$). There was a positive correlation (r = 0.59) between blastocysts per oocyte and percent of ovaries per batch with corpora lutea for ovaries derived from heifers but not from mature cows (r = .03, $P < 0.01$), likely because batches of ovaries with few corpora lutea were from heifers fed melengestrol acetate. In conclusion, oocytes from mature cows were greater than those from feedlot heifers.

Keywords: *in vitro* fertilization; cow; heifer; corpora lutea

INTRODUCTION

Bovine embryos frequently are produced in vitro starting with oocytes from ovaries from slaughterhouses. Some abattoirs slaughter fattened post-pubertal heifers (and steers) primarily, while others concentrate on culled cows. Confounding factors may include nutrition and hormonal supplementation. Heifers usually are fattened using high energy diets, frequently supplemented with the progestagen melengestrol acetate (MGA) to promote growth with the additional benefit of eliminating estrus (Zimbelman and Smith, 1966; Imwalle et al., 2002). This may affect oocyte quality. In our laboratory, we have observed that most of the ovaries available some days contain many medium size follicles, and few or none of the ovaries have corpora lutea; in contrast, about 30% of the ovaries from cows contain corpora lutea routinely.

For in vitro fertilization systems, it has been reported that at least 5,000 sperm per oocyte are necessary to obtain optimal fertilization rates (Yang et al., 1993). Therefore, in vitro capacitation of spermatozoa is not very efficient. Reducing the number of sperm per oocyte for in vitro fertilization is a way to optimize semen utilization, and decrease polyspermy (Barcelo-Fimbres et al., 2010).

The aim of exp. 1 was to compare developmental capacity and fertilization of oocytes obtained from heifers and mature cows. Exp. 2 was to evaluate oocyte collection and embryonic development in relation to the percentage of ovaries with corpora lutea in batches of ovaries derived from heifers and cows.

MATERIALS AND METHODS

Experimental design

For exp. 1, 2248 oocytes were subjected to a 2 x 3 x 2 factorial experimental design with two oocyte sources (oocytes harvested from cull cows, n=1151 and fattened beef heifers, n=1097); three sperm concentrations (0.25, 0.5, and 1.0 million sperm per mL), and semen from two bulls (A and B) two replicates for each bull, resulting in four replicates.

For exp. 2, embryonic development of oocytes derived from heifers and cows was assessed during the course of a year; 72 IVP cycles were evaluated, with oocytes (n=33,982) derived from heifers (53 IVP cycles) and mature cows (19 IVP cycles), using semen from 4 bulls (A, B, C, and D). All ovaries were from high throughput slaughterhouses (one specializing in cull cows and the other in fattened heifers), so we were unable to obtain specific histories for the animals slaughtered.

Oocyte collection and in vitro maturation (IVM)

Ovaries were transported to the laboratory in 1-3 h in 0.15 M NaCl saline at approximately 22 to 25°C. Ovaries were trimmed of extraneous tissue, and rinsed twice in 0.15 M NaCl. Cumulus-oocyte complexes (COCs) were aspirated from 3- to 8-mm antral follicles as described by De La Torre-Sanchez, et al. (2006). COCs were washed twice in hepes-buffered TCM-199 + 10% FCS (exp. 1), or in hepes-buffered chemically defined medium for maturation (CDM-M) (De La Torre-Sanchez et al., 2006) (exp. 2), and once in maturation medium. In both experiments, 50 COCs were matured per well of 4-well plates (Nunclon, Roskilde, Denmark), containing 1 mL of maturation medium (TCM-199 + 10% FCS in exp. 1, and CDM-M + 0.5% fatty acid-free BSA (Sigma A-6003) in exp. 2) with 50 ng/mL EGF (Sigma E-9644), 0.1 mM

cysteamine (Sigma M-6500), and hormones as described by De La Torre-Sanchez et al. (2006), all at 38.5°C in a humidified 5% CO_2 in air atmosphere for 23 h.

Sperm preparation and in vitro fertilization

Frozen bull semen (exp. 1 n=2 bulls; experiment 2 n=4 bulls) had at least 35% progressive motile sperm after straws were thawed in water at 35ºC for 30 sec. Semen was gently expelled onto a Percoll gradient (Sigma P-1644) and processed as described by De La Torre-Sanchez et al. (2006). In exp. 1, there were 3 final concentrations of 0.25, 0.5 and 1 x 10^6 spermatozoa/mL. For exp. 2, the final concentration was 0.5 x 10^6 spermatozoa per mL in Fert-CDM.

Following in vitro maturation, ~50 oocytes were placed in 450 µL of Fert-CDM medium per well of 4-well dishes, and 50 µL of sperm suspension were added to give final volume of 500 µL per well. Gametes were co-incubated for 18 h at 38.5ºC in an atmosphere of humidified 5% CO2 in air.

Next, presumptive zygotes were removed from wells and cumulus cells removed as described by De La Torre-Sanchez et al. (2006). Embryos were then rinsed three times in hepes-buffered handling medium Hepes CDM-1 (H-CDM-1). Early culture (d 0 to 2.0 post fertilization, exp. 1; 0 to 2.5, exp. 2) was done in a new 4-well dish, containing 500 µL of CDM supplemented with 0.5% fatty acid-free BSA, 0.5 mM glucose in exp. 1 and 0.5 mM fructose in experiment 2, non-essential amino acids (NEAA), and 10 µM EDTA (CDM-1) as described by De La Torre-Sanchez et al. (2006). Culture was done at 39°C under 90% N_2, 5% O_2, and 5% CO_2 humidified atmosphere; after 48 h, exp. 1; 60 h, exp. 2, embryos were examined with a stereomicroscope (15 to 20×) for cleavage, and all uncleaved ova and embryos less than 7 cells were discarded. The rest were cultured in new dishes of CDM-2 (CDM supplemented with 0.5% fatty acid-free BSA, NEAA and essential amino acids and 2 mM hexose (experiment 1=glucose and experiment 2= fructose), from d 2.5 to d 7 post fertilization.

In both experiments, cleavage rates were assessed at the end of CDM-1 culture; embryos at the 7- to 8-cell stage were then cultured in CDM-2; 2- to 6-cell embryos were considered cleaved, but were not cultured further. Development of embryos to the blastocysts stage was evaluated on 7 d of culture.

For exp. 1, at 168 h of culture, blastocysts were scored for stage (5: early, 6: full, 7: expanded, 8: hatched). Blastocysts also were evaluated for quality (1: excellent, 2: good/fair, 3: poor, and 4: dead), inner cell mass (ICM) quality (1: large, compacted ICM; 2: large, less compacted ICM; 3: medium, loose ICM; 4: scarce, loose ICM), and lightness (1: lighter,. . ., 4: darker). All subjective responses were evaluated by a single person 'blindly', with treatments coded differently for each replicate. After valuation, embryos were fixed and stained with Giemsa (Choi et al., 2001), and cells were counted.

For exp. 2, for each batch of ovaries derived from cows or heifers the number of corpora lutea, number of good quality oocytes aspirated and success of in vitro fertilization and embryonic development were recorded.

Statistical evaluations

Arcsin√% transformations were done on all percentage responses to achieve homogeneity of variance if necessary from evaluating residual plots. All responses were analyzed by analysis of variance (ANOVA, SAS statistical package GLM). Means were separated by the least significant difference method when applicable. For evaluating embryonic development according to the rate of corpora lutea per batch of ovaries, a regression model (proc REC) was used including the linear and quadratic terms. Sources of variation in the models included all main effects (all considered as fixed effects), all possible 2 way interactions, and residual error. Data are reported as untransformed least-squares means.

RESULTS

Experiment 1

There were no interactions ($P > 0.1$) between the factors studied, so only main effects are presented and discussed. There was a tendency of a higher cleavage rate for cow than heifer oocytes (Table 1). Eight-cell (74 ± 1.7 vs. 62 ± 2.2%, $P < 0.01$) and blastocyst rates (34 ± 1.4 vs. 16 ± 1.3%, $P < 0.01$) were superior for oocytes derived from mature cows than heifers (Table 1). Production rates of 8-cell embryos and blastocysts derived from cows were, respectively, 19% (12 percentage points) and 112% (18 percentage points) higher than those derived from heifers. In addition, numbers of cells per blastocyst were higher for blastocysts derived from cows than heifers (138 vs.116 cells, $P < 0.01$) (Table 1).

The blastocyst and inner cell mass quality at 132 h were not different between cow and heifer embryos ($P > 0.1$); however, blastocysts from mature cows had better blastocyst quality (1.4 ± 0.03 vs. 1.6 ± 0.05, $P < 0.01$) and inner cell mass quality (1.32 ± 0.03 vs. 1.47 ± 0.06, $P < 0.05$) at 168 h than heifer-derived blastocysts ($P < 0.01$); no significant differences were found for blastocyst stage and lightness at 132 and 168 h ($P > 0.1$) (Table 3).

There was a tendency for the 0.25 million sperm/mL group to result in lower cleavage and 8-cell rates than 0.5 and 1 million sperm/mL groups (78 ± 3.1 vs. 83 ± 1.4 and 84 ± 2.0%, respectively) and (63 ± 3.6 vs. 70 ± 2.0 and 70 ± 2.8%) ($P < 0.1$) (Table 1); however, no effect ($P > 0.1$) of sperm dose was found for blastocyst rate or blastocyst cell number. The blastocyst stage, blastocyst quality, inner cell mass quality and lightness at 132 and 168 h also were not different between sperm doses ($P > 0.1$) (Table 3). No differences ($P > 0.1$) were found between bulls A and B for any response (Tables 1 and 3).

Experiment 2

There were no interactions ($P > 0.1$) for any response, so only main effects are presented. Ovaries derived from heifers resulted in more morphologically normal oocytes per ovary than cows (10.0 ± 0.3 vs. 8.0 ± 0.5) ($P < 0.01$). No significant effect was found for cleavage (73.3 ± 1.8 vs. 74.0 ± 1.1%) and 8-cell rate (54.6

± 3.3 vs. 49.5 ± 2.0%) ($P > 0.1$) for cow- and heifer-derived oocytes respectively (Table 2). Superior blastocyst rates per oocyte (25.2 ± 1.9 vs. 13.2 ± 1.2%, $P< 0.01$) and per 8-cell embryo (45.5 ± 2.7 vs. 25.5 ± 1.6%, $P<0.01$) were found for oocytes derived from cows vs. heifers. Blastocyst production was 90% higher per oocyte (12 percentage points) and 78% higher per 8-cell embryo (20 percentage points) for oocytes derived from mature cows vs. heifers ($P < 0.01$) (Table 2).

Bull C was higher for cleavage than A, B and D ($P < 0.01$). No bull effect was found for 8-cell rate ($P > 0.1$), but blastocysts per oocyte were higher for bulls A and B than C and D ($P < 0.01$). Blastocysts per 8-cell was higher for bull A than bulls C and D ($P < 0.01$), but bull B was not different from A and D.

The proportion of ovaries with corpora lutea per batch of ovaries was higher from cow than heifer ovaries (25.8 ± 2.7 vs. 11.4 ± 1.6%, respectively, $P < 0.01$). There was a highly significant ($P < 0.01$) correlation of blastocyst development per oocyte and per 8-cell embryo with the rate of corpora lutea present per batch of ovaries for heifers (Fig. 1), $r^2 = 0.35$ but not for mature cows $r^2 = 0.001$ (Fig. 1). The correlation between corpora lutea rate per batch of ovaries and blastocyst rates per 8-cell for heifers was $r^2 = 0.26$, and for mature cows, $r^2 = 0.01$ (Fig. 1). Quadratic terms were not significant ($P > 0.1$) and dropped from the model.

DISCUSSION

Sperm dose effect on embryonic development

We routinely use 0.5 x 10^6 sperm per mL (5,000 sperm per oocyte) for in vitro fertilization; there is another report showing 5,000 sperm per oocyte are sufficient (Yang et al., 1993). In the first study we were able to reduce the sperm dose to 2500 sperm per oocyte (0.25 x 10^6 sperm per mL), without affecting rates of blastocyst development, so sperm dose can be reduced by half, at least for some bulls. This optimizes sperm use because more oocytes can be fertilized per straw. Previously, we reduced the sperm dose for in vitro fertilization to 0.3 x 10^6 sperm per mL (3000 sperm per oocyte) without affecting blastocyst development. However, decreasing the sperm dose to 0.11 x 10^6 sperm per mL (1,100 sperm per oocyte) negatively affected cleavage, 8-cell and blastocyst rates when compared to 0.33 x 10^6 sperm per mL (3,000 sperm per oocyte) and 1 x 10^6 sperm per mL (10,000 sperm per oocyte) (Barceló-Fimbres et al., 2010). Reducing the sperm dose can lead to decreased polyspermy (Enright et al., 2000; Ward et al., 2002; Barceló-Fimbres et al., 2010).

Bull effect on embryonic development

In exp. 2 no differences in embryonic development were observed between the two bulls used. In exp. 2, definite differences in embryonic development were observed due to bull effects; some bulls had better in vitro fertilization and blastocyst development than others. We have earlier reported differences in cleavage and blastocyst rates due to sires (Zhang et al., 2003; Barceló-Fimbres and Seidel, 2007). This effect may be due to the difference of sperm capacitation and kinetics of sperm penetration between sires during the in vitro fertilization process (Parrish et al., 1986; Ward et al., 2003).

Developmental differences of oocytes derived from cows and heifers

In both experiments, we confirmed that oocytes derived from cows are vastly more developmentally competent than those from fattened heifers. Interestingly no differences in cleavage and 8-cell embryo production were observed in the retrospective study ($P < 0.01$); but, in the first experiment a tendency of higher cleavage rate ($P < 0.1$) and a significant higher 8-cell embryo production was observed ($P < 0.01$) for cow- than heifer-derived oocytes. These differences may be due to ovary batches or differences in maturation media, since TCM-199 was used in the first study and CDM in second study, or differences in embryo culture. CDM-2 with glucose was used in the first study and fructose, in the second study because we showed fructose was superior to glucose (Barceló-Fimbres and Seidel, 2007).

Blastocyst production per oocyte matured was 112% (18 percentage points) higher in exp. 1 and 90% higher (12 percentage points) in exp. 2 for cow- than heifer-derived oocytes. Thus, in both experiments the blastocyst rate was doubled.

Besides higher development competence, in exp. 1 cow oocytes had higher total cell numbers per blastocyst than heifer-derived oocytes (138 vs. 116 respectively, $P<0.01$); this effect may help explain the better blastocyst quality ($P<0.01$) and better inner cell mass quality ($P<0.05$), in blastocysts from cows.

Influence of corpora lutea rate per batch of ovaries derived from heifers and mature cows on embryonic development

In exp. 2, the proportion of CL per batch of ovaries was higher in cows than heifers (25.8 ± 2.7 vs. 11.4 ± 1.6, $P < 0.01$). To validate these data, we conducted an additional small prospective experiment in which 4 batches of heifer ovaries with a high percentage of ovaries with CL (29.8 ± 8%) were compared with 4 batches of cow ovaries (32.6 ± 8% CL) ($P > 0.1$). Mean blastocyst rates per oocyte matured in the prospective experiment in cows and heifers (26.0 vs. 21.8%, respectively, $P<0.05$) from 8 replicates (4 each) corresponded closely to the regression line for each category with the proportion of CL per batch of ovaries (Fig. 1). For cows 32.6% of CL per batch corresponds to 25.8% of blastocyst rates per oocyte, and for heifers 29.8% corresponded to 19.0% blastocyst rate per oocyte, respectively. This is less than 2% difference between the calculated rate and the actual rates of the prospective study.

Mature cows are usually slaughtered because of age, physical or reproductive problems, usually without feedlot preparation; on the other hand, heifers are fed in feedlots with high energy diets and usually supplemented with melengestrol acetate (MGA), which may be the reason for lower blastocyst production per oocyte for heifers. Melengestrol acetate is an inexpensive, orally administrated progestagen, approved in 1968 for growth promotion by the US Food and Drug Administration, and

recently granted a zero-withdrawal status period to slaughter. MGA suppresses estrous behavior in feedlot heifers (Zimbelman and Smith, 1966; Imwalle et al., 2002), inhibits ovulatory LH surge (Zimbelman and Smith, 1966; Imwalle et al., 2002) blocking ovulation (Yelich et al., 1997), and increases the diameter of the largest follicle (Imwalle et al., 1998), leading to development of persistent dominant follicles (PDF) in up to 90% of MGA-treated cows by d 10 of treatment (Yelich et al., 1997). The MGA dose typically used to suppress estrus does not inhibit pulsatile release of LH; rather it is stimulated (Kojima et al., 1995; Imwalle et al., 1998), increasing growth of multiple follicles and estrogen biosynthesis. As these follicles regress, they are replaced by new follicles. Although oocytes from persistent dominant follicles can be fertilized, their subsequent development is compromised (Ahmad et al., 1995).

MGA is not used at all feedlots. The batches of ovaries to the right of Fig. 1 probably came from heifers not fed MGA; those to the left likely were all fed MGA; and those in the middle were mixtures of the two. This indicates that fattened heifers not fed MGA produce oocytes that are nearly as developmentally competent as oocytes from cows.

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Table 1. Main effect least-squares means of embryonic development of bovine embryos produced *in vitro* (±SE)--Experiment 1

Factors	No. oocytes	Cleaved (%)	8-cell rate (%)	Blastocysts per oocyte (%)	Blastocyst cell number
Ovary source					
Heifers	1097	79 ± 2.0 [c]	62 ± 2.2 [a]	16 ± 1.3 [a]	116 ± 5.8 [a]
Cows	1151	84 ± 1.6 [d]	74 ± 1.7 [b]	34 ± 1.4 [b]	138 ± 6.2 [b]
Sperm concentration					
0.25	770	78 ± 3.1 [c]	63 ± 3.6 [c]	24.0 ± 3.0	129 ± 7.1
0.5	760	83 ± 1.4 [d]	70 ± 2.0 [d]	26.0 ± 3.2	129 ± 8.6
1.0	718	84 ± 2.0 [d]	70 ± 2.8 [d]	24.0 ± 2.5	122 ± 8.0
Bull					
A	1097	83 ± 1.5	69 ± 2.3	24 ± 2.4	130 ± 6.8
B	1151	80 ± 2.3	67 ± 2.6	25 ± 2.4	123 ± 5.9

Values without common superscripts within factors within the same column differ, [a,b] $P < 0.01$; [c,d] $P < 0.1$.

Table 2. Main effect least-squares means of embryonic development of bovine embryos in vitro (±SE) -- Experiment 2

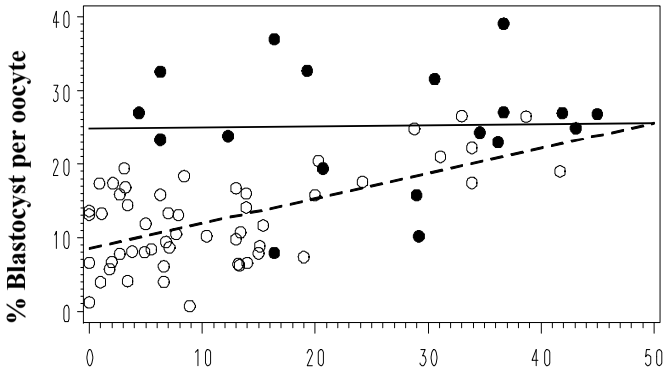
	No. oocytes	Cleaved (%)	8-cell rate (%)	Blastocysts per oocyte (%)	Blastocysts per 8-cell (%)
Ovary source					
Heifers	28815	74.0 ± 1.1	49.5 ± 2.0	13.2 ± 1.2 [a]	25.5 ± 1.6 [a]
Cows	5167	73.3 ± 1.8	54.6 ± 3.3	25.2 ± 1.9 [b]	45.5 ± 2.7 [b]
Bull					
A	11018	70.3 ± 1.4 [b]	51.4 ± 2.6	21.1 ± 1.4 [a]	39.5 ± 2.1 [a]
B	11287	72.0 ± 1.7 [b]	54.2 ± 3.2	21.3 ± 1.7 [a]	37.5 ± 2.6 [ab]
C	8228	79.9 ± 1.8 [a]	52.8 ± 3.2	15.7 ± 1.7 [b]	28.9 ± 2.6 [c]
D	3449	72.4 ± 2.8 [b]	47.7 ± 5.1	14.1 ± 2.6 [b]	30.4 ± 4.1 [bc]

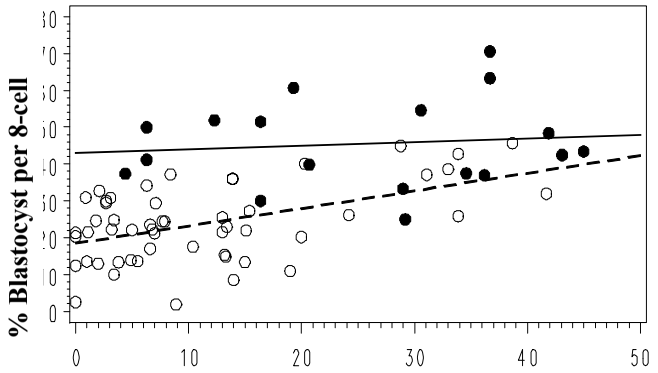
[a,b] Values without common superscripts within factors within the same column differ ($P < 0.01$).

Table 3. Main effect least-squares means of ovary source and bull on blastocyst stage, quality and lightness of bovine embryos produced in vitro (±SE)--Experiment 1

Factors	No. embryos	Blastocyst stage [1]		Blastocyst quality [2]		CM quality [3]		Lightness [4]	
		132 h	168 h	132 h	168 h	132 h	168 h	132 h	168 h
Ovary source									
Heifers	1097	6.1 ± 0.08	6.9± 0.06	1.2 ± 0.04	1.6 ± 0.05 [a]	1.2 ± 0.04	1.5 ± 0.06 [c]	2.2 ± 0.07	2.1 ± 0.06
Cows	1151	6.3 ± 0.05	7.0 ± 0.05	1.2 ± 0.04	1.4 ± 0.03 [b]	1.1 ± 0.04	1.3 ± 0.03 [d]	2.2 ± 0.05	2.0 ± 0.05
Sperm concentration									
0.25	770	6.2 ± 0.06	6.9 ± 0.06	1.2 ± 0.06	1.4 ± 0.06	1.2 ± 0.06	1.4 ± 0.09	2.3 ± 0.06	2.1 ± 0.07
0.5	760	6.1 ± 0.10	6.9 ± 0.08	1.1 ± 0.04	1.5 ± 0.04	1.2 ± 0.05	1.4 ± 0.05	2.2 ± 0.08	2.1 ± 0.06
1.0	718	6.2 ± 0.08	6.9 ± 0.07	1.2 ± 0.04	1.5 ± 0.07	1.2 ± 0.04	1.4 ± 0.05	2.1 ± 0.07	2.0 ± 0.07
Bull									
A	1097	6.2 ± 0.05	6.9 ± 0.06	1.2 ± 0.05	1.5 ± 0.04	1.2 ± 0.04	1.4 ± 0.03	2.2 ± 0.07	2.0 ± 0.06
B	1151	6.2 ± 0.08	6.9 ± 0.06	1.1 ± 0.03	1.5 ± 0.05	1.1 ± 0.03	1.4 ± 0.07	2.2 ± 0.05	2.1 ± 0.05

Values without common superscripts in the same column differ, [a,b] $P < 0.01$; [c,d] $P < 0.05$.
[1](5=early, …, 8=expanded), [2](1=excellent, …, 4=dead), [3](1= large, compact,. . .,4: few scattered cells), [4](1= lighter,. . .,4: darker).





●— (Cows) =24.758 + 0.016 (CL rate); r^2=0.001
○- - (Heifers) = 8.518 + 0.340 (CL rate); r^2=0.354

Ovary source effect (Cows vs. Heifers) ($P < 0.01$). r^2 for heifers ($P < 0.01$) and cows ($P > 0.1$).

●— (Cows) = 42.898 + 0.099 (CL rate); r^2=0.012
○- - (Heifers) = 18.394 + 0.477 (CL rate) ; r^2=0.255

Ovary source effect (Cows vs. Heifers) ($P < 0.01$). r^2 for heifers ($P < 0.01$) and cows ($P > 0.1$).

Figure 1. In vitro development of oocytes of different sources (heifers vs. cows) according to corpora lutea per batch of ovaries--Experiment 2

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GRAZING WHEAT PRE-BREEDING DID NOT REDUCE BEEF COW PREGNANCY RATES

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ABSTRACT: Complementary forage systems extend the grazing season of native rangelands, but anecdotal reports have been made concerning lowered fertility in beef cows bred when grazing lush forage such as wheat pasture. The objective of this study was to compare pregnancy rates (PR) of spring calving cows while consuming either wheat pasture or native mixed-grass rangeland before and during the early breeding season. Primiparous and second parity, crossbred cows were assigned to one of two grazing systems by age, sire breed and calving date in yr 1 (2001) of the 5 yr study. Grazing treatments were 1) grazing mixed-grass native rangeland from early spring until late fall in a season-long continuous grazing system (Native), or 2) grazing winter annual wheat in early spring followed by mixed-grass native rangeland until late fall in a seasonal complementary forage system (Wheat). The wheat grazing treatment consisted of 6 replicates of 8 to 10 cows each and the native treatment consisted of 3 replicates of 13 to 15 cows each. Cows grazed wheat 21 to 50 d prior to breeding, depending on the year. The first day of the breeding season consisted of fixed-time AI following a MGA-Select estrus synchronization protocol. Data were analyzed with mixed model procedures of SAS. Replication within treatment was considered a random effect and was used to test treatment differences. Cows that grazed wheat prior to breeding had a similar PR to fixed-time AI as cows that grazed native rangeland prior to breeding, 51.7% and 57.7% (P=0.41), respectively. Pregnancy rates averaged across grazing groups tended (P<0.11) to vary between years, mostly because of lower AI PR the first year of the study when cows were all 2 and 3 year-olds. Final PR was not different between the two grazing groups, and over years averaged 94.4 and 95.9% for the Wheat and Native groups, respectively. Cows in the Native group were heavier prior to breeding in years 2003, 2004 and 2005 (P<0.01), but weight had no effect on either AI or final PR (P≥0.47). Pregnancy rate was similar in beef cows grazing either native range or wheat pasture before and during the breeding season.

Key Words: Beef cow, pregnancy rate, wheat pasture, complementary forages

Introduction

Analysis of cow/calf enterprises indicates feed costs have accounted for as much as 63% of total annual cow costs (Miller et al, 2001). Extending the grazing period and reducing the need for harvested forages should lower feed costs. Complementary forage systems extend the grazing season from what is available from native range alone. Wheat (*Triticum aestivum* L.) pasture is used more commonly in the southern portion of the High Plains. Anecdotal reports have been made concerning lowered fertility in beef cows and heifers bred on lush forage such as wheat pasture; however, it is often difficult to rule out other possible causes of low fertility.

Wheat pasture is high in crude protein and grazing results in high blood urea nitrogen content (Horn et al., 1977; Beck et al., 2005) in steers and heifers. In lactating dairy cows, fertility is lowered during consumption of high protein diets that result in high blood urea nitrogen content (Butler, 1998). The general mechanism influencing fertility is believed to be a lower uterine pH that in turn impacts embryo survival. Little information is available on fertility of beef cows grazing wheat or consuming high protein diets. Therefore, the objective of this study was to compare pregnancy rates of spring calving cows consuming either wheat pasture or native range before and during the breeding season.

Materials and Methods

The procedures and facilities in this study were approved by the Kansas State University Institutional Animal Care and Use Committee and conducted at the Kansas State University Agricultural Research Center – Hays from 2001 to 2005. Primiparous and second parity Angus crossbred cows (n=100) were assigned to one of two grazing systems by age, sire breed and calving date. Cows remained in their respective treatment groups throughout the study. Whenever possible, contemporaries of the original group of cows were used to replace culled cows to maintain or increase group size. Grazing treatments were 1) grazing mixed-grass native rangeland from early spring until late fall in a season-long continuous grazing system (**Native,** n=40 to 45), or 2) grazing winter annual wheat in early spring followed by mixed-grass native rangeland until late fall in a seasonal complementary forage system (**Wheat**, n=48 to 60). Japanese brome (*Bromus arvensis* L.) and western wheatgrass [*Pascopyrum smithii* (Rybd.) A. Löve.] were cool-season grasses available in small proportions to the Native group early in the spring in warm-season grass dominated native rangeland pastures.

Cows in the Wheat group were placed on winter annual wheat pasture in late March or April each season in 6 replicates of 8 to 10 cows when plant growth had reached 15 cm in height. Wheat cows were allowed

access to free choice sorghum-sudangrass (*Sorghum* ssp.) hay the first two weeks of grazing wheat. Average initial wheat grazing date and removal date was April 11 and June 11, respectively. In 2001 and 2002, half of the cows in the Wheat group were moved from rangeland to graze sudangrass for 30 to 40 d in August and early September and then returned to native rangeland.

Cows in the Native group were placed on native pasture in three replicates of 13 to 15 cows on the same grazing initiation dates as the Wheat group being placed on wheat. Weights and BCS (Richards et al, 1986) of all cows were assessed at the initiation and end of wheat grazing after an overnight shrink. Native cows were stocked at 4.0 ha/pair from April through October, while the Wheat group was stocked at 0.6 ha/pair on wheat from April through June and 1.7 ha/pair through October on native rangeland.

At the end of the grazing season, all cows were placed in wintering pasture lots and fed a diet composed of sorghum-sudangrass hay and oat hay supplemented with vitamin and mineral fortified 26% protein range cubes as needed until being placed back into wheat pasture or native rangeland the following spring. Calving occurred in the wintering lots prior to wheat and native rangeland grazing.

The breeding season began between May 15 and May 20 each year. Cows in the Wheat group grazed on wheat 21 to 50 d prior to breeding, depending on the year. The first day of the breeding season consisted of a fixed-time insemination of all cows following an MGA-Select protocol. The MGA-Select protocol consisted of 0.5 mg melengesterol acetate (MGA; Pfizer Animal Health, New York, NY) in 1.8 kg of a 26% crude protein cube per cow per d from d -36 to d -22. Cubes were hand fed daily to all pasture groups. Cows received 100 µg GnRH (Factrel; Fort Dodge Animal Health, Fort Dodge, IA), i.m. on d -10 and 25 mg PGF (Lutalyse; Pfizer Animal Health, New York, NY) on d -3. Cows were inseminated to a single Angus sire on d 0, 72 hours following PGF, concurrent with 100 µg GnRH.

A total of three cleanup bulls were used each breeding season and turned in with cows 10 d after fixed-timed AI. One cleanup bull was used for the Native group, while cows in the Wheat group were divided into two groups of 30 cows, each with one cleanup bull per group. The total length of breeding season each year ranged from 46 to 53 d. Cows grazing wheat at the time of breeding remained on wheat pasture an average of 25 d following AI. Pregnancy was determined by transrectal ultrasonography 30 to 40 d after timed AI to determine pregnancy rate to AI and on d 76 to 141 to determine final pregnancy rate.

Due to an equipment failure, weight and body condition data were not collected on the Native group at the end of the wheat grazing period in 2003. Therefore, comparisons of changes in weight and body condition could not be calculated for 2003 and were omitted for that season.

Statistical analysis of binomial variables was performed with PROC GLIMMIX of SAS (Cary, NC). The model used for binomial variables included the factors of year, replication, grazing treatment, and their interactions. Replication within treatment was used as a random variable to test for treatment differences. Pre-grazing BCS, pre-grazing weight, and days postpartum were included in the model as covariates. Initially, these factors and their interactions were included as covariates, but only those interactions found to have $P<0.25$ remained in the final model. In further analysis, PROC MIXED was used to analyze dependent variables of pre-grazing weight, pre-grazing BCS, and their change over the wheat grazing period. The replication within treatment factor was used as the random effect to test differences, and days postpartum was also included as a covariate in the model. The 'ddfm=satterth' option was used to compute degrees of freedom for factors included in the models.

Data from 2001 and 2002 of cows in the Wheat group were initially analyzed to compare those that grazed wheat only or wheat and sudan through the season. Cow pregnancy data and cow weight and BCS at the beginning and end of the grazing season were similar between the two groups and therefore, data were combined for further analyses and reporting. The LSMEANS option was used to determine differences between Wheat and Native treatments when significant effects were present.

Results

Average cow age, weight and body condition at the start of wheat pasture grazing each year is shown in Table 1. Pre-grazing cow weights, body condition scores, and their change during wheat pasture grazing (Table 2) differed with year ($P<0.01$). Year and grazing treatment interacted ($P<0.01$) to affect both pre-grazing weight and body condition (Table 2 & 3). Cows were lowest in body condition and weight prior to breeding in 2001 as 2 y-olds. By 2003, pre-grazing weight and body condition in the Native group exceeded that of the Wheat group.

Native cows weighed more ($P<0.01$) and had more body condition ($P<0.01$) at the end of the wheat grazing period than Wheat cows. Average daily gain during the wheat grazing period was greater ($P<0.01$) for Native cows than Wheat cows, but body condition change was similar. Treatment and year interacted to affect body condition change during wheat grazing (Table 2, Figure 1). Within a year, magnitude of difference in body condition change between treatments was significant when wheat grazing was longest in 2002 ($P<0.01$). Part of this difference may be due to the fact that wheat pasture was maturing and declining in quality towards the end of the period while forage quality for Native cows was still high.

Average days postpartum at breeding did not differ between grazing treatment groups. The postpartum interval was longest ($P<0.01$) in 2001 since many were 2 y olds that had been bred ahead of the mature cow herd. Postpartum interval to AI was shorter ($P<0.05$) in 2002 and 2003 compared to 2004. There was no treatment by year interaction.

Pregnancy rate to fixed-time AI was similar for cows that grazed wheat prior to and during breeding compared to cows that grazed native rangeland, 51.7% and 57.7%, respectively. Pregnancy rates averaged across grazing groups tended (P<0.11) to vary between years, mostly because of lower AI pregnancy rates the first year of the study when cows were all 2 and 3 y-olds (Table 3). Neither the treatment by year interaction (P=0.14) nor pre-grazing body weight had an effect on AI pregnancy rate; however, pre-grazing body condition did affect (P<0.01) AI pregnancy rate. A separate simple regression analysis showed that a one unit increase in pre-breeding BCS improved AI pregnancy rate 10% (Figure 2). Final pregnancy rate was similar between the two grazing groups, and over years averaged 94.4 and 95.9% for the Wheat and Native groups, respectively (Table 3).

Discussion

Failure of wheat pasture to reduce pregnancy rates of cows in this study agrees with Bryant et al., (2011) who reported that replacement heifers had similar pregnancy rates whether fed in a drylot or grazing wheat pasture prior to breeding. This was despite the fact blood urea nitrogen concentrations were 22 mg/dL two days before breeding in wheat pasture heifers. In dairy cattle, serum urea nitrogen > 20 mg/dL has been found detrimental to fertility (Butler, 1998). Beck and coworkers (2005) found average conception date was earlier for heifers bred while in the drylot than those grazing a wheat and ryegrass pasture.

Timing of peak protein content would have varied with pasture growth conditions each year and could not be held constant for each breeding season. Evidence of embryonic losses occurred when heifers received a diet high in degradable protein yet energy restricted (Elrod and Butler, 1993). Energy did not appear to be limited in the current study since body condition and weight change of cows was positive with the exception of 2001. Fertility of cows grazing wheat pasture prior to and during breeding was equal to cows grazing native range when energy for cows grazing wheat was not limiting.

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Table 1. Characteristics of cows at the beginning of the wheat grazing period each year and length of wheat grazing.

		Days on	Age	Days Postpartum			Body weight, kg			Body Condition Score		
Year	n	wheat	mean	mean	SD	range	mean	SD	range	mean	SD	range
2001	100	40	2.2	88	31	21-123	440	51	349-616	4.5	0.62	3.0-6.2
2002	105	83	3.5	68	18	36-91	540	63	384-709	5.2	0.91	2.3-7.0
2003	105	56	4.7	68	17	22-89	597	51	477-755	6.2	0.77	4.3-7.8
2004	105	49	5.7	74	15	34-95	630	54	515-743	6.5	0.82	4.5-8.7
2005	93	74	6.6	72	15	34-90	601	53	490-763	6.0	1.26	3.5-8.5

Table 2. Least squares means of weight and body condition and analysis of treatment[a] effects for cows grazing either wheat pasture or native mixed grass rangeland for 21 to 50 d prior to fixed-time AI.

	BW, kg					BCS				
Item	Native	Wheat	trt[a]	y	trt x y	Native	Wheat	trt[a]	y	trt x y
Pre-Grazing	567±6	557±5	NS	**	**	5.9±0.1	5.5±0.1	*	**	**
Post- Wheat Grazing	603±7	569±7	*	**	NS	6.5±0.1	6.0±0.1	*	**	NS
ADG, kg/d	0.65±0.10	0.24±0.09	*	**	NS					
BCS change						0.72±.08	0.58±.07	NS	**	**

*P<0.05, **P<0.01

Table 3. Least squares means for pre-grazing weight and body condition score and pregnancy rates for cows grazing either wheat pasture or native mixed grass rangeland for 21 to 50 d prior to fixed-time AI. Values within a row category with different superscripts differ (P<0.05).

	Pre-grazing BW, kg		Pre-grazing BCS		AI Pregnancy Rate (%)		Final Pregnancy Rate (%)	
Year	Native	Wheat	Native	Wheat	Native	Wheat	Native	Wheat
2001	456±11[a]	450±10[a]	4.5±0.2[a]	4.6±0.1[a]	35.0	43.3	97.5	91.7
2002	522±10[a]	545±8[a]	5.2±0.1[a]	5.2±0.1[a]	66.7	50.8	93.3	91.5
2003	603±9[a]	585±8[b]	6.5±0.1[a]	5.9±0.1[b]	64.4	52.5	100.0	94.8
2004	653±9[a]	615±7[b]	7.0±0.1[a]	6.1±0.1[b]	60.0	63.3	91.1	95.0
2005	614±9[a]	586±7[b]	6.5±0.1[a]	5.5±0.1[b]	60.0	47.9	97.8	100.0
Mean	570 [a]	565 [a]	5.9 [a]	5.5 [b]	57.7	51.7	95.9	94.4

Figure 1. Body condition score change during wheat pasture grazing (*within year, treatments differ at P<0.01).

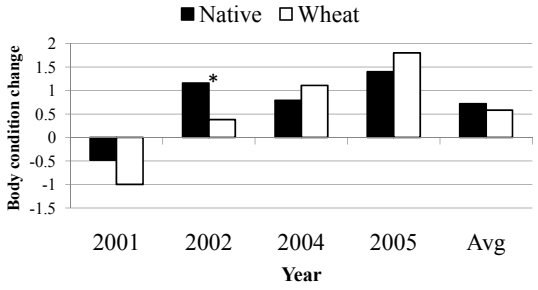
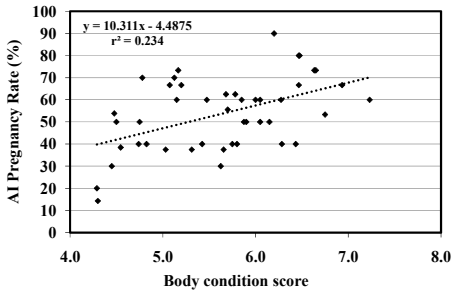


Figure 2. Relationship between cow BCS and pregnancy rate to fixed-time AI for beef cows grazing wheat or native mixed grass rangeland for 21 to 50 d prior to AI.



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SERUM CONCENTRATIONS OF PROGESTERONE, IGF-I, INSULIN, AND GLUCOSE AND PREGNANCY RATES OF EWES TREATED WITH DEXAMETHASONE BEFORE BREEDING

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ABSTRACT: Sixty-six Rambouillet ewes (59.1 ± 2.3 kg) were used to examine effects of injecting dexamethasone (DEX) before breeding on serum hormone profiles and pregnancy rates. Before initiation of a fall breeding period, ewes received an intravaginal insert containing 0.3 g of progesterone (P4) for 12 d to synchronize onset of estrus. At insert removal (d 0) ewes received 10 mg of $PGF_{2\alpha}$ (Lutalyse, Pfizer, i.m.) and either 0 (saline control, n = 22), 5 (n = 22), or 10 (n = 22) mg of DEX (Vet One, Vetpharm Group Ltd, i.m.). Ewes were joined with fertile rams after treatment for 2 breeding cycles. Blood samples were collected from all ewes daily from d 0 through 18. Serum P4 concentrations were similar ($P = 0.55$) among groups for 6 d after treatment (treatment x day, $P = 0.85$) indicating that DEX had no adverse impact on CL development. Serum IGF-I was lower ($P < 0.01$) on d 1 and 2 in DEX-treated ewes compared to control ewes. Serum insulin concentrations were 0.64, 1.78, and 1.90 (± 0.11) ng/mL on d 1 in ewes receiving 0, 5, and 10 mg of DEX, respectively (L, Q, $P < 0.01$). A similar linear and quadratic response in insulin was observed on d 2 ($P < 0.01$). Serum glucose values on d 1 were 52, 101, and 116 (± 5.7) mg/dL in ewes administered 0, 5, and 10 mg of DEX (L, Q, $P < 0.02$). Pregnancy rates immediately after DEX treatment (determined by P4 profiles) were 27, 54, and 23% for ewes receiving 0, 5, and 10 mg of DEX ($P = 0.057$) while percentages of 54, 68, and 68, respectively, resulted after 2 breeding cycles ($P = 0.75$, estimated by P4 measured 4 mo after treatment). Treatment with DEX induced elevated serum insulin and glucose levels, decreased IGF-I for 2 d, and resulted in an increase in pregnancy rate in the 5 mg group after the first breeding period. However, pregnancy rates among treatment groups were not different across the entire breeding period.

Key words: dexamethasone, reproduction, sheep

INTRODUCTION

Glucocorticoids have been suggested to inhibit reproductive function in animals; but, in the ewe, preliminary reports suggest that glucocorticoids may have little to no effect (Phillips and Clarke, 1990). The stress-induced release of glucocorticoids inhibits certain reproductive (Kanchev et al., 1976; Echternkamp, 1984; Liptrap, 1993) and endocrine (Vighio and Liptrap, 1990) functions. Dexamethasone (DEX) has been used in these studies because of its potent glucocorticoid activity (Norris and Kohler, 1977; Sutanto and de Kloet, 1987) and relatively long (3 to 4 h) half-life (Ferguson and Hoenig, 1995; Reding et al., 1997). In cyclic ewes, DEX treatment at rates of up to 2 mg/d did not affect the natural or PMSG-stimulated ovulation rate or timing and incidence of behavioral estrus (Phillips and Clarke, 1990). Results from a study by Maciel et al. (2001) in dairy cattle indicated that daily injections of DEX (44 µg/kg of BW, i.m.) 1 d after ovulation until the first dominant follicle stopped growing or up to d 12 postovulation increased serum glucose and insulin concentrations and decreased IGF-I concentrations but did not affect growth of dominant follicles. These researchers also showed that DEX decreased plasma progesterone concentrations without affecting gonadotropin levels. Dexamethasone also had significant effects on metabolism without a major impact on growth of the first-wave dominant follicle (Maciel et al., 2001). However in fluorogestone acetate-synchronized (14 d), PMSG-treated (500 IU at sponge removal) ewes receiving 2, 3, or 4 mL of DEX 72 h before mating, conception rates were greater in those receiving 3 or 4 mL of DEX than in those treated with 0 or 2 mL of DEX (Koyuncu et al., 2008). Therefore, whether small dosages of DEX result in a detriment or benefit to reproduction in ewes is not clear. The objectives of this study were to determine whether DEX administration influences serum concentrations of progesterone, IGF-I, insulin, and glucose and conception rates and lamb production of Rambouillet ewes.

MATERIALS AND METHODS

All procedures involving animals were approved by the New Mexico State University Institutional Animal Care and Use Committee.

Animals and Treatments. Sixty-six Rambouillet ewes (59.1 ± 2.3 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 1.8 kg/animal daily and had free access to water, salt, and shade. Before initiating a fall breeding period, ewes received a progesterone-impregnanted intravaginal insert (CIDR, 0.3 g progesterone; Pharmacia and Upjohn Pty Limited, Rydalmere, NSW) to synchronize onset of estrus. The CIDR was removed after 12 d and ewes received an i.m. injection of 10 mg of $PGF_{2\alpha}$ (Lutalyse, 5 mg/mL, Pfizer, Pharmacia and Upjohn Company) and were joined with 3 fertile Rambouillet rams. Ewes were stratified by BW and age and randomly assigned to 1 of 3 treatments on the day of CIDR removal (d 0). Twenty-two control ewes received an i.m. injection containing 5 mL of 0.9% saline solution at CIDR removal. The second group of 22 ewes received 5

mg of DEX (Vet One, 2 mg/mL, Bimeda-MTC Animal Health Inc., Cross Vetpharm Group Ltd) i.m. and the third group of 22 ewes received 10 mg of DEX i.m. at CIDR removal.

Blood Collection and Analysis. Beginning on d 0 and continuing through d 18, blood was collected daily from all ewes before morning feeding by jugular venipuncture into serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO) and allowed to clot at room temperature for 30 min. Samples were centrifuged at 4ºC for 15 min at 1,500 x g and serum was stored frozen in plastic vials until assayed. Serum IGF-I (Berrie et al., 1995) was quantified by double antibody RIA. Insulin (Riemers et al., 1982) and progesterone (Schneider and Hallford, 1996) were quantified by solid phase RIA using components of commercial kits (Coat-A-Count, Siemens Medical Solutions Diagnostics; Los Angeles, CA). Serum glucose was determined using a glucometer (One Touch UltraMini, Life Scan, Johnson and Johnson, Milpitas, CA) using procedures reported by Camacho (2009). Within and between assay CV were less than 15.9% for all hormone and glucose determinations.

Statistical Analysis. Effects of DEX on serum concentrations of IGF-I, insulin, progesterone, and glucose during the treatment period were examined by ANOVA appropriate for a split-plot design. Effects of treatment were included in the main plot and tested using animal within treatment as the error term. Effects of day and treatment by day interaction were included in the subplot and tested using the residual error. If a significant treatment by day interaction was detected, effects of treatment were examined within day. When treatment effects were detected, treatment means were separated using linear and quadratic contrasts. Analyses were computed using the mixed procedure of SAS (SAS Inst. Inc., Gary, NC) with compound symmetry as the covariance structure. Number of ewes pregnant at the first estrus after treatment was determined by serum progesterone profiles monitored for 24 d after treatment. Likewise, pregnancy rate over the entire breeding period was determined by serum progesterone values determined twice (10 d apart) approximately 4 mo after treatment. Pregnancy rate was subjected to Chi-square analysis using the frequency procedure of SAS. Weight responses were subjected to ANOVA for a completely random design and the analysis was computed using the GLM procedure of SAS.

RESULTS AND DISCUSSION

Serum Hormone Profiles

Serum progesterone was measured in samples collected daily from the time of CIDR removal and DEX administration through the subsequent 18-d period. No differences were observed ($P = 0.55$) among groups for the 6 d following treatment (treatment by day, $P = 0.85$). This similarity in serum progesterone values across treatments implies that DEX administration at breeding has no adverse impact on normal CL development. This is in contrast with previous research by Vighio and Liptrap (1990) in cattle that showed that progesterone concentrations remained significantly high and the appearance of estrus after DEX treatment was delayed or not observed when administered at 2 mg twice daily from d 13 through 17 of the estrous cycle. However in a study by Maciel et al. (2001), progesterone concentrations were lower in DEX-treated vs. control cows from d 4 after ovulation when treated with daily injections of 44 µg/kg of BW.

Serum IGF-I concentrations were also determined in the daily post-treatment samples and are shown in Figure 1. A treatment by day interaction was detected ($P < 0.001$) necessitating examination of treatment effects within sampling day. Before DEX treatment on d 0, serum IGF-I was similar ($P = 0.61$) in the 3 groups of ewes. However, serum IGF-I was lower ($P < 0.01$) on d 1 and 2 in DEX-treated ewes compared to control ewes with concentrations of 166, 86, and 75 (± 7.1) ng/mL on d 1 in ewes receiving 0, 5, and 10 mg of DEX, respectively. Both linear and quadratic responses in serum IGF-I were observed on d 1 after treatment ($P < 0.01$) while the response was a linear decline on d 2 ($P < 0.01$). A similar decrease in IGF-I was observed by Maciel et al. (2001) as IGF-I concentrations were lower ($P < 0.05$) in cows treated with daily injections of DEX (44 µg/kg of BW, i.m.) until the first dominant follicle stopped growing or up to d 12 after ovulation compared to control cows. On d 3, 4, and 5 after treatment with DEX, serum IGF-I in the current study was similar in the 3 treatment groups ($P > 0.35$). These data demonstrate that both 5 and 10 mg of DEX caused decreased IGF-I levels in ewes for 2 d after injection.

Serum insulin profiles in control and DEX-treated ewes after CIDR removal and DEX administration are shown in Figure 2 (treatment by day, $P < 0.001$). Before treatment on d 0, serum insulin values were less than 0.50 ng/mL in all 3 groups. One day after treatment began, however, insulin in control ewes was 0.64 ng/mL compared with 1.78 and 1.90 (± 0.11) ng/mL in 5 and 10 mg DEX-treated females (linear and quadratic, $P < 0.001$). A similar linear increase in insulin ($P = 0.004$) in response to increasing amounts of DEX was also observed on d 2 although the actual concentrations of insulin were reduced. Therefore, a single injection of DEX resulted in very large increases in serum insulin that continued through d 2. In a study by Cox et al. (1987), gilts receiving injections of either short-acting or long-acting insulin had increased ovulation rates suggesting that elevated insulin concentrations might be beneficial to reproduction. Similar benefits of insulin relative to reproduction have been reported in cows by Harrison and Randel (1986).

However, if elevated insulin values after DEX administration are accompanied by a decrease in insulin sensitivity, benefits to reproduction may be lessened. Therefore, we also examined serum glucose in samples through d 3 after treatment and these values are presented in Figure 3 (treatment by day, $P < 0.001$). Before treatment on d 0, glucose values were 38 ± 1.6 mg/dL in all 3 groups.

At 24 h after treatment, control ewes had a glucose concentration of 52 mg/dL compared with 101 and 116 (± 5.7) mg/dL (L, Q, $P < 0.02$) for those receiving 5 and 10 mg of DEX, respectively. This increase in glucose after DEX treatment reflects the well-known effect of glucocorticoids on serum glucose. However, glucose concentrations in treated ewes subsequently declined and remained similar ($P > 0.10$) to control females on d 2 and 3 after treatment. Similar glucose responses were reported by Maciel et al. (2001) who showed that serum glucose concentrations were greater in DEX-treated cows than control cows. These data demonstrate clearly that a single injection of DEX results in a rapid increase in serum glucose by the day after treatment. However, the decline to baseline levels on d 2 and 3 suggests that the elevated insulin on d 1 quickly restored blood glucose profiles. Therefore, DEX-treated ewes were apparently not insulin insensitive.

Pregnancy Rates

As stated previously, one of the objectives of this experiment was to determine the influence of DEX on pregnancy rates of ewes. Pregnancy rate at the first estrus after treatment was determined by serum progesterone profiles that were monitored for 24 d after treatment. Six of 22 (27%) control ewes were pregnant compared with 12 of 22 (54%) and 5 of 22 (23%) ewes in the 5 and 10 mg DEX-treated groups, respectively ($P = 0.057$). These data suggest an increase in conception rate in Rambouillet ewes treated with 5 mg of DEX at the time of CIDR removal. An experiment by Phillips and Clarke (1990) showed that daily i.m. injections of DEX at 2 mg/d had no effect on ovulation rate and timing and incidence of behavioral estrus in sheep indicating that DEX does not significantly modify reproductive function in the ewe. More recently, results from a study by Koyuncu et al. (2008) demonstrated greater conception rates in fluorogestone acetate-synchronized (14 d), PMSG-treated (500 IU at sponge removal) ewes receiving 3 or 4 mL of DEX 72 h before mating (fertility rates of 100 and 100%, respectively) than in females treated with 0 or 2 mL (fertility rates of 88 and 92%, respectively).

Pregnancy rate over the entire breeding period was determined by serum progesterone values measured twice (10 d apart) approximately 4 mo after treatment with percentages of 54, 68, and 68 for ewes receiving 0, 5, and 10 mg of DEX, respectively ($P = 0.55$). These data suggest that administration of DEX before the first estrus has no beneficial effect on conception rates over the entire breeding period.

In conclusion, treatment with DEX resulted in a decrease in serum IGF-I and large increases in serum insulin and glucose concentrations which may have contributed to effects observed on reproduction. Pregnancy rates were increased by administering 5 mg of DEX immediately before breeding. However, pregnancy rate across the entire breeding period was not affected by the treatment regimen.

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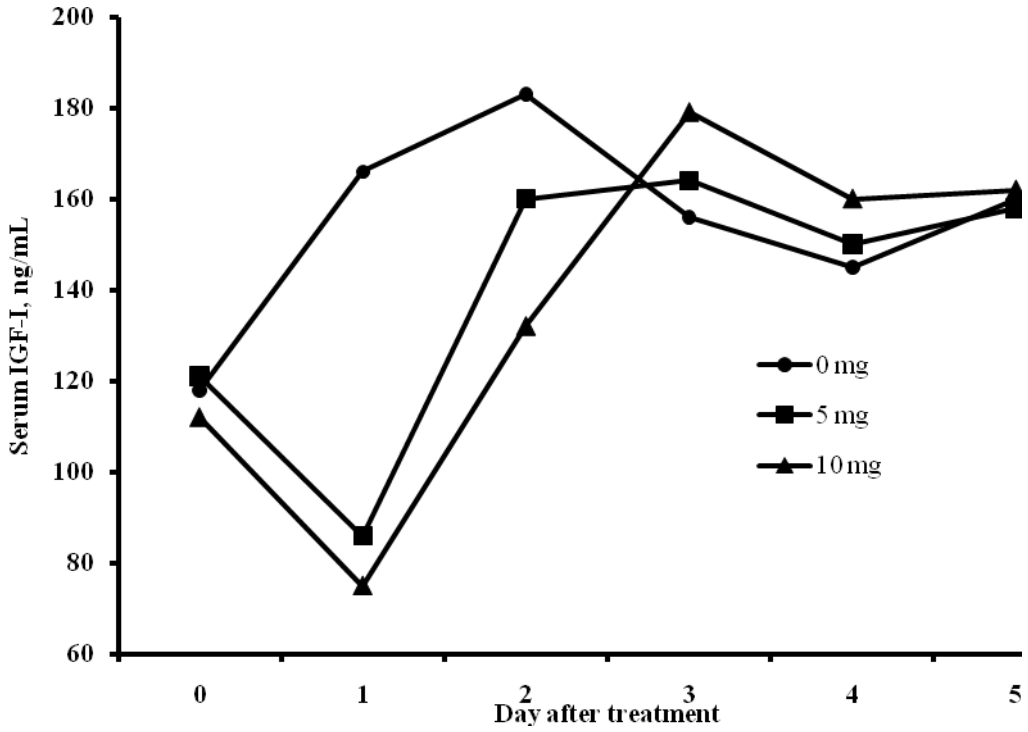

Figure 1. Serum IGF-I concentrations in Rambouillet ewes treated with 0 (control, n = 22), 5 (n = 22), or 10 (n = 22) mg of dexamethasone beginning on the day of removal of a progesterone-containing intravaginal insert (d 0) during a fall breeding season. Values differed among treatments on d 1 (linear and quadratic, $P < 0.001$) and 2 (linear, $P = 0.002$). The SE ranged from 6.8 to 11.8 ng/mL.

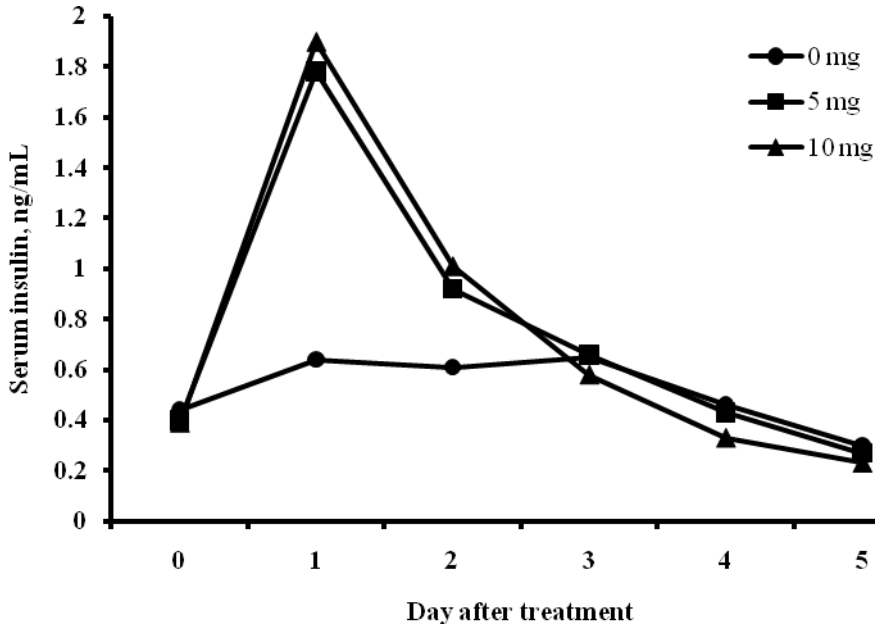

Figure 2. Serum insulin concentrations in Rambouillet ewes treated with 0 (control, n = 22), 5 (n = 22), or 10 (n = 22) mg of dexamethasone beginning on the day of removal of a progesterone-containing intravaginal insert (d 0) during a fall breeding season. Values differed among treatments on d 1 (linear and quadratic, $P < 0.001$) and 2 (linear, $P = 0.004$). The SE ranged from 0.03 to 0.11 ng/mL.

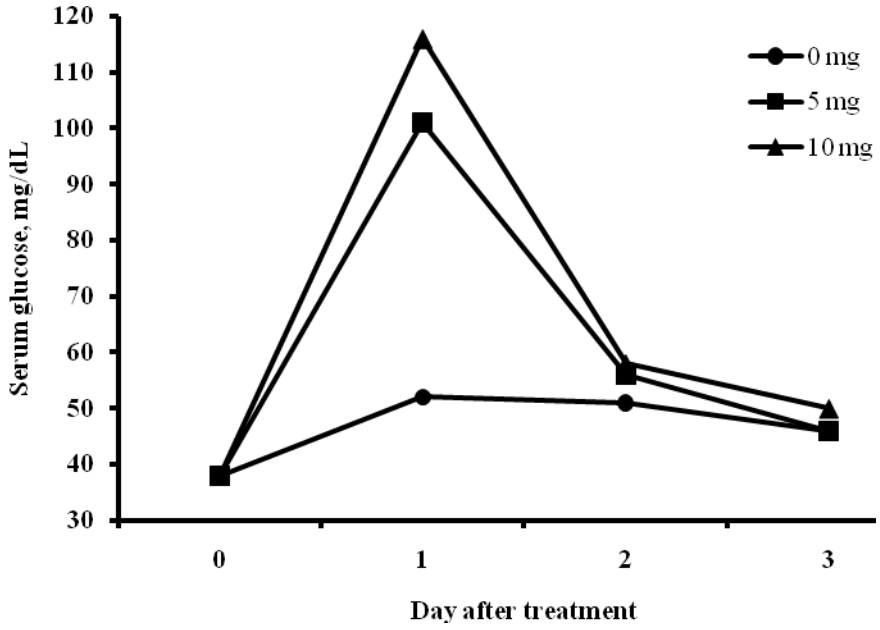

Figure 3. Serum glucose concentrations in Rambouillet ewes treated with 0 (control, n = 22), 5 (n = 22), or 10 (n = 22) mg of dexamethasone beginning on the day of removal of a progesterone-containing intravaginal insert (d 0) during a fall breeding season. Values differed among treatments on d 1 (linear and quadratic, $P < 0.02$). The SE ranged from 1.6 to 5.7 mg/dL.

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EFFECTS OF DIETARY SELENIUM AND NUTRITIONAL PLANE DURING GESTATION ON MAMMARY GLAND GROWTH, CELLULAR PROLIFERATION, AND VASCULARITY IN EWE LAMBS[1]

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ABSTRACT: Objectives were to examine the effects of Se supply and maternal nutritional plane during gestation on mammary gland growth, cellular proliferation, and vascularity at parturition and d 20 of lactation. Rambouillet ewe lambs (n = 84) were allocated to treatments in a 2 x 3 x 2 factorial. Factors were dietary Se (adequate Se [**ASe**, 11.5 µg/kg BW] or high Se [**HSe**, 77.0 µg/kg BW]), nutritional plane (60% [**RES**], 100% [**CON**], or 140% [**HIH**]), and necropsy period (parturition [**PRT**] or d 20 of lactation [**LCT**]). At parturition, lambs were removed, and 42 ewes (7/treatment) were necropsied. Remaining ewes were fed a common diet meeting requirements for lactation and mechanically milked twice daily until necropsy on d 20. At both necropsy periods, mammary glands were dissected and tissues harvested. Samples were analyzed for RNA, DNA, protein, proliferation, and vascularity. Where interactions were present ($P \leq 0.05$), least squares means from the highest-order interaction are presented. Ewes necropsied at parturition had greater ($P < 0.001$) mammary gland weights than LCT. High Se ewes had greater ($P = 0.05$) mammary gland weights compared to ASe. Mammary gland weight was decreased ($P = 0.002$) in RES compared to HIH, with CON intermediate. Concentration of RNA (mg/g) and total RNA (mg) were greater ($P < 0.03$) in LCT compared to PRT. Total DNA (mg) was greater ($P < 0.001$) in PRT compared with LCT. A tendency ($P = 0.07$) for a nutritional plane effect on total DNA was found where HIH was greater ($P = 0.03$) than RES with CON intermediate. Increased ($P < 0.001$) RNA:DNA was found in LCT compared to PRT. No differences ($P > 0.40$) were found in protein:DNA. Proliferation of cells was greater ($P < 0.001$) in LCT compared to PRT. Vascular area was greater ($P < 0.001$) in PRT compared to LCT. There was a nutritional plane by necropsy period interaction ($P = 0.05$) for alveoli per area where RES-LCT ewes were decreased ($P \leq 0.03$) compared to all others. Results of this study indicate that proper maternal nutritional plane during gestation is important for mammary gland development, even out to 20 d of lactation.

KEYWORDS: Mammary Gland, Nutrition, Selenium

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Introduction

Maternal nutrition directly influences milk quantity and quality available to offspring (Miranda et al., 1983; Meyer et al., 2011). Previously, Swanson et al (2008) reported decreased colostrum production due to maternal under- and over-nutrition during pregnancy which corresponded with a decreased mammary gland weight in the undernourished ewes. Furthermore, these authors (Swanson et al., 2008) report decreased cellular proliferation in the alveoli, but increased alveolar area, from undernourished ewe lambs. Others have noted altered mammary gland growth in sows in response to changing maternal nutritional status (Mahan, 1990). Mammary gland growth, milk yield, and DNA quantity was influenced by energy and protein intake in sows during lactation (Kim et al., 1999). Moreover, increased dietary lipids in ewe lambs resulted in increased mammogenesis (McFadden et al., 1990).

Development of the mammary gland in ewes from birth through puberty, gestation, and into 5 d of lactation was outlined by Anderson et al. (1975) where the authors concluded that unlike many species, sheep do not exhibit post-parturient growth of the mammary gland (i.e., during lactation). Our laboratory has recently demonstrated that capillary density of mammary alveoli at parturition is increased in ewes that received high selenium throughout gestation, whereas impacts of differing nutritional levels were less dramatic (Vonnahme et al., 2011).

We hypothesized maternal Se supplementation would increase vascular density at different periods of mammary growth. Moreover, we hypothesized that under- and over- nutrition during pregnancy would negatively affect mammary gland growth, development, and vascularity. Therefore, the objectives of this study were to determine how maternal Se supplementation and nutritional plane during gestation influence mammary tissue growth, cellular proliferation, and vascularity and if realimentation to a common diet during lactation can reverse the effects.

Materials and Methods

Animals and Diets. This experiment was approved by the Institutional Animal Care and Use Committee at North Dakota State University. Ewes were bred and managed as described in Meyer et al. (2010; 2011). Breeding occurred at the U.S. Sheep Experiment Station, at this time, selenium treatments [adequate Se (ASe; 3.5 µg Se•kg BW^{-1}•d^{-1}) or high Se (HSe; 65 µg Se•kg BW^{-1}•d^{-1})] were initiated. After shipping to North Dakota State University at d 36 of

gestation, pregnant Rambouillet ewe lambs (n = 84; 52.1 ± 6.2 kg) were individually housed. Ewes remained on their Se treatments (actual intakes: **ASe**, 11.5 µg Se•kg BW^{-1}•d^{-1}; **HSe**, 77.0 µg Se•kg BW^{-1}•d^{-1}), and on d 40 of gestation were assigned randomly to 1 of 3 nutritional treatments supplying 60% (**RES**), 100% (**CON**), or 140% (**HIH**) of NRC (1985) recommendations for 60 kg pregnant ewe lambs during mid to late gestation (weighted ADG of 140 g) except for Se. Within each Se and nutritional plane treatment, half of the ewes were assigned to be euthanized and necropsied at parturition (**PRT**; 3 to 24 h post-partum) or lactation (**LCT**; d 20). Resulting in a completely randomized design with a 2 × 3 × 2 factorial of Se supply x nutritional plane x physiological stage at necropsy (ASe-RES, ASe-CON, ASe-HIH, HSe-RES, HSe-CON, HSe-HIH; n = 7 for PRT and LCT).

All diets were fed once daily in a complete pelleted form (based on wheat middlings, beet pulp, alfalfa meal, and ground corn). Three pellet formulations (basal, high Se, and concentrated Se pellets) were blended to meet Se and ME intake based upon the Se treatment and nutritional plane of each ewe. The basal pellet contained 15.9% CP and 2.81 Mcal/kg ME DM basis. Selenium sources used were Se-enriched wheat mill run to replace wheat middlings in the basal diet to make a high Se pellet (16.6% CP and 2.82 Mcal/kg ME DM basis) and purified seleno-methionine added to achieve 37.1 ppm Se in the concentrated Se pellet (16.2% CP and 3.01 Mcal/kg ME DM basis). Every 14 d, BW was measured and diets were adjusted accordingly.

Parturition and Lactation. All births were attended and lambs were removed immediately for artificial rearing. Ewes assigned to necropsy on d 20 of lactation were transitioned over 5 d to a diet providing 100% of NRC (1985) requirements for early lactation, delivered by a combination of the basal pellet fed during gestation and a protein pellet (50.2% CP and 2.6 Mcal ME/kg; soybean meal, wheat middlings, urea, and supplement) and fed in 2 portions, 1 after each milking. Ewes were mechanically milked (Meyer et al., 2011) at 0500 and 1700 until necropsy after the 0500 milking on d 20.

Slaughter Procedures. Ewes assigned to necropsy at parturition and lactation were slaughtered 3 to 24 h post-partum or after the 0500 milking and feeding on d 20 of lactation, respectively. Immediately before slaughter, ewes were weighed and mammary glands were stripped of residual milk accumulated since the last milking. Animals were stunned by captive bolt (Supercash Mark 2; Acceles and Shelvoke Ltd., England), exsanguinated, and detailed necropsies performed. The mammary gland was dissected from the skin, weighed, and processed. From one half of the mammary gland, 5 samples (approximately 1 g each) of glandular tissue were snap frozen in super-cooled isopentane (submerged in liquid nitrogen) and stored at -80˚C until analysis for RNA, DNA, and protein. The remaining half of the mammary gland was perfusion fixed with Carnoy's fixative (70% ethanol, 30% acetic acid, 10% chloroform) by cannulating the cranial mammary artery with a polyethylene (PE-60; o.d. = 1.22 mm; i.d. = 0.77 mm; Intramedic, Becton Dickinson & Company, Sparks, MD) beveled catheter that was secured to surrounding tissue. The mammary gland was initially perfused with PBS, then Evan's blue dye (to define the vasculature), then PBS again, and finally, was perfusion fixed with Carnoy's fixative. Tissue was then cut into ~1-cm cubes and was further immersion fixed in Carnoy's fixative for an additional 24 h. Thereafter, mammary gland tissues were dehydrated in a series of ethanol, Histo-clear (National Diagnostics, Atlanta, GA) rinses, and embedded in paraffin wax.

Cellularity Estimates. Tissue homogenates were analyzed for concentrations of DNA and RNA using the diphenylamine (Johnson et al., 1997) and orcinol procedures (Reynolds et al., 1990), respectively. Protein concentrations were 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). Concentration of DNA was used as an index of hyperplasia, and RNA:DNA and protein:DNA ratios were used as an index of hypertrophy (Scheaffer et al., 2003; Soto-Navarro et al., 2004).

Cellularity and Vascularity. Paraffin-embedded tissues were sectioned at 4 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 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). Vascularity was performed using an antibody to Factor VIII, a specific endothelial cell marker, as previously described by our laboratory (Grazul-Bilska et al., 2010) and counter-stained with using periodic acid-Schiff's staining procedures to provide contrast to the vascular tissue, as previously described (Borowicz et al., 2007; Vonnahme et al., 2007). Photomicrographs were taken at 400× magnification using a Nikon Eclipse E800 microscope equipped with Nikon DXM 1200F digital camera (n = 10 pictures per slide, 85,734.7 µm2 per picture). Vascularity was then determined by image analysis (Image-Pro Plus; Borowicz et al., 2007; Vonnahme et al., 2007). Briefly, for each ewe, 10 images per mammary gland (in the alveolar area) were analyzed for tissue area, luminal area, alveoli, and total vascular area (i.e. area stained for Factor VIII; capillary area per total tissue area).

Calculations. Digesta weight was calculated by difference (total full viscera weight – visceral tissues after stripping of digesta contents). Empty BW was considered to be digesta weight subtracted from the final BW before slaughter. Total tissue DNA, RNA, and protein contents were calculated by multiplying DNA, RNA, and protein concentration by fresh tissue weights (Swanson et al., 2000; Scheaffer et al., 2003; Scheaffer et al., 2004). Vascular area was calculated as (((total picture area – luminal area) – total vascular area) / (total picture area – luminal area)) * 100. Alveoli per area was calculated as the number of alveoli / total picture area * 1000.

Statistics. Data were analyzed as a completely randomized design with a 2 × 3 × 2 factorial arrangement using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Model contained effects for Se (ASe vs. HSe), nutritional plane (RES, CON, and HIH), necropsy period (PRT vs. LCT) and all interactions. Means were separated using least significant difference when $P < 0.05$ and tendencies discussed when $P > 0.05$ and ≤ 0.10. In the absence of interactions ($P > 0.05$), main effects are reported; otherwise interactive means are discussed.

Results

In data published previously (Meyer et al., 2010), final and empty BW (**EBW**) were least ($P < 0.001$) in RES and greatest ($P < 0.001$) in HIH. High-Se ewes had heavier ($P = 0.05$) mammary glands than ASe ewes (Table 1). When mammary gland weight is expressed proportional to EBW (g/kg EBW) there was a tendency ($P = 0.09$) for HSe to have heavier mammary glands then ASe. Mammary gland weight (g) was decreased ($P = 0.002$) in RES compared to HIH with CON intermediate ($P \geq 0.08$). Ewes necropsied at parturition had greater ($P < 0.001$) mammary gland weights (g and g/kg EBW) than those at LCT.

There were no effects ($P > 0.16$; Table 1) on DNA concentration (mg/g), however, a tendency ($P = 0.07$) for a nutritional plane effect on total DNA (mg) was found where HIH was greater ($P = 0.03$) than RES with CON intermediate ($P \geq 0.10$). Additionally, total DNA (mg) was greater ($P < 0.001$) in PRT compared to LCT. There was a tendency ($P = 0.09$) for nutritional plane effect on concentration for RNA (mg/g) to be decreased ($P = 0.05$) in RES compared to CON with HIH intermediate. Additionally, nutritional plane tended ($P = 0.07$) to affect total RNA (mg) resulting in decreased ($P = 0.03$) RNA in RES compared to CON and HIH. Concentration of RNA (mg/g) and total RNA (mg) were greater ($P \leq 0.02$) in LCT compared to PRT. Increased ($P < 0.001$) RNA:DNA was found in LCT compared to PRT. Concentration of protein (mg/g) was not different ($P > 0.49$), however total protein tended ($P = 0.06$) to have a nutritional plane effect where total protein was decreased ($P = 0.02$) in RES compared to HIH with CON intermediate, and a tendency ($P = 0.06$) for increased total protein in PRT compared to LCT. No differences ($P > 0.57$) were found in protein:DNA.

Mammary cell proliferation was greater ($P < 0.001$; Table 1) in LCT compared to PRT. There was a tendency ($P = 0.07$) for vascular area to be increased in HSe compared to ASe. Vascular area was greater ($P < 0.001$) in PRT compared to LCT. Alveoli per unit tissue area showed a nutritional plane by necropsy period interaction ($P = 0.05$) where RES-LCT ewes were less than ($P \leq 0.03$) all other groups.

Discussion

The decreased mammary gland weight observed for under- compared to over-fed ewes is similar to results published by Swanson et al. (2008) with these authors additionally noting a decreased weight in under-fed compared to control. In both the current study and Swanson et al. (2008), mammary glands of control and over-fed ewes were of similar weight, contrary to data by Rattray et al. (1974) where ewes consuming ad libitum diets had greater mammary gland weights then ewes consuming a maintenance diet. Mammary gland growth was also impaired within 3 d of late gestation nutrient restriction where decreased gland mass was recorded at parturition (Mellor and Murray, 1985) and even when ewes were realimented during the last 5 d of pregnancy (Mellor et al., 1987).

Total DNA measured in the mammary glands of the current study was reduced in under- compared to over-fed ewes, whereas Swanson et al. (2008) reported under-fed were reduced compared to control ewes. While we are reporting similar DNA concentration between physiological stage and increased total DNA in ewes necropsied at parturition compared to those at lactation. This is opposite of Anderson et al. (1975) where DNA concentration was highest in ewes near term yet there were no differences in total DNA between the end of pregnancy and d 5 of lactation. Our results of total RNA are similar to those of Swanson et al. (2008) where decreased RNA content is reported in under-fed ewes compared to control and over-fed ewes. We are reporting increases in RNA concentration and total RNA at 20 d of lactation whereas Anderson et al. (1975) reported similar RNA concentrations between end of pregnancy and early lactation, but greater total RNA at 5 d of lactation. The differences in DNA and RNA resulted in a greater RNA:DNA in ewes at 20 d of lactation which is similar to the results of ewes at 5 d of lactation (Anderson et al., 1975).

We saw no dietary treatment effects on proliferation of cells, but Swanson et al. (2008) reported an increase in proliferation in over- compared to under-fed ewes. We report an increased proliferation of cells at 20 d of lactation compared to those at parturition. Other researchers (Colitti and Farinacci, 2009) noted increased proliferation index in ewes from 10 d prior to lambing to lactation (30, 60, and 150 d of lactation all had similar proliferation). Current vascular area data are supported by previous research where, at parturition, capillary vascularity was greatly enhanced in high-Se fed ewes (Vonnahme et al., 2011). The increased milk production data may be due to augmented vascular differences in high-Se ewes. Additionally, it appears that maternal nutritional status and Se impact mammary gland development. Furthermore, mammary gland development through 20 d of lactation is dependent upon proper gestational nutrition.

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Table 1: Effects of gestational Se supply and nutritional plane on mammary gland weight, cellularity, cell proliferation, and vascularity when necropsied after parturition or at d 20 of lactation

	Se Supply[1]			Nutritional Plane[2]				Stage[3]			*P*-value[4]		
Item	ASe	HSe	SEM[5]	RES	CON	HIH	SEM[6]	PRT	LCT	SEM[7]	Se	Nut	Stage
Final BW[8], kg	55.2	56.1	0.9	47.4[a]	55.3[b]	64.2[c]	1.2	54.7	56.6	1.0	0.52	<0.001	0.15
Empty BW[8,9], kg	46.8	47.9	0.9	39.6[a]	47.1[b]	55.3[c]	1.1	47.9	46.8	0.9	0.44	<0.001	0.41
Mammary gland, g	665.9	742.6	27.6	628.5[a]	700.8[ab]	783.4[b]	34.5	802.4	606.1	28.3	0.05	0.008	<0.001
g/kg EBW[9]	14.3	15.8	0.7	16.0	15.1	14.1	0.8	17.1	13.1	0.7	0.09	0.25	<0.001
DNA, mg/g	3.51	3.43	0.13	3.39	3.55	3.46	0.16	3.59	3.34	0.13	0.64	0.77	0.17
DNA, g	2.32	2.55	1.29	2.14	2.51	2.66	1.62	2.83	2.04	1.32	0.22	0.07	<0.001
RNA, mg/g	6.99	6.76	0.28	6.53	7.48	6.62	0.34	5.46	8.30	0.28	0.55	0.09	<0.001
RNA, g	4.44	4.88	2.70	4.03	5.08	4.89	3.38	4.22	5.11	2.58	0.25	0.07	0.02
RNA:DNA	2.03	2.01	0.06	1.98	2.12	1.96	0.08	1.55	2.49	0.07	0.76	0.29	<0.001
Protein, mg/g	28.54	28.24	1.87	26.60	29.06	29.51	2.34	27.51	29.27	1.92	0.91	0.64	0.50
Protein, g	19.47	20.66	1.53	16.76	20.25	23.18	1.91	22.14	17.98	1.57	0.58	0.06	0.06
Protein:DNA	8.53	8.76	0.67	8.52	8.35	9.07	0.84	8.39	8.90	0.69	0.80	0.81	0.58
Proliferation, %	6.60	6.65	0.75	6.09	7.04	6.73	0.94	2.76	10.48	0.77	0.96	0.76	<0.001
Vascular area, %	49.15	51.47	0.93	51.19	49.19	50.27	1.16	53.64	46.98	0.94	0.07	0.55	<0.001
Alveoli per area[10]	0.57	0.54	0.02	0.52	0.55	0.59	0.02	0.58	0.53	0.02	0.18	0.15	0.07
PART	--	--	--	0.59[d]	0.55[d]	0.59[d]	0.04	--	--	--	--	--	--
LACT	--	--	--	0.45[e]	0.55[d]	0.59[d]	0.03	--	--	--	--	--	--

[a,b,c] Within a row, nutritional plane means differ ($P \leq 0.05$).

[d,e] Within a parameter, interactive means differ ($P \leq 0.05$).

[1]Ewes fed 11.5 µg/kg BW Se (ASe) or 77.0 µg/kg BW Se (HSe) during gestation.

[2]Ewes fed 60% (RES), 100% (CON), or 140% (HIH) of nutrient requirements during gestation.

[3]Ewes necropsied within 24 h of parturition (PRT) or on d 20 of lactation (LCT).

[4]Probabilities of difference for Se supply (Se), nutritional plane (Nut), and physiological stage at necropsy (Stage). All interactions were included in the model; a Nut x Stage interaction ($P = 0.05$) was present for alveoli per area, but all others were not significant ($P \geq 0.06$).

[5]Standard error means for ASe n = 39 and HSe n = 40.

[6]Standard error means for RES n = 25, CON n = 28, and HIH n = 26.

[7]Standard error means for PRT n = 37 and LCT n = 42.

[8]Data previously published Meyer et al. (2010).

[9]Empty BW (EBW) = Final BW – digesta weight.

[10]Alveoli per area = (number of alveoli / total picture area) *1000.

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HEIFER RESPONSE TO GNRH IN A 7-DAY CIDR SYNCHRONIZATION PROTOCOL

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ABSTRACT: GnRH is commonly administered at the time of CIDR insertion in 7-day CIDR synchronization protocols for both beef heifers and cows. The necessity of GnRH administration in heifer synchronization is questionable. Our objective was to compare heat response and fertility in heifers with or without GnRH administration at the time of CIDR insertion. In 2009 (2 locations) and 2010 (1 location), yearling beef heifers were randomly assigned within breed to either receive GnRH (Select Synch+CIDR) or not receive GnRH (7-day CIDR-PG) at the time of CIDR insertion (d 0). Seven days later $PGF_{2\alpha}$ was given and the CIDR was removed (d 7). At both d 0 and d 7, follicles larger than 5mm were measured by transrectal ultrasonography and a blood sample was taken for progesterone assays. Heifers at location 1 (n=147) were artificially inseminated approximately 12 hours after onset of estrus or were given an injection of GnRH at a fixed-time insemination 54 hours after CIDR removal (n=93; location 2; 2009 only). Conception (# pregnant / # inseminated) and pregnancy (# pregnant / # treated) rates were determined by a transrectal ultrasonography scan 30 to 35 days after insemination. More than 90% of heifers had progesterone concentrations > 1.0 ng/ml at d 0 or d 7. At location 1, onset of estrus was similar between treatments with more than 85% displaying estrus from 48 to 72 hours after $PGF_{2\alpha}$. Synchronization rate (% of heifers that displayed estrus; 88% vs. 89%), conception rate (58% vs. 59%) and pregnancy rate (56% vs. 58%) did not differ between the Select Synch+CIDR and 7-day CIDR-PG treatments respectively. Pregnancy rates to a 54 hour fixed-time insemination for heifers at location 2 were similar between the Select Synch+CIDR and 7-day CIDR-PG treatments (56% vs. 53%, respectively). These results demonstrate no advantage in administering GnRH to beef heifers at the beginning of a 7-day CIDR synchronization protocol.

Key Words: Beef heifer, CIDR, Estrous synchronization.

Introduction

In the Select Synch + CIDR or 7-day CO-Synch + CIDR protocols, GnRH is routinely given at the time an intravaginal progesterone (P4)-releasing insert (**CIDR**) is administered. Seven days later $PGF_{2\alpha}$ (**PG**) is administered at CIDR removal and females are inseminated after observed estrus or at a fixed-time. Whether the use of GnRH at CIDR insertion improves pregnancy rates in beef cattle remains unclear. Richardson et al. (2002) reported higher pregnancy rates in beef heifers that received GnRH at CIDR insertion compared with those that received a CIDR only but in dairy heifers higher pregnancy rates were observed in the group that did not receive GnRH. In other studies there was no difference in conception rates or pregnancy rates between groups of beef heifers (Lamb et al., 2006; Howard et al., 2009) and suckled beef cows (Larson et al., 2006) that received or did not receive GnRH at the beginning of CIDR-based synchronization protocols. Whether ovulation in heifers occurs in response to GnRH administration has been shown to be dependent on the day of the estrous cycle when the GnRH was administered (Atkins et al., 2008).

The objective of this study was to compare heat response and fertility in beef heifers with or without GnRH administration in a 7-day CIDR synchronization protocol.

Materials and Methods

Experiment 1. Exp. 1 was conducted over 2 yr using Angus, Hereford, and Simmental purebred heifers (n = 147) at a single location. Heifers were maintained in dry lot pens and fed 3.6 kg/hd/day of sweet bran and had free access to grass hay. Heifers were assigned randomly within breed to one of two treatments (Figure 1). At d 0, heifers in the Select Synch+CIDR treatment were administered a 100-µg i.m. injection of GnRH (2 mL Cystorelin, Merial Limited, Duluth, GA) at the time of a Eazi-Breed CIDR insertion (Pfizer Animal Health, New York, NY). On d 7, a 25-mg i.m. injection of PG (5 ml Lutalyse, Pfizer Animal Health) was administered and the CIDR removed. Heifers assigned to the 7-day CIDR-PG treatment was administered a CIDR but no GnRH at d 0 followed by PG on d 7. A Estrotect Heat Detector (Rockway, Inc., Spring Valley, WI) patch was placed on the tail head of each heifer at d 7 and a vasectomized bull was placed in each pen of heifers. Heifers were observed twice daily for 108 h beginning on d 7 and artificially inseminated approximately 12 h after the onset of estrus by one of two inseminators. A total of 22 sires were used over the two years. Heifers continued to be monitored daily for approximately 40 d after the synchronization period and inseminated 12 h after the onset of estrus.

Experiment 2. Exp. 2 was conducted using Angus crossbred heifers (n = 93) that were maintained on native range. Heifers were assigned randomly to one of the same two treatments as Exp 1. Fifty-four hours after PG, each heifer was administered GnRH and was timed-inseminated by one of two inseminators using a single sire. Heifers were exposed to bulls for approximately 60 d beginning 7 d after the timed-insemination.

Ultrasonography and Blood Collection. Ovaries of all heifers in Exp. 1 and 60 heifers in Exp. 2 were

scanned by transrectal ultrasonography (Aloka 500V with a 5.0-MHz linear array probe; Aloka, Wallingford, CT) on d 0 and d 7 of treatment. Measurements of follicles > 5mm and presence of luteal tissue were recorded. Conception (# pregnant / # inseminated; experiment 1 only) and pregnancy rates (# pregnant / # synchronized) were determined by transrectal ultrasonography 30 to 35 d after insemination. Blood samples were collected via coccygeal venipuncture on d 0 and a minimum of 1 h after CIDR removal on d 7 to estimate cyclicity and treatment response. Blood was allowed to clot overnight at 4°C serum separated by centrifugation the following day. Serum was frozen at -20°C until assayed for P4 concentration by RIA. Heifers with P4 concentrations > 1 ng/ml at either d 0 or d 7 were considered to be cycling.

Statistical Analyses. Analyses were performed using the Glimmix procedure of SAS (SAS Inst., Inc., Cary, NC) for binomial data (binomial distribution; link function of logit) and adjusting the denominator degrees of freedom with the Kenward-Rodgers option. For Exp. 1, synchronization rate and proportion of heifers that were cycling were modeled with treatment, location and the treatment by location interaction as fixed effects. For conception and pregnancy rate, AI technician and sire nested within year were included as random effects. In Exp. 2, location and sire were dropped from the model. Follicle observations from both experiments were included in the same analyses. Proportion of heifers with a follicle > 8mm at d 0 and d 7, proportion of heifers with luteal tissue at d 0 and d 7, and incidence of ovulation were analyzed using the Glimmix procedure for binomial data. Size of the largest and next largest follicles at d 0 and d 7 were analyzed as continuous trait in the Glimmix procedure. Treatment, location by year, and their interaction were included as main effects. The Catmod procedure of SAS was used to analyze time of estrus response (Exp. 1) by a weighted least squares analysis of mean response with treatment, year, and their interaction as main effects.

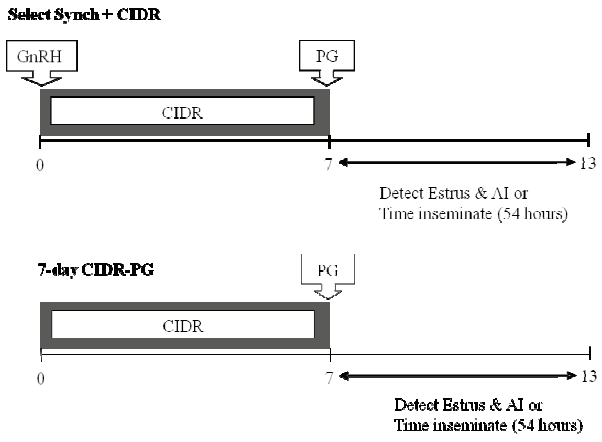

Figure 1. Schematic diagram for Exp. 1 (detect estrus & AI) and Exp. 2 (54 h timed-insemination) protocols for heifers treated with GnRH (Select Synch + CIDR) or no GnRH (7-day CIDR-PG).

Results

Experiment 1. No difference ($P = 0.43$ to 0.69) in the proportion of heifers with high P4 at either d 0 or d 7 was detected between treatment, year, or treatment by year. Synchronization rate (88%) did not differ between treatments ($P = 0.82$) but tended to differ ($P = 0.06$) between yr 1 (92%) and yr 2 (81%). Average time of estrus (Figure 2) did not differ ($P = 0.56$) between treatments but did differ ($P < 0.01$) between yr 1 (56 h) and yr 2 (66 h). Conception (58%) and pregnancy rate (56%) did not differ ($P = 0.90$ to 0.96) between yr or between the Select Synch+CIDR and 7-day CIDR-PG treatments.

Experiment 2. No difference ($P = 0.32$) was detected between treatments in the proportion of heifers (> 93%) that had high P4 at either d 0 or d 7. Pregnacy rate between the Select Synch+CIDR (56%) and 7-day CIDR-PG treatments (53%) did not differ ($P = 0.75$).

Follicle Characteristics. The proportion of heifers that had a follicle > 8 mm did not differ on d 0 or d 7 between treatment ($P = 0.42$ and 0.76 respectively) or location by year ($P = 0.60$ and 0.10 respectively). Size of the largest follicle at d 7 tended to differ ($P = 0.08$) between Select Synch+CIDR (11.2 mm) and 7-day CIDR-PG treatments (10.3 mm) but was similar at d 0 ($P = 0.58$). More ($P < 0.01$) ovulations occurred between d 0 and d 7 in the Select Synch+CIDR (29%) treatment compared with the 7-day CIDR-PG treatment (8%).

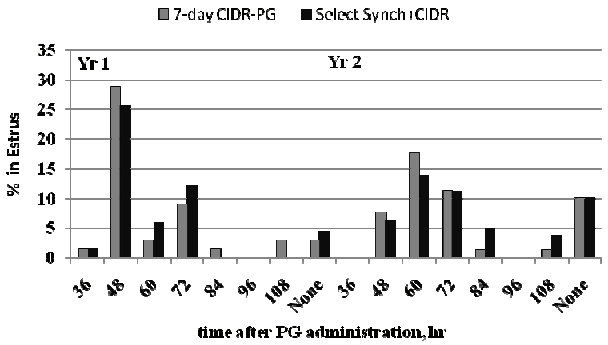

Figure 2. Distribution of estrus after PG on d 7 in Exp1. Effects of treatment ($P = 0.56$) and year ($P < 0.01$)

Discussion

No differences between treatments were observed for time of estrus, conception rate, and pregnancy rate in Exp. 1 but differences were observed between yr. Estrus was detected in fewer heifers after administration of PG and the time to estrus was longer in yr 2. The reasons for these differences are unknown. There were no differences between yr in heifers that appeared to have reached puberty based on progesterone concentrations at either d 0 and d 7 or in the proportion of follicle > 8 mm and size of the largest follicle at d 0 and d 7.

Lamb et al. (2006) also published results using the Select Synch+ CIDR protocol on beef heifers. Their study

included 12 locations and they performed a timed-insemination at 84 h on heifers not detected in estrus the previous 72 h. They reported a time of 49 h from PG administration to estrus which is similar to the time we observed in yr 1 (56 h). Overall we detected 88% of heifers in estrus in a 108 h period which was higher than 74% (range 51 to 93%) reported by Lamb et al. (2006). Their conception rate of 63% was higher than the conception rate of 58% we observed but overall pregnancy rates were similar (57% vs. 56% respectively).

More ovulations and larger follicles at d 7 were observed in the the Select Synch+CIDR treatment compared to the 7-day CIDR-PG treatment but no differences were observed in proportion of heifers detected in estrus, time to estrus after PG administration, or conception rates.

Implications

These findings suggest that there is no advantage in administering GnRH to cycling beef heifers at the beginning of a 7-day CIDR synchronization protocol.

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Table 1. Synchronization, conception and pregnancy rates for Select Synch+CIDR and 7-Day CIDR-PG treatments.

Item	Select Synch+CIDR	7-day CIDR-PG
	% (Total no.)	% (Total no.)
Synchronization Rate [a]		
Exp. 1		
Yr 1	90.9 (33)	93.9 (33)
Yr 2	82.9 (41)	80.0 (40)
Overall	87.5 (74)	88.7 (73)
Conception Rate[b]		
Exp. 1		
Yr 1	63.3 (30)	54.8 (31)
Yr 2	52.9 (34)	62.5 (32)
Overall	58.2 (64)	58.7 (63)
Pregnancy Rate[c]		
Exp. 1		
Yr 1	60.6 (33)	53.1 (33)
Yr 2	51.4 (41)	61.3 (40)
Overall	56.4 (74)	56.9 (73)
Exp. 2	56.0 (47)	52.7 (46)

[a]Total no. inseminated / total number synchronized
[b]Total no. conceived to AI / total no. inseminated
[c]Total no. conceived to AI / total no. synchronized

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EFFECTS OF REALIMENTATION AFTER NUTRIENT RESTRICTION DURING EARLY TO MID-GESTATION ON UMBILICAL BLOOD FLOW IN PREGNANT BEEF COWS

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ABSTRACT: The objective was to examine the effect of maternal realimentation after nutrient restriction during early to mid-gestation on fetal cardiovascular hemodynamics. Multiparous beef cows (30 d pregnant; initial BW = 667.5 ± 13.4 kg, BCS = 6.2 ± 0.1) were assigned to 1 of 3 treatments: 1) 100% NRC requirements from d 30 to 156 of gestation (CCC; n = 6); 2) 60% NRC from d 30 to 85, then realimented to 100% NRC to d 156 (RCC; n = 5); or 3) 60% NRC from d 30 to 140, then realimented to 100% NRC to d 156 (RRC; n = 6). Cows were individually fed once daily at 1500 h. Cows were weighed every 14 d to adjust diets throughout the experiment, and BCS were assigned to cows once a month. Umbilical measurements were obtained using Doppler ultrasonography at 0700 h on d 85, 87, 89, 91, 100, 140, 142, 146, and 148. Measurements included fetal heart rate (HR), umbilical blood flow (BF), pulsatility index (PI), and resistance index (RI). There was a treatment by day interaction for cow BW ($P < 0.01$), with cows exhibiting different patterns of weight change throughout gestation. Only day affected ($P < 0.01$) BCS, as BCS dropped to 5.5 ± 0.5 by d 156. There was no treatment by day interaction for BF, PI, and RI measurements ($P \geq 0.26$). However, there was a day effect for PI ($P < 0.01$; d 85 = 1.61 ± 0.05 mL/min, d 148 = 0.96 ± 0.03 mL/min) which decreased as gestation proceeded and a day effect for BF ($P < 0.01$; d 85 = 46.0 ± 3.6 mL/min, d 148 = 244.7 ± 21.5 mL/min) which increased as gestation proceeded. There was a tendency for a treatment by day interaction ($P = 0.09$) for HR, where fetuses from RRC cows had a greater HR ($P \leq 0.07$) than RCC and CCC fetuses on d 85. From d 87 to 140 HR did not differ ($P \geq 0.17$) for the 3 treatments. On d 142 and 144 fetuses from CCC cows had a decreased ($P \leq 0.09$) HR compared to RCC and RRC fetuses. There was no treatment effect ($P = 0.55$) for fetal biparietal distance (3.02 ± 0.09 cm) on d 85. Maternal diet restriction during early to mid-gestation did not affect umbilical blood flow; however, there was evidence to indicate that it may alter fetal cardiac output. **Key words:** nutrient restriction, pregnancy, umbilical blood flow

Introduction

Beef cows are commonly managed in grazing systems where the quality of forage varies according to the regional conditions. Forage often has a poor quality affecting nutritional and physiological status of the animal (Wu et al., 2006). Maternal nutrition during pregnancy plays an important role for fetal and placental growth and development. Previous research has demonstrated that intrauterine growth restriction is associated with altered fetal organ development and subsequent performance of offspring (Godfrey and Barker, 2000; Wu et al., 2006). During early stages of embryo development, when nutrient requirements appear trivial for conceptus growth, maternal nutrient intake has an effect on prenatal growth and development (Robinson et al., 1999). Therefore, research elucidating the effect of maternal nutrition on placental and fetal development is very important from a production and management standpoint.

The placenta plays a major role in the regulation of fetal growth. Placental nutrient transport efficiency is directly related to utero-placenta blood flow (Reynolds and Redmer, 1995). Gases, nutrients, and metabolic end products are exchanged between maternal and fetal systems via the placenta (Bleul et al., 2007; Reynolds and Redmer, 1995; Reynolds and Redmer, 2001). Previous research demonstrated that large increases in transplacental exchange, which supports the exponential increase in fetal growth during the last half of gestation, depends primarily on growth of the placenta during early gestation followed by dramatic development and reorganization of the uteroplacental vasculature during the last half of gestation (Meschia, 1983; Reynolds and Redmer, 1995).

The specific objective of this study was to examine the effect of maternal realimentation after nutrient restriction during early to mid-gestation on fetal cardiovascular hemodynamics. Therefore, we hypothesized that the duration of nutrient restriction would impact umbilical blood flow and vascular resistance. Moreover, we further hypothesized that upon realimentation, umbilical blood flow from restricted cows would ultimately surpass blood flow in control animals.

Materials and Methods

All procedures involving animals were approved by the North Dakota State University (**NDSU**) Institutional Animal Care and Use Committee.

Animals and Management. Seventeen gestating, non-lactating, multiparous beef cows of similar genetic background were transported from the NDSU Beef Research and Teaching Unit to the Animal Nutrition and Physiology Center (**ANPC**) located on NDSU campus within 3 d post-insemination. Prior to initiation of the experiment, cows were trained to use the Calan gate system that is located at ANPC. Cows were grouped with 3 to 4 head per pen. All cows were fed a common diet from

ANPC until day 30 of pregnancy, (100% of NRC recommendations; NRC, 2000). Pregnancy was confirmed on d 25 or 26 post-insemination via transrectal ultrasonography. On day 30 of pregnancy (initial BW = 667.5 ± 13.4 kg, BCS = 6.2 ± 0.1), cows were assigned randomly to one of 3 treatments: 1) 100% NRC requirements from d 30 to 156 of gestation (CCC; n = 6); 2) 60% NRC from d 30 to 85, thereafter being re-alimented to 100% NRC to d 156 (RCC; n = 5); 3) or receive 60% NRC from d 30 to 140, thereafter being re-alimented to 100% NRC to d 156 (RRC; n = 6). Cows were individually fed once daily in a Calan gate system at 1500 h. Cows were weighed every 14 d to adjust rations for changes in BW throughout the experiment and BCS were assigned to cows once per month by 4 separate evaluators. Percentage of BW change was calculated as:
[(Final BW - Initial BW) / Initial BW] x 100, where Initial BW was BW on d 30 of gestation.

Diets. Cows were fed a diet containing a mixture of grass hay (98.7% DM, 5.7% CP, 0.46% Ca, and 0.08% P) and alfalfa hay (99.0% DM, 16.2% CP, 1.33% Ca, and 0.28% P). Intake was adjusted every 2 wk for BW. Diet DMI was adjusted monthly to meet NRC requirements according to stage of gestation. Cows from the restricted group received 60% of the total requirements. Minerals (Easylix® 12-12-12 Mineral pressed block; Hubbard Feeds, Inc., Mankato, MN) were supplemented 3 times a week to meet 7 d requirements according to manufacture label (11% Ca, 13% P, 12% salt, 180,000 IU/lb vitamin A, 18,000 IU/lb vitamin D-3, 50 IU/lb vitamin E).

Ultrasonography Evaluation. Umbilical measurements were obtained via color-Doppler ultrasonography (Aloka SSD-3500; Aloka America, Wallingford, CT, USA) at 0700 h on d 85, 87, 89, 91, 100, 140, 142, 146, and 148. Measurements included fetal heart rate (**HR**), umbilical blood flow (**BF**), pulsatility index (**PI**), and resistance index (**RI**). Biparietal measurements were obtained on d 85 using B mode. Briefly, a transrectal-finger probe (~5 x 2 cm; Aloka UST-672; 5.0 MHz) was inserted into the rectum and the umbilical cord was located. In B-mode using the linear transducer, a longitudinal section of the umbilical cord was visualized by manually turning the transducer of the probe. The probe was aligned with the umbilical cord at the correct angle of insonation (i.e. ~45 degrees) and once an adequate portion of umbilical cord was identified, the sample gate cursor was placed over the umbilical artery. At least 3 similar waveforms from 3 different measurements were obtained with Spectral Doppler. Fetal HR, PI, RI, and blood flow were calculated by pre-programmed Doppler software where PI = (Peak Systolic Velocity [PSV]-End Diastolic Velocity [EDV])/Mean velocity [MnV]; RI = (PSV-EDV)/PSV; BF (mL/min) = MnV (cm/s) x (π/4) x Diameter^2 (cm^2) x 60 s.

Statistical Analysis. Body weight, % BW, BCS, PI, RI, BF, and HR were examined by repeated measures analysis and biparietal measurements analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Fixed effects included in the model were treatment, day, and the treatment by day interaction. Appropriate covariance structures were utilized for each parameter tested. When significant treatment by day interactions were detected, treatment effects were examined within day using the PDIFF option of the LSMEANS statement.

Results

There was a treatment by day interaction for cow BW ($P \leq 0.001$; Figure 1), with cows exhibiting different patterns of weight change throughout gestation. When examining percentage BW change from d 44 of pregnancy there was a tendency for a treatment effect ($P = 0.11$). Percentage BW change for CCC cows was -0.29 ± 0.85%, -1.14 ± 0.93% RCC cows, and -2.92 ± 85% for RRC cows. Moreover, there was a day effect ($P < 0.001$; data not shown) for percentage BW change where cows, regardless of treatment, had a decrease percentage BW change from the beginning of the experiment until d 128 and by d 150 cows had an increase in percentage BW change. Only day affected ($P < 0.001$; data not shown) BCS, were cows initiated the experiment with a BCS of 6.2 ± 0.1 and by d 156 dropped to 5.5 ± 0.1.

There was no treatment effect ($P = 0.55$) for fetal biparietal distance (average = 3.02 ± 0.09 cm) on d 85. There was no treatment by day interaction for umbilical BF, PI, and RI measurements ($P \geq 0.26$). However, there was a day effect for PI ($P < 0.001$) which decreased as gestation proceeded. On d 85 the PI was 1.61 ± 0.05, by d 100 PI decreased to 1.45 ± 0.04, and on d 148 PI continued decreasing to 0.96 ± 0.03. As anticipated with increasing fetal growth, a day effect for BF ($P < 0.001$) was found which increased as gestation proceeded.. Umbilical BF on d 85 was 46.0 ± 3.6 mL/min, by d 100 umbilical BF was 76.3± 7.1 mL/min, which continued increasing to d 148 where umbilical BF was 244.7 ± 21.5 mL/min. There was a tendency for a treatment by day interaction ($P = 0.09$; Figure 2) for HR, where fetuses from RRC cows had a greater HR ($P \leq 0.07$) than fetuses from RCC and CCC cows on d 85. From d 87 to 140 HR was similar ($P \geq 0.17$) for the 3 treatments. On d 142 and 144 the fetuses from CCC cows had a decreased ($P \leq 0.09$) HR compared to fetuses from RCC and RRC cows.

Discussion

We reject our hypothesis that upon realimentation, umbilical blood flow from restricted cows would exceed blood flow in control cows. Previous research in maternal nutrient restriction from early to mid-gestation in ewes (50% of NRC requirements from d 28 to 78 of pregnancy), caused a percentage weight increase of 7.4% in control ewes and a 7.5% decrease in restricted ewes. These treatments resulted in intrauterine growth restriction for restricted vs. control fed ewes (Vonnahme et al., 2003). In cows, nutrient restriction (50% of requirements) from d 30 to d 125 of gestation reduced fetal, caruncular, and cotyledonary wt compared to control cows at d 125. However, when cows where realimented fetal and caruncular wt were similar between restricted and control cows at d 245 (Zhu et al., 2006). Vonnahme et al. (2007)

demonstrated that restriction from d 30 to 125 did not affect placenta vascularity. Conversely, upon realimentation, placental vascularity was altered near term, indicating that the placenta compensated after restriction.

A paucity of information exists on the use of Doppler ultrasonography to monitor chronic umbilical blood flow in cattle, particularly during nutrient restriction. Our laboratory has previously reported (Lekatz et al., 2009) when restricting ewes to 60% of NRC requirements PI, RI, and BF were not affected. However, when percentage change was calculated, PI in restricted ewes increased throughout pregnancy, while it decreased in the control ewes. Further studies are needed to determine if the placenta can compensate for varying durations of maternal nutrient restriction by altering umbilical blood flow after a duration of restriction in the beef cow.

Implications

Maternal diet restriction during early to mid-gestation did not affect umbilical blood flow. However, tendencies for altered fetal heart rate may depict alterations in fetal cardiac output during maternal diet restriction. Additional research is needed to understand the relationship between maternal diet restriction and fetal hemodynamics. Future studies focusing on frequent umbilical blood flow ultrasonography and fetal measurements throughout gestation warrant further investigation in the beef cow.

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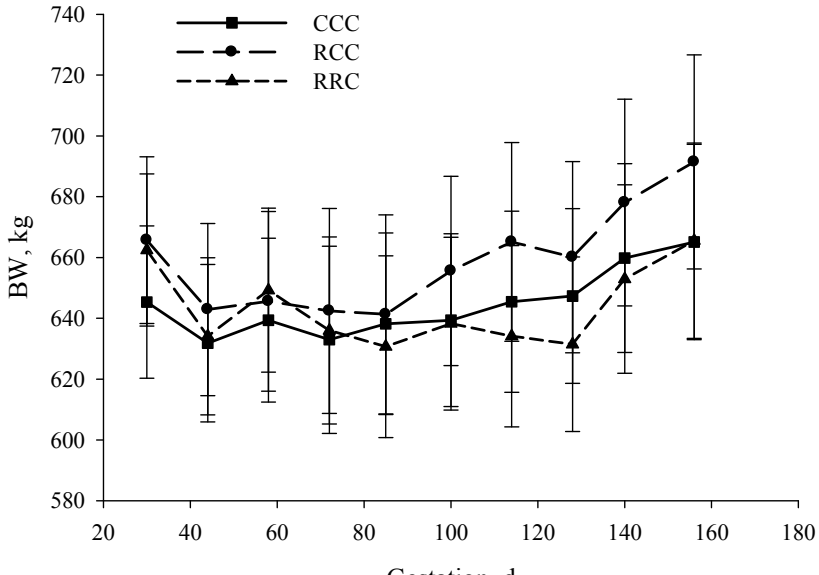

Figure 1. Cow BW (kg) every 14 d from d 30 to 156. Cows received either control diet (100% NRC; CCC), restricted from d 30 to 85 (60% NRC; RCC), or restricted from d 30 to 140 (60% NRC; RRC). Treatment × day $P \leq 0.001$

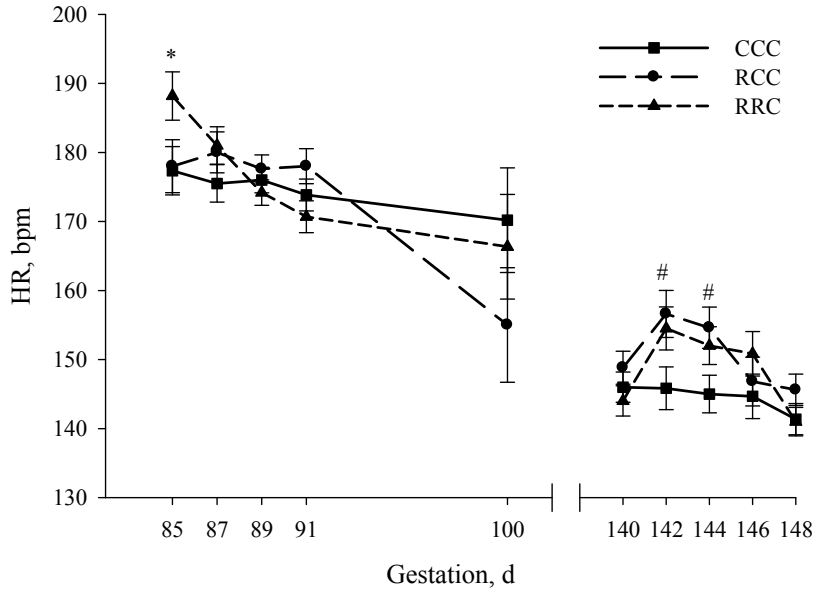

Figure 2. Fetal heart rate (HR) from cows receiving either control diet (100% NRC; CCC), restricted from d 30 to 85 (60% NRC; RCC), or restricted from d 30 to 140 (60% NRC; RRC). Treatment × day $P = 0.09$. Asterisk (*) signifies RRC greater than CCC and RCC ($P \leq 0.07$). Number sign (#) signifies CCC decreased compare to RCC and RRC ($P \leq 0.09$).

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PROGESTERONE CONCENTRATIONS AND LAMBING RATES IN EWES GIVEN HUMAN CHORIONIC GONADOTROPIN

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ABSTRACT: The objective was to determine if hCG injected on d 4 or 7 after mating would increase serum progesterone (P4) concentrations in ewes and increase number of lambs born. Sixty-two mixed aged Suffolk ewes (mean BW= 75.15 kg ± 9.36 kg) received an intravaginal P4-containing pessary (CIDR; 0.3 g P4) for 10 d. Ewes were mated with fertile rams on the second estrus after CIDR removal and were randomly assigned to one of three treatments. Ewes received 600 IU (4.8 mL) of hCG i.m. on d 4 (n= 21) or d 7 (n= 21) of the estrous cycle (d 0 = mating); control ewes (n=20) received 4.8 mL of saline on d 4. Jugular blood samples were taken from 10 ewes of each treatment group starting on d 1 and through 7 d after administration of treatments and continued twice weekly through d 34. Ewes treated with hCG on d 4 had greater ($P < 0.05$) P4 concentrations, beginning on d 6 than control ewes and remained elevated through d 14. Ewes treated on d 7 with hCG had greater ($P < 0.05$) P4 concentrations than controls beginning on d 8 and through d 14. Ovulation rates, corpora lutea (CL) counted laproscopically on the ovaries 25 d after mating, differed ($P > 0.05$) among treatments. Fifty-five and 88% of ewes given hCG on d 4 and 7, respectively, had > 2 CL; whereas, 0% of control ewes had > 2 CL. Fetal numbers ($P > 0.36$) and lambs born per ewe ($P > 0.19$) were similar among treatments. In conclusion, hCG administered to ewes on 4 or 7 d after mating elevated serum P4 concentrations through d 14, increased number of CL on d 35 but did not appear to alter fetal numbers or the incidence of multiple births in Suffolk ewes.

Keywords: corpus luteum, human chorionic gonadotropin, lamb crop, progesterone

INTRODUCTION

Lamb crop enhancement is a factor with which commercial sheep producers struggle from year to year. Estimated lambing percentage in the US in 2010 was 108% (NASS, 2010) where as lamb crop percentage in New Mexico for the same time was 67%. A number of factors, including embryonic mortality, can contribute to a decrease in lamb crop percentage. Maternal recognition and adhesion of blastocyst must occur in order for pregnancy to occur. Supplying exogenous luteotropic hormones during early pregnancy has been shown to increase progesterone (P4) concentrations with a tendency toward greater pregnancy rates (Willard et al., 2003). Human chorionic gonadotropin (hCG), which is a major embryonic signal in primates, is a glycoprotein synthesized and secreted by the trophoblast, increasing P4 concentrations during the first 6 to 8 wks of pregnancy (Srisuparp et al., 2001). Human chorionic gonadotropin has been shown to increase circulating P4 concentrations in domestic livestock and acts similarly to LH (Szmidt, et al., 2008; Lankford et al., 2010). The objective of this study was to determine the effects of hCG administered on d 4 or d 7 post mating on serum P4 concentrations and embryonic survival.

MATERIALS AND METHODS

General

All procedures involving animals were approved by the New Mexico State University Animal Care and Use Committee.

Animals and Treatment

Sixty-two mixed-aged Suffolk ewes (75.2 kg ± 9.4 kg) received an intravaginal P4-containing pessary (EAZI-BREED CIDR, 0.3 g P4; Pharmacia and Upjohn, Co., Hamilton, New Zealand) to synchronize estrus. Ten days later, CIDR were removed and ewes were sorted into 1 of 3 breeding groups. A vasectomized ram, fitted with a marking harness, was placed with each group of ewes to detect estrus. Upon removal of vasectomized rams, 5 fertile rams, with marking harnesses, were placed with ewes, which were randomly split into 5 breeding groups.. The onset of estrus, determined as the day a ewe was initially mounted by a fertile ram (d 0), was monitored and recorded daily. Ewes were randomly assigned to 1 of 3 treatment groups; 600 IU of hCG (ProSpec-Tanny TechnoGene, LTD, Rehovot, Israel, CAS: HOR-250) administered on d 4 (n =21) or d 7 (n = 21) and control (n =20). Treated ewes were injected (i.m.) with 4.8 mL of saline plus hCG whereas control ewes received 4.8 mL saline on d 4 of estrous cycle.

Blood Collection and Progesterone Assay

Beginning on d 1 blood was collected daily through 7 d after treatment, and continued twice weekly to d 34, by jugular venipuncture into serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO). Tubes were held at room temperature for a minimum of 30 min prior to being centrifuged at 4°C for 20 min at 1,500 x g. Serum was then poured into plastic vials and stored frozen until assayed.

Serum progesterone concentrations were determined using RIA (Coat-A-Count Siemens Medical Solutions Diagnostics, Los Angeles, CA; Schneider and Hallford, 1996) and were conducted by the New Mexico State University Endocrinology Laboratory. The inter- and intra-assay CV was 7.8% and 6.0%, respectively.

Laparoscopies

On d 35, ovulation rates were determined in 10 ewes from each treatment group via laparoscopy. Ovulation rate was determined by the number of CL present on the ovaries. Ewes were held off feed for 24 h prior to surgery. As a general anesthesia, 1.5 mL Ketamine (Vedco Inc., St. Joseph MO) was

administered i.v. and ewes were placed in a supine position. The surgical area was clipped and cleaned thoroughly. Ewes were then given 6 mL Lidocaine (Vedco Inc., St. Joseph, MO) s.c. as a local anesthetic, approximately 3 cm on each side of midline and 2 cm from the mammary gland. A 1-cm incision was made and a 10-mm end view scope using 300 watts Xenon Fiber-Optic Light Source (Gyrus Acmi, Southborough, MA) was inserted. The abdominal cavity was inflated using carbon dioxide gas. Corpora lutea were counted on each ovary and recorded for each of the 30 ewes. Following the procedure, 4 mL Liquamycin (Pfizer Animal Health, New York City, NY) was administered s.c. and topical antiseptic, (Nitrofurazone, Neogen Corp for Hess & Clark, Inc. Lexington, KY), was applied to the surgical site. Animals were returned to pens and closely monitored for 24 h.

Pregnancy Determination and Postpartum Measurements

Pregnancy was determined by external flank ultrasound (3.5.MHz probe; Aloka, SSD-500V, Japan) on d 67. At parturition, lambs born to a ewe were recorded, as well as gender and weight of lambs.

Statistical Analysis

All data were analyzed by SAS (SAS Inst. Inc., Cary, NC). Corpora lutea number, fetal counts, and number of lambs born per ewe were analyzed using PROC FREQ with Chi-Square. Progesterone concentrations were analyzed by PROC MIXED with repeated function. Treatment and ewe were in the whole plot and day and day by treatment were in the subplot.

RESULTS

A treatment x day interaction ($P < 0.05$) was noted for serum P4 concentrations (Figure 1). Serum P4 concentrations were similar ($P > 0.05$) among treatments through d 5. Beginning on d 6, ewes receiving hCG on d 4, had greater ($P < 0.05$) serum P4 concentrations than control and remained elevated through d 14; while beginning on d 8, ewes receiving hCG on d 7 had greater ($P < 0.05$) serum P4 concentrations than controls and remained elevated through d 14. Beginning on d 6 through d 14, administration of hCG to ewes on d 4 displayed higher ($P < 0.09$) serum P4 concentrations than ewes treated with hCG on d 7. On d 14, administration of hCG on d 4 differed from d 7 hCG administration ($P < 0.02$).

Number of CL differed ($P < 0.05$) among treatments (Table 1). For ewes lambing, those receiving hCG on d 4, 55% had ≥ 3 CL; 88% of ewes receiving hCG on d 7 had ≥ 3 CL, whereas none of control ewes had ≥ 3 CL. The trend was similar for all ewes treated. The percentages of ewes carrying multiple fetuses were similar among treatments ($P > 0.36$) on d 67 and were 64, 35, and 67, for ewes receiving hCG on d 4, d 7, and control, respectively (Table 2). In addition, 78% of ewes receiving hCG on d 4 gave birth to multiples ($P > 0.19$), with 41% of ewes receiving hCG on d 7, compared to 66% control ewes.

DISCUSSION

Data from this study supports the hypothesis that hCG administration to ewes during early pregnancy can increase serum P4 concentrations, as shown by increased serum P4 in ewes injected with hCG. The response of treatment shows to be dependent on the day of hCG administration, with ewes treated with hCG on d 4 having a higher serum P4 concentrations than ewes treated with hCG on d 7 (Figure 1). A likely cause of increased serum P4 is the development of small and large luteal cells. In the ewe an increase in CL size and weight is due to an increase in the volume of large luteal cells and number of small luteal cells (Senger, 2003). Treatment of hCG induced a change in the cellular composition of the CL (Kelly et al., 1988). Small luteal cells will differentiate into large luteal cells increasing serum P4 with administration of hCG (Farin et al., 1988).

Administration of hCG to ewes at different times of the estrous cycle can enhance endogenous P4 production as shown within our laboratory (Redden et al., 2006; Yates et al, 2009; Lankford et al; 2010). The current study concurs with the P4 findings in Lankford et al (2010), whereas Yates et al. (2009) found that serum P4 differs starting on d 9. A difference in dosage and use of multiple injections between studies should not be overlooked. The current study used 600 IU hCG, whereas Yates et al (2009) used 100 IU and Lankford et al (2010) used 200 IU. The increased dosage may explain the difference in response of serum P4 concentrations.

Ewes that received hCG did not show an increase in fetal numbers as reported by Lankford et al. (2010). Ewes in the current study were previously used in by Lankford et al (2010). Therefore one possible explanation can be that ewes used in the previous studies may have developed antibodies against hCG. Duchamp et al. (1987) reported that multiple injections of hCG, this phenomenon can occur in mares and Draincourt et al., (1990) observed a similar response in ewes. Gamboni et al. (1984) reported that a 500 IU hCG injection 7 d after induced ovulation had no effect on pregnancy rates in anestrous ewes. Even when exogenous progesterone was administered within 1 wk after mating, it either had no effect (Diskin and Niswender, 1989) or decreased (Hecker et al., 1974) pregnancy rates in sheep. Conceptuses recovered from hCG-treated ewes were longer and had a higher interferon tau, which is the primary mediator of maternal recognition of pregnancy (Nephew et al., 1994) but did not show a difference in number of lambs born.

Implications

Administration of human chorionic gonadotropin to ewes on d 4 or d 7 after mating increased serum P4 concentrations and number of CL, but did not increase fetal numbers. The likelihood of sheep building immunity to hCG should be further investigated along with alternatives for increasing pregnancy rates in sheep.

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Table 1. Percent of ewes with ≥ 3 corpora lutea (CL) after administration of 600 IU human chorionic gonadotropin (hCG) on d 4 or 7 post-mating[1].

	Treatment[2]		
Item	Control	hCG d4	hCG d7
% of ewes treated	10	60	77
% of ewes lambing	0	55	88

[1]Estrus was synchronized using intravaginal progesterone containing pessary (CIDR, 0.3 g P4) for 10 d and were mated with fertile rams on the second estrus after CIDR removal. Ewes were randomly assigned to one of three treatments, hCG on d 4, d 7 or control. Chi-Square (P <0.05).

[2]Treatments consisted of 600 IU (4.8 mL) hCG i.m. on d 4 of estrus or d 7 of estrus. Control ewes received 4.8 mL saline i.m. on d 4 of estrus.

Table 2. Fetal numbers (shown in percent of ewes) in response to administration of 600 IU human chorionic gonadotropin (hCG) on d 4 or d 7 post-mating[1].

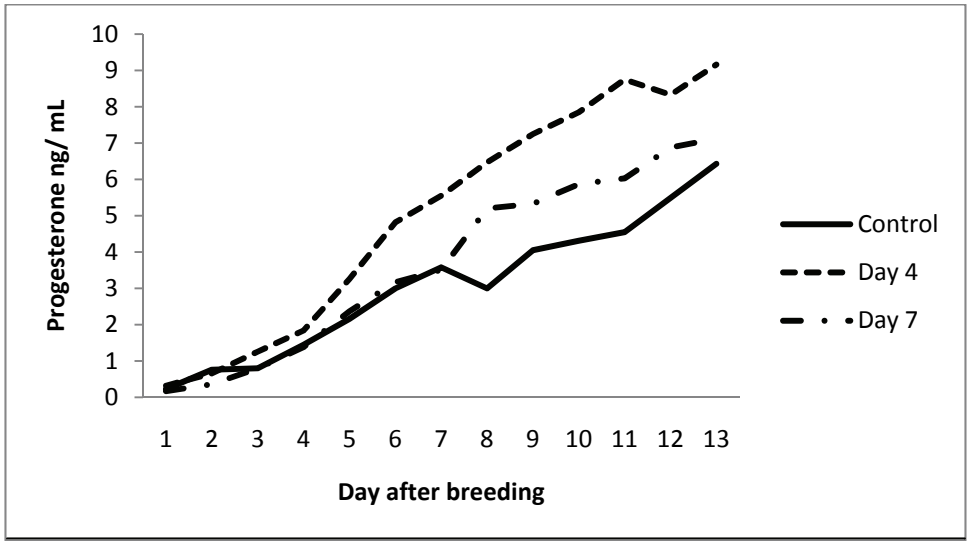
	Treatment[2]		
Fetal numbers [3]	Control	hCG d 4	hCG d 7
0	0	0	6
1	33	35	58
2	66	64	35

[1]Estrus was synchronized using intravaginal progesterone containing pessary (CIDR, 0.3 g P4) for 10 d and were mated with fertile rams on the second estrus after CIDR removal. Ewes were randomly assigned to one of three treatments, hCG on d 4, d 7 or control. Chi-Square (P < 0.36).

[2]Treatments consisted of 600 IU (4.8 mL) hCG i.m. on d 4 of estrus or d 7 of estrus. Control ewes received 4.8 mL saline i.m. on d 4 of estrous.

[3]Fetal numbers were determined via external flank ultrasound on d 67 post breeding.

Figure 1. Average serum progesterone concentrations in response to administration of 600 IU human chorionic gonadotropin (hCG) on d 4 or d 7 post-mating.



Estrus was synchronized using intravaginal P4 containing pessary (CIDR, 0.3 g P4) for 10 d and were mated with fertile rams. Data was analyzed as a split plot. A day by treatment interaction was distinguished ($P > 0.05$). Therefore, data were analyzed within d. Progesterone concentrations were similar ($P > 0.05$) through d 5. Beginning on d 6 through d 14, ewes treated with hCG on d 4 were different ($P < 0.05$) than ewes treated with hCG on d 7. Ewes treated with hCG on d 4 or d 7 had elevated ($P < 0.05$) serum P4 concentrations through d 14 compared to control ewes. On d 14, administration of hCG on d 4 differed from d 7 hCG administration ($P < 0.02$).

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ESTRUS SYNCHRONIZATION IN SHEEP USING GONADOTROPIN-RELEASING HORMONE, PROSTAGLANDIN, AND CONTROLLED INTERNAL DRUG RELEASE INSERTS

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ABSTRACT: The objective of this experiment was to evaluate the effects of combinations of controlled internal drug release (**CIDR**) inserts, prostaglandin (**PG**), and GnRH on days to estrus and concentrations of progesterone ($\mathbf{P_4}$) in Columbia and Hampshire ewes (n = 38 and 47, respectively). Treatment and period were randomly assigned to ewes during the anestrous transition period (August). Ewes were assigned to 1 of 3 periods 1 week apart, then assigned to 1 of 4 treatments; 1) Untreated (**U**, n = 21); 2) CIDR (0.3 g progesterone) inserts for 5 d (**C**, n = 22); 3) 5 d CIDR and PG (dinoprost, 10 mg i.m.) at CIDR removal (**P**, n = 21); and 4) GnRH (gonadorelin, 0.02 mg i.m.) at CIDR insertion and PG at CIDR removal (**G**, n = 21). Rams equipped with marking harnesses were introduced at CIDR removal and ewes were checked at 0800 h and 1700 h daily for marks. Blood samples were collected via jugular venipuncture on d -7, 0, 5, 7, 9, 11, 13, 15, and 17 relative to CIDR insertion (d 0). Serum was analyzed for progesterone concentration in the Hampshire ewes. There was a treatment by breed interaction ($P < 0.05$) for days to estrus. Days to detected estrus were greater ($P < 0.01$) for U (14.6 ± 2.42) than C (5.7 ± 1.77), P (5.6 ± 1.85), and G (2.90 ± 2.02) ewes within the Hampshire breed. In contrast, no differences were detected ($P = 0.22$) among treatments for days to detected estrus within the Columbia breed. There was a treatment by time effect ($P < 0.005$); for P_4 concentrations; therefore, means were compared within time. No differences ($P > 0.05$) were detected among treatments on d -7, 0, 13, 15, or 17. On d 5, C, P, and G treated ewes had greater ($P < 0.005$) concentrations of P_4 than U ewes. Ewe P_4 concentrations were lower ($P < 0.05$) in G than U ewes on d 7, 9, and 11; whereas P and C treated ewes did not differ ($P > 0.05$) from U or G. In conclusion, efficacy of CIDR based estrus synchronization techniques varied depending upon breed. Furthermore, in Hampshire ewes, those treated with GnRH at insertion and PG at removal appeared to most consistently synchronize estrus among 5 d CIDR treatment groups.

Keywords: Ewe, Estrus Synchronization, CIDR

Introduction

Great variability of hormone levels and time of ovulation occurs in females; hence efficacy in regards to various estrus synchronization protocols has been challenging to achieve (as reviewed by Dutt, 1953). Several strategies have included: providing exogenous progesterone ($\mathbf{P_4}$) to extend the luteal phase or the administration of prostaglandin (**PG**) to shorten the phase by causing regression of the corpus luteum (as reviewed by Wildeus, 2000). In addition, seasonally anestrous ewes can be stimulated into estrus after exposure to synthetic progestins and/or rams. Effective ewe synchronization protocols could provide a more continuous supply of lamb and condense lambing season(s) to reduce labor, feed, and facilities (Carlson et al., 1989).

Current industry practice is to use the controlled internal drug release (**CIDR**) insert for 12 – 14 d followed by a gonadotropin for estrus synchronization during the estrus period, especially when used for advanced reproductive techniques. Furthermore, 5 d CIDR treatments in association with FSH, 24 h before CIDR removal, are equally effective compared with a 12 d CIDR (Knights et al., 2001) and was sufficient to induce cyclicity in anestrous ewes (Knights et al., 2000). In 2009, CIDR inserts were approved for use in sheep by the FDA and labeled for a 5 d period to induce estrus in seasonally anestrous ewes. Moreover, when the CIDR was used in combination with PG, a greater percent of ewes were observed in estrus and a greater lambing rate to the first service period was achieved (Dixon et al., 2006). Additionally, GnRH given prior to 5 d treatment of exogenous progesterone and PG has been shown to be an effective synchronization protocol and has improved prolificacy (Titi et al., 2010). The objective of this study was to evaluate the effects of 5 d CIDR inserts, GnRH, and PG on estrus synchronization, pregnancy rates, and prolificacy.

Materials and Methods

The North Dakota State University Institutional Animal Care and Use Committee approved all procedures involving animals.

Animals and Treatments. In August 2010, we conducted a study at North Dakota State University Sheep Unit to examine synchronization treatments applied to ewes prior to fall seasonal estrus cyclicity. Eighty five Columbia (n = 38) and Hampshire (n = 47) ewes were randomly assigned to treatment and period. Three periods, 1 wk apart, were used to control for effect of season. One wk prior to treatment application, ewes were moved to paddocks that had not been previously grazed for one month. Once treatments began, the ewes were moved to a drylot and fed alfalfa hay (3 kg/ewe) and a 14% CP concentrate ration (1 kg/ewe) daily.

Ewe estrus synchronization treatments were: untreated (**U**, n = 21), CIDR insert (EAZI-BREED CIDR, 0.3 g progesterone, Pfizer Animal Health) for 5 d (**C,** n =

22), 5 d CIDR and PG (Lutalyse, Pfizer Animal Health, 10 mg i.m.) at CIDR removal (**P**, n = 21), and GnRH (Cystorelin, Merial, 0.02 mg i.m.) given at CIDR insertion (d 0) and PG at removal (**G**, n = 21). Rams equipped with marking harnesses were introduced to ewes at CIDR removal. Ewes were checked daily for breeding marks (detected estrus) at 0800 h and 1700 h. Ewes remained with rams for at least 60 d.

Blood Collection and Analysis. To evaluate P_4 concentration levels, blood samples were collected via jugular venipuncture into 10 mL serum tubes (BD Vacutainer Serum, Becton, Dickinson and Company) and were immediately placed on ice. Samples were centrifuged at 4˚C for 30 min at 1,500 x g and serum was collected in plastic 2.0 mL microcentrifuge tubes and frozen until assayed. Blood samples were drawn 1 wk prior to treatment initiation (d -7) and at d 0 to obtain baseline levels of P_4 concentrations. Further samples were collected at CIDR removal (d 5) and every other day thereafter until d 17. Serum was analyzed using hormonal chemiluminescence technology (IMMULITE, Siemens, Los Angeles, CA) for P_4 concentrations. Low, medium, and high P_4 pools within each assay were run in duplicate. The intra- and inter-assay CVs were 7.0 and 7.3%, respectively. A progesterone concentration greater than 2 ng/mL was interpreted to indicate ovarian luteal activity.

Statistical Analysis. Time to estrus and days to lambing were examined using the General Linear Model procedure of SAS (SAS Inst. Inc., Cary, NC). Models included effects of treatment, breed, period, and treatment × breed for all production data. When treatment × breed interactions were detected ($P < 0.05$) means were separated within breed. Pregnancy and lambing rates were analyzed with the Chi-Square procedures of SAS. Repeated measures of the Mixed procedure of SAS were used to analyze P_4 concentration levels. The model included the main effects of treatment, period, and time. Data are presented as least squares means with differences considered significant at $P \leq 0.05$.

Results

Days to estrus and lambing. Treatment × breed interactions were detected for d to estrus post ram introduction ($P < 0.05$; Table 1); therefore, effects of treatment were identified by breed. Within the Hampshire breed, d to detected estrus were greater ($P < 0.01$) for U treated ewes than C, P, and G treated ewes. In contrast, no differences were detected ($P = 0.22$) within the Columbia breed among treatments for d to detected estrus. No treatment × breed interaction was detected for d to lambing. Greater d to lambing ($P < 0.04$; Table 1) were observed in the U treated ewes when compared with P and G treated ewes while C treated ewes were intermediate.

Pregnancy and lambing rates and prolificacy. Pregnancy, lambing, and prolificacy rates for synchronized estrus and overall breeding season are presented in Table 1. No treatment × breed interactions were detected for pregnancy rates, lambing rates, or prolificacy ($P > 0.05$). Pregnancy rate to the first service was lower ($P < 0.01$) in U treated ewes compared with C, P, and G ewes. Overall pregnancy rate did not differ ($P = 0.83$) among treatment groups. Lambing rate to the first service was greater ($P < 0.01$) in C, P, and G ewes compared with U treated ewes. No differences ($P = 0.67$) in overall lambing rate were observed among treatments. Prolificacy to the first service and overall did not differ among ewes ($P = 0.67$ and $P = 0.36$, respectively).

Progesterone Concentration Levels. Treatment × time interactions were detected ($P < 0.005$; Figure 1) for ewe P_4 concentrations; therefore, treatment means were compared within time. On d -7, 0, 13, 15, and 17 no differences ($P > 0.05$) were detected in P_4 concentration. Ewe P_4 concentrations were greater ($P < 0.005$) on d 5 for C, P, and G treated ewes than U ewes. On d 7, 9, and 11, ewe P_4 concentrations were lower ($P < 0.05$) in G than U ewes; whereas P and C treated ewes did not differ ($P > 0.05$) from U or G.

Discussion

A majority of P_4 concentrations were less than 0.2 ng/mL on d -7 and 0 among all treatments indicating females were still in seasonal anestrous. The ram effect has been shown to be effective in bringing ewes from an anestrous state into a synchronized estrus period after an isolation period greater than two weeks. Following exposure to rams, ewes typically exhibit a silent heat. This is a period in which the ewe is not receptive to breeding; however a fertile estrus will generally occur 10 – 14 d later (as reviewed by Martin et al., 1986). The ram effect was prevalent in U treated ewes; fore it appeared U treated ewes had a steady increase in P_4 levels which decreased abruptly 10 d after CIDR removal.

Untreated ewes required more time to exhibit estrus, become pregnant, and lamb than all CIDR treatments. Similarly, Knights et al. (2000) reported that anestrous ewes treated with a CIDR had a greater pregnancy rate to the first service (53% and 45%, respectively) than untreated ewes. Similar to Knights et al. (2000), prolificacy was not different among groups.

As expected, all CIDR treatments had higher P_4 levels compared to U treated ewes at CIDR removal. Decreased P_4 levels were seen in G treated ewes on d 7, 9, and 11 compared with U treated ewes. However, no differences were detected for C and P treated ewes on d 7, 9, and 11. This data suggests that G treatment was more effective than C and P to increase the number of ewes in estrus shortly after CIDR removal. Consequently, d to estrus was numerically lowest among all Hampshire treated ewes. However, difference in response to estrus synchronization between breeds warrants further investigation.

Implications

These findings imply that efficacy of CIDR based estrus synchronization techniques vary depending upon breed. Furthermore, Hampshire ewes treated with GnRH at CIDR insertion and PG at CIDR removal appeared to be more consistently synchronized for estrus among 5 d CIDR treatment groups. Lastly, ewes treated with a progestagen

source had greater pregnancy and lambing rates to the first service period compared with untreated ewes; yet overall lambing rate and prolificacy were unaffected.

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Table 1. Reproductive performance of synchronized ewes to estrus after 5 d synchronization treatment protocols

	Treatment[1]					
Variable	U	C	P	G	SEM	*P*-value
d to Estrus, Columbia[2]	7.5	3.1	6.1	5.8	1.53	0.22
d to Estrus, Hampshire[2]	14.6[a]	5.7[b]	5.6[b]	2.9[b]	2.42	0.001
d to Lambing[3]	166[a]	162[ab]	158[b]	156[b]	2.56	0.04
Pregnancy rate, %						
First service period[4]	0[a]	42[b]	63[b]	63[b]	11	0.001
Overall	87	79	89	82	10	0.83
Lambing rate[5]						
First service period	0.00[a]	0.68[b]	1.00[b]	0.88[b]	.20	0.003
Overall	1.53	1.37	1.37	1.18	0.21	0.67
Prolificacy[6]						
First service period	-	1.63	1.58	1.40	0.21	0.67
Overall	1.77	1.73	1.53	1.43	0.16	0.36

[ab]Values within the same row with different superscripts differ ($P < 0.05$).

[1]Treatments were (U) untreated, (C) CIDR inserted on d 0 and removed on d 5, (P) 5 d CIDR and PG given at removal, (G) GnRH given on d 0, 5 d CIDR, and PG given at removal with ram exposure starting after the 5 d protocol.

[2]Days to estrus post ram introduction; n ranges from 7 to 13.

[3]Days to lambing post ram introduction; n ranges from 13 to 17.

[4]Detection of estrus within 7 d of ram introduction and/or lambing to synchronized estrus; n ranges from 15 to 19.

[5]Lambs born per ewe exposed to rams; n ranges from 15 to 19.

[6]Lambs born per ewe lambing; n ranges from 8 to 17.

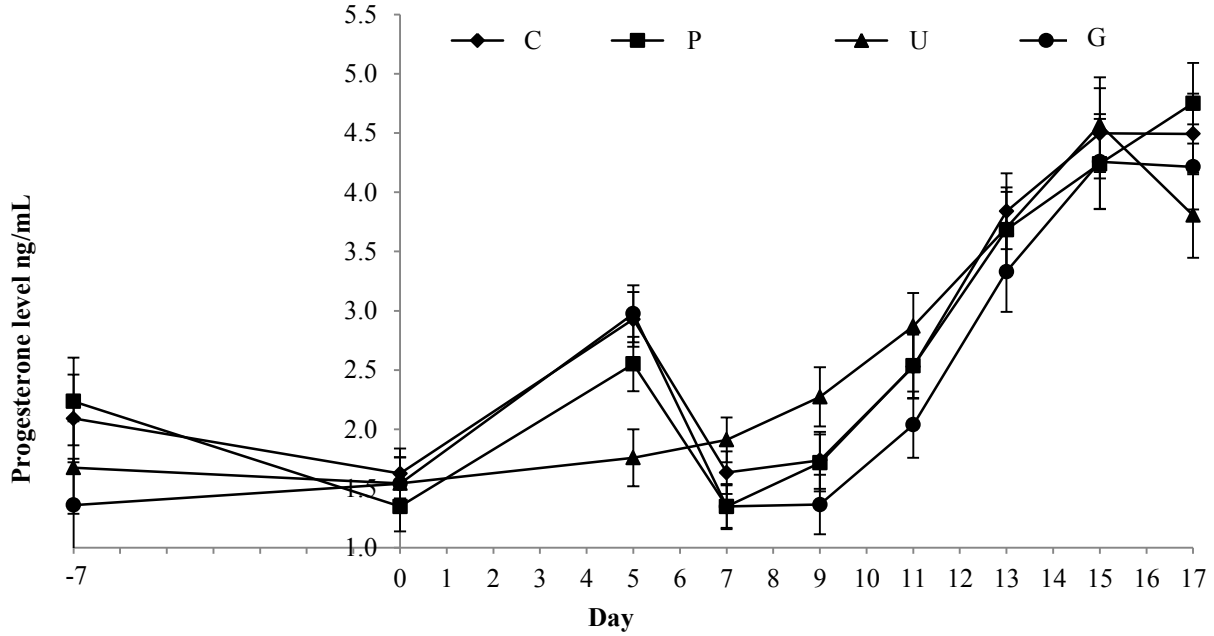

Figure 1. Concentrations of progesterone in Hampshire ewes before and after 5 d synchronization treatment protocols: (U) untreated, (C) CIDR inserted on d 0 and removed on d 5, (P) 5 d CIDR and PG given at removal, (G) GnRH given on d 0, 5 d CIDR, and PG given at removal with ram exposure starting after the 5 d protocol.

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THE ROLE OF RUMEN-PROTECTED METHIONINE ON AMINO ACID METABOLISM IN LATE GESTATION BEEF HEIFERS IN THE NORTHERN GREAT PLAINS

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ABSTRACT: This study evaluated changes in plasma amino acids in late-gestating (beginning 58 ± 1.02 d prior to calving), primiparous, winter-grazing range heifers receiving a wheat middling based supplement without (**CON**) or with rumen-protected methionine (**MET**). Plasma was collected on d -2 and d 0 (start of MET supplementation just prior to individually receiving supplement at 0700 h). Plasma was sampled again on d 40, 42 and 44 prior to supplementation at 0700 h and 1100 h (4 h after receiving daily supplement). Data were analyzed with cow as the experimental unit. Continuous variables were analyzed by the main effects of treatment, date, or time and their interaction when appropriate. Comparable BW (P = 0.74) and BCS (P = 0.65) over the 44-d metabolism trial were found between both CON- and MET-fed heifers. MET-supplemented heifers had greater (P < 0.0001) plasma concentrations of methionine indicating that the rumen-protected technology successfully presented methionine to the small intestine for absorption. Notable decreases in plasma AA concentrations in MET-fed heifers after 44 d included leucine (P = 0.04), valine (P = 0.03), and serine (P = 0.05). Glycine, the most abundant amino acid in maternal blood, was greater (P = 0.05) in CON-fed heifers than MET-fed heifers. Lower glycine concentrations in MET heifers were due to the role of glycine in catabolism of methionine. Branched chain amino acids (**BCAA**) are instrumental in metabolism of maternal protein and there were several significant differences in BCAA between CON and MET fed heifers. Isoleucine, leucine and valine resulted in notable decreasing percent changes from d 0 to 44 (P = 0.06, P = 0.04, P = 0.03, respectively) for MET-fed heifers. These results imply methionine is a limiting amino acid in late-gestating heifers grazing dormant range forages due to improved utilization of other amino acids when supplemental methionine was provided.

Key Words: Amino acids, primiparous heifers, rumen-protected methionine

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Introduction

Late gestating cattle grazing rangelands in the northern Great Plains often receive crude protein supplementation in order to reduce nitrogen limitations on ruminal fermentation function. Protein supplementation efficiency may be improved if specific limiting amino acids are provided post ruminally. Research indicates that the first limiting AA is most likely methionine **(Met),** especially when rumen microbial protein is the primary source of AA to the small intestine (Richardson and Hatfield, 1978; Rulquin and Delaby, 1997; Greenwood and Titgemeyer, 2000). Forage and supplementary proteins are generally degraded extensively in the rumen, incorporated into microbial protein, and delivered to the small intestine as metabolizable protein. Waterman et al., (2007) showed that pregnant beef cows exhibited improved nitrogen retention when consuming a mature forage based diet with added urea to minimize N deficiency in the rumen while infusing DL-Met directly into the abomasum.

The objectives of this field metabolism study were to primarily determine if plasma AA concentrations were altered due to inclusion of rumen-protected, DL-Met when supplemented to range 21 month old heifers in late gestation. The second objective was to determine if inclusion of methionine reflected improved protein accretion resulting in a more desirable body weight change. By supplementing rumen-protected, DL-Met, we hypothesize that heifers will improve utilization of other non-limiting or less limiting AA.

Materials and Methods

Study Location and Environment

Research was conducted at Fort Keogh Livestock and Range Research Laboratory (LARRL) about 1.6 km West of Miles City, MT (46°22'N 105°5'W). The LARRL encompasses 22,500-ha and has an average elevation of 730 m. Figure 1 illustrates average weekly precipitation and temperature patterns for the period which the study was implemented. The high temperature reached 0.56°C, and the low temperature dropped to -22.78°C. Predominant grass genera at the study site includes grama (*Bouteloua*), needlegrass (*Hesperostipa*), and wheatgrass (*Pascopyron*) within a mixed-grass dominated rangeland

(Küchler, 1964). The average annual forage standing crop at the study site is 870 ± 14 kg/ha (Grings et al., 2005). Animals were stocked at a rate of 14.9 AUD/ha such that only 16% of the available forage would be utilized. Quantity of mature forage availability during the experimental period was in excess of cattle needs, and the 75.9 ha pasture grazed by experimental heifers had not previously been grazed during the previous growing season.

Experimental Design

The LARRL Institutional Animal Care and Use Committee approved the procedures and use of animals for this study. The trial consisted of a 44 d period from d 13 through 57 in the year 2010. Twenty-four late gestating heifers (BW; 418 ± 6.48 kg) of predominantly Angus (≥75%) breeding were used to evaluate the effects of rumen-protected, DL-Met.

Prior to the trial, all heifers were grouped as a single herd and grazed native range. Heifers had been previously AI and diagnosed pregnant via rectal palpation at approximately d 75 of gestation. As heifers approached the final third of gestation, 24 heifers were stratified by BW and 3 AI sires before being randomly assigned to treatment groups (12 per experimental treatment). Experimental treatments were individually fed daily to provide (Table 1): (1) 26.3% CP of wheat mid based supplement (**CON**; 300 g/d) or (2) 26.3% CP of a wheat mid based supplement (276.5 g/d) with 23.5 g/d of rumen-protected, DL-Met **(MET**; Mepron M85, Evonik-Degussa Corp., Kennesaw, GA). The DL-Met supplement used in this study is reported to be between 33% (Koenig and Rode, 2001) and 60% (Berthiaume et al., 2001) available to the small intestine. Therefore, 23.5 g/d of DL-Met provided in the supplement supplied approximately 6.5 to 12 g/d of absorbable Met to the small intestine. This amount of DL-Met targeted was selected based upon research findings by Waterman et al., (2007) that observed a linear response for N retention when concentrations of DL-Met were infused into the abomasum up to 15 g/d.

Heifers received treatments for 44 consecutive days during the final third of gestation beginning approximately 58 ± 1.02 d before parturition. Heifers were gathered daily at approximately 1000 h, sorted into a hearing bone shaped individual feeding stalls where dietary treatments were offered. Heifers consumed their daily feed allotment and were promptly released. After feeding, all heifers were managed as a single herd and were allowed to graze dormant winter rangeland. Due to snow cover restricting available forage, a 500 kg (approximately 18 kg/hd) round bale of grass hay (91.5% DM, 11.9% CP, 64.0% TDN) was fed on d 27 of the study and was offered free choice twice weekly until the end of the study (Table 2).

Heifer BW and BCS was measured and recorded on and off trial. Body condition scores (1 = emaciated to 9 = extremely obese) were assigned by 2 experienced technicians as described by Herd and Sprott (1986) and Wagner et al. (1988). On d 44, heifers were relocated to dry lots and separated according to treatment group. Supplement was offered by means of top-dressing over a corn silage-based diet from d 45 to 54 (Table 3). Experimental groups remained separated through calving. At calving, calf BW was measured, and cow calf pairs were moved into a pair pen where they remained until being released onto native rangeland.

Plasma Amino acids

On d (-2) and d 0, initial plasma and serum samples were collected from all heifers. Samples were taken prior to feeding (t = 0) at approximately 0900 h.

On d 40, 42 and 44, final plasma samples were collected from each heifer prior to supplementation. During all collection dates, plasma samples were obtained via coccygeal venipuncture. Upon collection, plasma [7-mL tubes with EDTA for plasma (Corvac, Sherwood Medical, St. Louis, MO)] samples were allowed to coagulate at 20 °C, and samples were centrifuged at 1,500 x g for 30-min, decanted, and stored at -20ºC until analysis.

Plasma samples were composited according to collection date and sent to a commercial laboratory for further analysis of AA (Evonik-Degussa Corp., Kennesaw, GA). Amino acids were analyzed using a Biochrom 30 Amino Acid Analyzer (Biochrom Ltd, Cambridge, UK). Determination of AA used procedures previously reported (Llames and Fontaine, 1994; Fontaine et al., 1998; Fontaine and Eudaimon, 2000). The first composite consisted of plasma from d (-2) and d 0 at t = 0. Secondly, a composite plasma sample was made from samples collected on d 40, 42, and 44 just prior to offering of supplements. Composite samples were made by combining 1 mL of plasma per heifer for each specified time period.

Statistical Analysis

Data were analyzed as a completely randomized design using MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with heifer as the experimental unit. Continuous variables were analyzed by the main effects of treatment, date, and their interaction when appropriate. Covariates used in the model were birth weight and weaning weight. These values are important biological variables that indicate metabolic size. They are related to AA partitioning and potential utilization. One heifer was removed from the MET group after the trial was complete because she failed to have a calf. Therefore, data from this heifer was not included in any of the statistical analysis. Results were considered significant if $P \leq 0.05$.

Results and Discussion

Performance

Heifer BW ($P \geq 0.39$) and BCS ($P \geq 0.48$) were similar throughout the 44-d supplemental period (Table 1). No differences ($P = 0.66$) were observed for calf birth weight between treatments (35.9 and 35.1 ± 1.24 kg, respectively for CON and MET treated heifers).

Plasma Amino Acids

Supplementation with rumen-protected Met caused a significant elevation in plasma concentration of Met ($P < 0.0001$) indicating that the bypass technology successfully presented Met to the small intestine for absorption (Table 2). When adequate energy is available, protein deposition is linearly related to the supply of the most limiting AA until another factor becomes more limiting (Titgemeyer and Loest, 2001) Differences in on-trial and off-trial plasma AA concentrations imply that protein was being absorbed.

Since methionine supply limitations were lifted with supplementation, other AA could be used more efficiently to build protein-rich fetal tissues. McNeill et al., (1997) using traditional N balance and comparative slaughter techniques and estimated the utilization of apparently digested CP in ditocous ewes that were fed to required energy and protein for late-gestation, (calculations from Bell and Ehrhardt, (1998)) to be 80% of apparently digested CP can be used in the gravid uterus. The remainder is used to support metabolism and net deposition of AA in the developing mammary glands and visceral organs.

Since no differences were found for calf birth weights ($P = 0.66$) it cannot be determined how the additional Met was partitioned. Placental growth is most rapid during mid gestation (Ehrhardt and Bell, 1995). Moderate undernutrition of ewes during early to mid pregnancy has caused conflicting positive (Faichney and White, 1987; McCrabb et al., 1992) and negative (McCrabb et al., 1992); (Clarke et al., 1998) effects on placental size. This reported variation can be explained partially by dam body condition. Fatter ewes responded to undernutrition by increasing placental size, whereas the opposite occurred in lean ewes (McCrabb et al., 1992) These results suggest that if maternal energy stores are unavailable, the dam will use a majority of dietary energy and AA for placental growth. However, no placental data was collected in the present study.

The greatest plasma AA concentration increase was Met ($P < 0.0001$). The accumulation of Met can be partially explained by the use of DL form of methionine. Although both isomers are absorbed through the small intestine at the same rate, Campbell et al., (1996) concluded that D-Met may be metabolized more slowly than the naturally occurring L-Met. In this instance, D-Met will accumulate (due to slow metabolism) and can result in less efficient utilization (Campbell et al., 1997)

Notable AA that decreased in concentration from on-trial to off-trial include leucine ($P = 0.04$), valine ($P = 0.03$), and serine ($P = 0.05$). On the other hand, glycine increased in concentration in both the CON and MET treated heifers ($P = 0.05$; Table 9). Glycine is the most abundant AA in maternal blood (Bell and Ehrhardt, 2000). Excess AA from the diet, in this case Met, must be catabolized. Glycine (Gly) is required for catabolism of excess Met. Even small increases in Met can reduce the Gly supply during gestation (Rees et al., 2006). As depicted in Table 2, Gly is found at a lower concentration in the MET treatment group since MET heifers have a greater supply of Met to breakdown. The placenta converts serine, mostly taken up from maternal blood, to Gly (Chung et al., 1998). This explains the decrease in Ser concentrations in the MET treatment group compared to the CON heifers.

With Met supplementation, utilization of nearly all other AA should increase until another factor becomes limiting. There were significant differences in percent change. Branched chain AA, Ile, Leu, and Val, posted notable changes ($P= 0.06$, $P = 0.04$, $P = 0.03$, respectively). In summary, addition of methionine to supplements allowed for better utilization of branch chain AA. It is not determined if BCAA were utilized by the placenta, fetus, or maternal tissues.

Acknowledgement

Authors greatly appreciate Dr. R. Payne (Evonik-Degussa Corp., Kennesaw, GA) for donation of the rumen protected DL-methionine and analysis of plasma amino acids. Furthermore, Authors greatly appreciate W. Kelly, L. Voigt, M. Woods, D. Armstrong, T. Johnson, and A. Mason for the technical and animal handling assistance.

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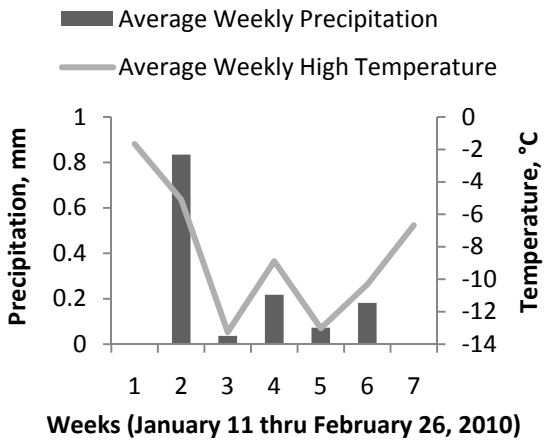

Figure 1. Average weekly precipitation (bars) and daily high temperature (line) from January 11 thru February 26 (wk 1 to 7), 2010 for Miles City, MT. Information obtained from Western Regional Climate Center (WRCC, 2010)

Table1. Least square mean ± SEM for BW and BCS at the beginning and end of a 44-d supplementation period during late gestation.

	Treatment*			
Item	CON	MET	SEM	P - value
Heifers(n=)	12	12	--	--
On test BW, kg	417	423	6.5	0.54
On test BCS	4.6	4.6	0.14	0.89
Off test BW, kg	418	425	5.7	0.39
Off test BCS	4.4	4.3	0.11	0.48

*Experimental treatments fed individually daily to provide: 1) 26.3% CP of wheat mid based supplement (**CON**; 300 g/d) and 2) 26.3% CP of a wheat mid based supplement (276.5 g/d) with 23.5 g/d of rumen-protected, DL-Met **(MET**; Mepron M85, Degussa Hüls Corp., Allendale, NJ 07401).

Table 2. Least square mean ± SEM for plasma AA at the beginning and end of a 44-d supplementation period during late gestation.

	On Test*				Off test*				% Change*			
Amino Acid, mM	CON	MET	SEM	*P*- value	CON	MET	SEM	*P*- value	CON	MET	SEM	*P*- value
Heifer (n=)	12	12	--	--	12	12	--	--	12	12	--	--
Met	18.5	17.6	1.04	0.5517	16.6	29.0	1.72	<0.0001	-0.07	0.67	0.08	<0.0001
Lys	103.1	102.7	5.00	0.9618	103.5	97.5	3.65	0.2553	0.04	-0.05	0.05	0.2156
Thr	84.0	84.1	4.44	0.8734	60.1	52.4	4.62	0.2531	-0.29	-0.39	0.04	0.0878
Arg	51.9	55.9	3.16	0.3824	55.0	52.9	3.19	0.6500	0.09	-0.05	0.06	0.1487
Ile	105.6	108.0	3.50	0.6361	103.8	96.4	3.79	0.1836	-0.01	-0.12	0.04	0.0625
Leu	116.9	121.6	3.70	0.3856	124.5	114.9	4.46	0.1415	0.08	-0.07	0.04	0.0396
Val	195.9	201.1	6.10	0.5542	186.8	170.3	6.08	0.0692	-0.04	-0.16	0.03	0.0250
His	46.6	46.4	2.08	0.9674	47.0	43.7	1.57	0.1535	0.02	-0.06	0.03	0.0945
Phe	50.3	48.7	1.58	0.4789	50.1	47.4	1.59	0.2396	0.00	-0.03	0.03	0.3914
Tyr	46.3	45.47	2.00	0.7891	42.1	39.5	1.06	0.0939	-0.07	-0.14	0.03	0.2030
Gly	300.4	311.6	14.47	0.5910	381.8	360.5	17.72	0.4052	0.27	0.17	0.03	0.0405
Ser	55.2	58.0	2.53	0.4327	53.8	50.7	2.92	0.4689	-0.01	-0.13	0.04	0.0495
Ala	224.5	207.7	7.95	0.1498	217.7	210.0	8.28	0.5178	-0.02	0.02	0.03	0.3712
Pro	97.7	96.1	3.87	0.7752	96.7	96.3	3.30	0.9371	-0.01	0.01	0.04	0.7733
Asp	10.2	10.5	0.52	0.6253	10.2	9.1	0.98	0.4252	0.05	-0.10	0.10	0.3271
Glu	68.1	65.3	3.30	0.5558	60.7	54.5	3.04	0.1654	-0.01	-0.14	0.04	0.5549
Asn	20.9	19.8	1.80	0.6641	21.5	20.6	1.11	0.5504	0.07	0.09	0.09	0.8737
Aln	224.4	228.8	9.07	0.7321	229.3	219.1	8.11	0.3866	0.02	-0.03	0.03	0.1720
Essential	819.1	833	26.3	0.7205	789.5	743.9	24.3	0.1986	-0.03	-0.11	0.03	0.0782
Non-Essential	1,001	998	32.7	0.9401	1,071	1,020	31.6	0.2681	0.07	0.03	0.02	0.2400
Glucogenic	1,600	1,606	45.9	0.9313	1,633	1,552	46.2	0.2311	0.02	-0.03	0.02	0.1100
Ketogenic	422.2	426.4	14.26	0.8338	424.0	395.6	13.0	0.1375	0.02	-0.08	0.04	0.0970
Glucogenic and Ketogenic	202.2	202.2	6.39	0.9986	196.0	183.3	5.61	0.1239	-0.02	-0.10	0.03	0.1112

*Experimental treatments fed individually daily to provide: 1) 26.3% CP of wheat mid based supplement (**CON**; 300 g/d) and 2) 26.3% CP of a wheat mid based supplement (276.5 g/d) with 23.5 g/d of rumen-protected, DL-Met **(MET**; Mepron M85, Degussa Hüls Corp., Allendale, NJ 07401).

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METHANE EMISSIONS FROM CATTLE DIFFERING IN FEED INTAKE AND FEED EFFICIENCY FED A HIGH CONCENTRATE DIET

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ABSTRACT: Methane gas released by cattle is a product of fermentation of feed in the digestive tract and represents a loss of feed energy. In addition to being a dietary energy loss, methane is considered a greenhouse gas. Developing strategies to reduce methane emissions from cattle have the potential to increase production efficiency as well as reduce the impact of cattle on the environment. We hypothesized that steers with a higher feed efficiency would have a lower methane production. One hundred thirteen steers were fed a dry-rolled corn-based ration to determine feed intake and growth over a 64-d period. Steers had ad libitum feed access (82.75% corn, 12.75% corn silage, and 4.5% Biegerts, Bradshaw, NE (contains 0.066% monensin)), and feed intake was calculated as the sum of feed intake over the 64-d period. Steers for methane emissions measurement were selected by regressing gain on feed intake and selecting the 40 steers that were outside the 60% elliptical confidence interval. Thirty-seven of the 40 selected steers were chosen to measure methane emissions based on their temperaments. Five d following the collection of growth and feed intake data, methane emissions were determined over a two-wk period using indirect calorimeters (headboxes) to determine gas exchange. A six-hr sampling period was chosen to mitigate the potential reduction in feed intake during sample collection. Collection began at 0800 when fresh feed was offered. Steers had ad libitum access to feed and water during gas collection. Residual feed intake was determined as the residuals resulting from the regression of feed intake on ADG, and middle metabolic body size ($R^2 = 0.70$). Steers that were evaluated had a methane emission of 2.6 ± 0.1 g/h, a BW of 573 ± 12 kg, a DMI of 696 ± 17 kg/64 d, a gain of 104 ± 3 kg/64 d, and a residual feed intake of -0.1 ± 0.1 kg. Methane emissions did not differ with DMI ($P = 0.52$) or gain ($P = 0.26$). Methane emissions did not differ with residual feed intake ($P = 0.88$). Our findings do not support that variation in methane emissions from cattle fed high-corn diets containing monensin can be explained by DMI and gain.
Key Words: Cattle, Efficiency, Methane

Introduction

Methane gas released by cattle is a product of fermentation of feed in the digestive tract. In our previous research, we have shown that 3% of intake energy consumed by steers fed a high-corn diet is lost as methane energy (Archibeque *et al.,* 2007). In addition to dietary energy loss, methane is considered a greenhouse gas. Developing strategies to reduce methane emissions from cattle have the

potential to increase production efficiency as well as reducing the impact of cattle on the environment. Hegarty *et al.* (2007) found reduced methane emissions from steers that have a low residual feed intake. Zhou *et al*. (2010) determined that cattle that differ in feed efficiency also differ in prevalence of methanogenic rumen species which may be a possible mechanism for the reduced methane emissions. Numerous factors contribute to the relative methane emissions amongst groups of cattle. Cattle eating a high forage diet typically release a greater percentage of their dietary energy as methane and cattle with a lower feed intake emit less methane (Flatt *et al*., 1965; Reynolds *et al.*, 1991; Freetly and Nienaber, 1998). This experiment is designed to determine the contribution of methane toward the inefficiency of feed utilization for growth in steers fed a high-corn diet differing in ad libitum feed consumption.

Materials and Methods

The experiment was conducted to conform to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999) and was approved by the U.S. Meat Animal Research Center Animal Care and Use Committee. One hundred thirteen fall-born crossbred steers were evaluated for feed efficiency. When the study began, steers were 355 ± 1 d of age and weighed 456 ± 10 kg when they were began on study. Feed intake was measured for a 64-d period using an Insentec Roughage Intake Control Feeding System (Insentec B.V., Marknesse, The Netherlands). Steers had ad libitum feed access (82.75% corn, 12.75% corn silage, and 4.5% Biegerts supplement, Bradshaw, NE (contains 0.066% monensin)), and feed intake was calculated as the sum of feed intake over the 64-d period. Body weights were measured on d 0, 1, 22, 43, 63, and 64 of the feeding period. Individual BW gain was calculated by regressing BW on day on study and subtracting the intercept from predicted BW at 64 d.

Residual feed intake was determined as the residuals resulting from the regression of feed intake on ADG and average metabolic body size ($R^2 = 0.70$); where metabolic body size was equal to BW at 32 d as estimated with individual animal regressions raised to 0.75 power and ADG was the slope of the regression.

Steers for methane emissions measurement were selected by regressing gain on feed intake and selecting the 40 steers that were outside the 60% elliptical confidence interval. Thirty-seven of the 40 selected steers were chosen to measure methane emissions based on their temperaments.

Five d following the collection of growth and feed intake data, methane emissions were determined over a two-wk period using indirect calorimeters (headboxes) to determine gas exchange over a six-hr sample period. A six-hr sampling period was chosen to mitigate the potential reduction in feed intake during sample collection. Collection began at 0800 when fresh feed was offered. Steers had ad libitum access to feed and water during gas collection. Gas samples were analyzed for methane as described by Nienaber and Maddy (1985).

The effect of gain and DMI during the growth evaluation period on methane emissions were tested using regression analysis where gain and DMI were considered to be continuous effects. The effect of residual feed intake on methane emissions was tested in a separated model by regression analysis where residual feed intake was considered to be a continuous effect.

Results and Discussion

Steers that were evaluated for methane emissions had a methane emission of 2.6 ± 0.1 g/h, a BW of 573 ± 12 kg, a DMI of 696 ± 17 kg/64 d, a gain of 104 ± 3 kg/64 d, and a residual feed intake of -0.1 ± 0.1 kg.

Hegarty et al. (2007) reported that cattle selected for low residual feed intake have a reduced daily methane production. Their findings suggest that there is potential to select against methane production in cattle; however in that study, cattle selected for a low residual feed intake also had a reduced feed intake. The methane produced as a fraction of DM intake did not differ between low and high selected lines. The reduction in methane production may have been a function of intake level rather than a selection against methane production. We did not observe a difference in methane produced as a fraction of DM intake with changes in RFI (Figure 1).

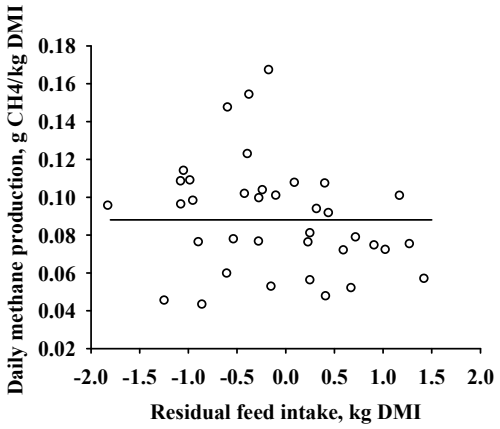

Figure 1. Relationship between daily methane production expressed as a ratio of daily dry matter intake and residual feed intake (RFI). f(RFI) = -0.0089 ± 0.006(RFI)+0.088 ± 0.005; $R^2 = 0.06$, $P = 0.16$

We observed a positive correlation between DMI and RFI in steers that were selected for methane measurement ($R^2 = 0.30$; $P < 0.001$); however, we did not observe a relationship between methane emissions and RFI ($P = 0.88$) or DMI ($P = 0.13$).

Neither gain ($P = 0.26$) nor DMI ($P = 0.52$) during the 64-d feeding trial accounted for variation in methane emissions when they were included in the same model as covariates.

In the current study, the ratio of energy lost in methane accounted for 2.3 ± 0.1% of the DE consumed which is lower than observed in our previous studies with steers fed high dry-rolled corn diets (~4.5%; Archibeque, 2007). The two studies differ from each other in that monensin was fed in the current study. Monensin is an inhibitor of rumen methane emissions (Thornton and Owens, 1981; Odongo *et al*., 2007).

Our findings do not support that variation in methane emissions as a fraction of dry matter intake in cattle fed high-corn diets containing monensin are associated with differences in residual feed intake. In this experimental model, differences in feed efficiency are most likely associated with mechanisms other than variation in methane emissions.

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EFFECTS OF FIELD PEAS FED WITH DISTILLERS GRAINS WITH SOLUBLES AND DRY-ROLLED CORN ON FINISHING PERFORMANCE AND CARCASS TRAITS OF FEEDLOT CATTLE

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ABSTRACT: A finishing study was conducted to evaluate feeding field peas in dry-rolled corn-based diets with or without wet distillers grains with solubles (**WDGS**). Crossbred steers (n = 352, initial BW 356 ± 27 kg) were used in a RCBD using a 2 × 2 factorial treatment structure. Cattle were stratified by BW and assigned within strata to 32 pens and fed for 140 or 159 d. Pens were assigned randomly to one of four treatments (8 pens/treatment) with 11 steers/pen. Factors consisted of 0 or 20% dry, whole field peas and either 0 or 30% WDGS. Diets also contained 7.5% alfalfa hay and 6% supplement. There was a small (3 kg) difference in initial BW for the main effect of peas (P = 0.04), therefore initial BW was used as a covariate in the model. There was an interaction for DMI (P < 0.01), in which WDGS had no effect (P = 0.07) in diets with no peas, but increased DMI by 1.2 kg in diets containing peas (P < 0.01). Peas decreased DMI by 0.6 kg in diets with no WDGS (P < 0.01), but had no effect (P = 0.10) on DMI in diets containing WDGS. Feeding WDGS increased ADG by 0.3 kg/d (P < 0.01), while peas had no effect on ADG (P = 0.33). A peas × WDGS interaction was also observed for G:F (P < 0.01), with WDGS increasing G:F by 12% in diets without peas (P < 0.01), but having no impact (P = 0.12) in diets containing peas. Feeding peas increased G:F (P = 0.04) in diets with no WDGS, but decreased G:F (P = 0.03) in the presence of WDGS. Feeding WDGS increased final BW and HCW by 41 kg and 27 kg, respectively (P < 0.01). Dressing percentage, 12th rib fat thickness, and calculated yield grade were greater for cattle fed WDGS (P < 0.01), but LM area was not different (P = 0.99). A peas by WDGS interaction (P = 0.01) was observed for marbling score, as WDGS resulted in a numerical decrease in marbling score in cattle fed no peas, but increased marbling score in the presence of peas (P = 0.04). The impact of WDGS on G:F was diminished in the presence of peas from 40 to 24% improvement relative to corn. However, the effect of WDGS on ADG was similar with or without peas.

Keywords: distillers grains, feedlot, field peas

Introduction

Field pea production is increasing in the Northern Plains (NASS, 2009). The portion of the crop that does not meet quality standards for human consumption can be priced competitively enough to be utilized as a livestock feed. Previous research has focused on increasing inclusion of field peas in corn-based diets in which field pea inclusion has resulted in either no impact (Lardy et al., 2009 and Jenkins et al., 2011), or an increase (Flatt and Stanton, 2000) in G:F. To date, no research has evaluated the impact of combining field peas with grain milling co-products in finishing diets, even though the vast majority of cattle on feed (Vasconcelos, 2007) are being fed diets that take advantage of the availability and relatively high feeding value of distillers grains (Klopfenstein, 2008). Thus, the objective of this study was to determine the effects of feeding field peas as a partial replacement for corn in diets that contain WDGS.

Materials and Methods

Animals and Treatments. Procedures were approved by the University of Nebraska's Institutional Animal Care and Use Committee. Three hundred fifty-two crossbred steers (356 ± 27 kg initial BW) were received from multiple sources and used in a RCBD experiment at the UNL Panhandle Research and Extension Center Feedlot located near Scottsbluff, Nebraska. Animals were limit fed for 5 days and then weighed and blocked by BW into 4 blocks, stratified by BW within block, and assigned randomly to treatment within block. Treatments were assigned randomly to 32 open soil-surface pens, with 8 pens per treatment and 11 steers per pen. Treatments were arranged in a 2 × 2 factorial arrangement with one factor being presence or absence of 20% whole grain field peas and the other being presence or absence of 30% wet distillers grains plus solubles (**WDGS**) (DM basis, Table 1). Field Peas and WDGS replaced dry-rolled corn in the diets.

Sample Analysis. Weekly feed ingredient samples were collected, composited, and analyzed for nutrient composition. The nutrient composition (DM basis) of the field peas used in this study was: 89.6% DM, 23.4% CP, 14.0% NDF, 1.2% crude fat, 49.7% starch, and 0.24% sulfur. The WDGS used in this study were (DM basis): 33.1% DM, 30.9% CP, 37.4% NDF, 10.9% crude fat, and 0.52% sulfur.

Statistics. Animal performance and carcass data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC) as a randomized complete block design with pen as the experimental unit. The model included the effects of block, peas, WDGS, and peas × WDGS. There was a small (3 kg) significant difference in initial BW for the main effect of peas, so initial BW was used as a covariate in the model. Differences were considered significant at P < 0.05.

Results and Discussion

Performance. A significant peas × WDGS interaction (P < 0.01; Table 2) was observed for DMI, in which WDGS had no effect (P = 0.07) in diets with no

peas, but increased DMI by 1.2 kg in diets containing peas ($P < 0.01$). Peas decreased DMI by 0.6 kg in diets with no WDGS ($P < 0.01$), but had no effect ($P = 0.10$) on DMI in diets containing WDGS. The impact of field pea inclusion on DMI in finishing diets has been mixed. In agreement with the current study, decreases in DMI due to pea inclusion have been observed by Lardy (2009), when peas replaced a combination of dry-rolled corn, high-moisture corn, and canola meal, and by Flatt and Stanton (2000), when peas replaced whole corn. Loe (2004) in lamb finishing diets, Lardy (2009) in both dry-rolled corn and barley based diets, and Jenkins (2011) in dry-rolled corn diets, each saw no change in DMI due to pea inclusion. Finally, Fendrick (2005) saw an increase in DMI at up to 40% inclusion of peas, but then a decrease at 59% of dietary DM replacing dry-rolled corn, and Anderson (1999) saw an increase in DMI when peas replaced dry-rolled barley. Similar to previous field pea research (Lardy et al., 2009; Jenkins at al., 2011), feeding peas had no effect on ADG ($P = 0.33$). As expected, WDGS improved ADG ($P < 0.01$), which is a common observation (Klopfenstein, 2008). A significant peas × WDGS interaction ($P < 0.01$) was observed for G:F, with WDGS increasing G:F by 12% in diets without peas ($P < 0.01$), but having no impact ($P = 0.12$) in diets containing peas. Feeding peas increased G:F ($P = 0.04$) in diets with no WDGS, as observed by Flatt and Stanton (2000), but decreased G:F ($P = 0.03$) in the presence of WDGS. However, more often, there has been no effect of peas on G:F observed (Anderson, 1999; Loe et al., 2004; Fendrick et al., 2005; Lardy et al., 2009; Jenkins et al., 2011). The reason for the interaction between peas and WDGS on G:F in this study is not clear.

Carcass Characteristics. A significant peas × WDGS interaction ($P = 0.01$) was observed for marbling score, as feeding WDGS decreased marbling score when peas were not included in the diet, but increased marbling score in the presence of peas. However, the magnitude of these differences was relatively small, with cattle in all treatments averaging USDA Choice quality grade. Field pea inclusion had no effect ($P > 0.30$) on carcass characteristics. There was a significant main effect of WDGS ($P < 0.01$) for final BW, HCW, dressing percent, 12th rib fat depth, and calculated yield grade. These results agree with previous work (Klopfenstein et al., 2008), in which cattle fed WDGS gained more rapidly, and thus were fatter at equal days on feed.

Implications

Field peas can be utilized as a replacement for a portion of the corn in finishing diets. Inclusion of 20% field peas increased gain efficiency by 4% in corn-based diets. Even though the positive impact of WDGS on gain efficiency is apparently diminished in the presence of 20% field peas, performance was acceptable when 50% corn is replaced with peas and WDGS.

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Table 1. Diet and nutrient composition of treatments.

	0 Peas		20 Peas	
Item	0 WDGS	30 WDGS	0 WDGS	30 WDGS
Ingredient, % DM				
DRC	86.5	56.5	66.5	36.5
Field Peas	-	-	20.0	20.0
WDGS	-	30.0	-	30.0
Alfalfa Hay	7.5	7.5	7.5	7.5
Urea	1.1	-	0.4	-
Supplement[1]	4.9	6.0	5.6	6.0
Analyzed Composition, %				
CP	11.5	15.2	12.6	18.2
NDF	10.7	19.7	12.0	21.0
Crude Fat	2.8	5.1	2.4	4.7

[1] Supplement included: 500 g/907 kg DM monensin and 130 g/907 kg DM tylosin.

Table 2. Growth performance and carcass characteristics.

	0 Peas		20 Peas			*P*-values		
Item	0 WDGS	30 WDGS	0 WDGS	30 WDGS	SEM	Peas	WDGS	Peas × WDGS
Growth Performance								
Initial BW, kg	358	357	355	355	2.1	0.04	0.77	0.48
Final BW, kg	635	677	632	672	8.1	0.32	<0.01	0.83
DMI, kg/d	11.3[b]	11.6[b,c]	10.7[a]	11.9[c]	0.28	0.30	<0.01	0.001
ADG, kg	1.87	2.15	1.85	2.12	0.054	0.33	<0.01	0.82
G:F	0.165[a]	0.185[c]	0.172[b]	0.177[bc]	0.002	0.96	<0.01	0.003
Carcass Characteristics								
HCW, kg	400	427	398	424	5.1	0.33	<0.01	0.80
Dress yield, %	62.4	63.5	62.2	63.5	0.002	0.60	<0.01	0.52
LM area, cm^2	85.3	85.6	84.9	84.6	0.76	0.37	0.99	0.66
12^{th}-rib fat, cm	1.52	1.65	1.52	1.70	0.009	0.40	<0.01	0.25
Calc. yield grade	3.54	3.86	3.51	3.95	0.049	0.54	<0.01	0.24
Marbling Score[1]	595[a]	576[a,b]	563[b]	588[a]	8.7	0.30	0.72	0.01

[a,b,c] Means in a row with different superscripts are different ($P < 0.05$).
[1]Marbling Score: 500 = $Small^{00}$, 600 = $Modest^{00}$.

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SUPPLEMENTAL BRANCHED-CHAIN AMINO ACIDS IMPROVE PERFORMANCE AND IMMUNE RESPONSE OF NEWLY-RECEIVED FEEDLOT CALVES[1]

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ABSTRACT: Supplemental branched-chain AA (BCAA) improved N balance of steers during a simulated pathogen challenge. The objective of this study was to determine the effect of supplemental BCAA on growth and health of newly-received feedlot steers. Steers (n = 120; initial BW = 376 ± 5 kg) were blocked by BW and assigned to 12 pens and 2 treatments in a randomized complete block design. Treatments were no supplemental AA (CON) or rumen-protected BCAA, which was top-dressed to a receiving diet that was fed for 28 d after initial processing (d 0). On d 0 and 14, steers were vaccinated against ovalbumin (OVA). On d 0, 14, 28, and 56, blood samples and BW were collected. Morbidity was recorded throughout the experiment. No BCAA × day interactions ($P \geq 0.29$) were detected for serum anti-OVA IgG, insulin, white blood cell count, or plasma Ile, Leu, or Val. Serum anti-OVA IgG was greater ($P = 0.02$) for BCAA vs. CON steers. Serum insulin and plasma Ile, Leu, and Val concentrations were not different between treatments ($P \geq 0.30$). White blood cell count was not different ($P = 0.56$) between treatments, but differential proportion of neutrophils among total white blood cells was greater ($P = 0.04$) for BCAA than CON. From d 0 to 14, DMI, ADG, and G:F were not different between treatments ($P \geq 0.44$). From d 15 to 28, DMI was less ($P = 0.03$), and ADG and G:F tended to be greater ($P \leq 0.11$) for steers fed BCAA than CON. From d 29 to 56, DMI was not different ($P = 0.50$), and ADG and G:F were greater ($P < 0.05$) for BCAA than CON steers. From d 57 to finish, DMI, ADG, and G:F of steers were not different between treatments ($P \geq 0.60$). Overall, DMI and ADG were not different ($P \geq 0.25$), and G:F was greater ($P = 0.05$) for steers supplemented with BCAA compared with CON. Morbidity was not different ($P = 0.99$). Transportation and translocation stresses predispose newly-received feedlot calves to infection, which may increase the metabolic demand for BCAA. Our results demonstrate that BCAA supplementation may improve the adaptive immune response and efficiency of gain in feedlot receiving steers.

Key words: branched-chain AA, feedlot, steer

INTRODUCTION

The feedlot receiving period, where cattle transition from ranch to feedlot, involves transportation, comingling, and handling stress, and increases the calf's susceptibility to pathogens. Waggoner et al. (2007) observed that steers treated for disease in the feedlot tended to have lower carcass prices and lighter carcasses than untreated steers, which resulted in up to a 20% decrease in gross value at slaughter.

The immune system is comprised of a variety of proteins, including acute-phase proteins, immune cells and their products (pro-inflammatory cytokines and antibodies). In a review, Calder (2006) indicated that immune challenge shifts nutrient partitioning from growth to the immune response, increasing tissue protein catabolism to supply AA for the immune response. Waggoner et al. (2009) demonstrated that increasing dietary CP (16 vs. 14.5%) decreased N loss in steers during a simulated immune challenge with lipopolysacharride. We theorized that supplementing specific AA needed by the immune system would have similar effects to increasing dietary CP.

Branched-chain AA (**BCAA**; Ile, Leu, Val) improved N retention in cancer and surgical patients (Choudry et al., 2006). Additionally, Löest et al. (2001) demonstrated that BCAA are limiting in low RUP diets for growing steers. Furthermore, Gilliam et al. (2008) demonstrated that calves supplemented with BCAA during a lipopolysacharride challenge exhibited less N loss compared with calves receiving no supplemental BCAA. Based on this evidence, we hypothesize that supplementing BCAA during times of stress will improve subsequent performance and enhance immunocompetence. Therefore, the objective of this study was to determine the effect of supplemental BCAA on growth and health of newly-received feedlot steers.

MATERIALS AND METHODS

Experimental Design and Treatments

All procedures were approved by the Institutional Animal Care and Use Committees of New Mexico State University and University of Arizona (IACUC No. 2007-013 and 08-021, respectively). Yearling Brangus cross steers (n = 120; 376 ± 5 kg initial BW) of ranch origin were housed in partially shaded soil-surfaced pens (6 × 23 m) at the University of Arizona's research feedlot. The experiment was a randomized complete block design and pen (n = 12) was the experimental unit. Upon arrival, steers

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were housed in open lots with *ad libitum* access to water and were fed chopped alfalfa hay in feed bunks. Two days after arrival (d 0), all steers were individually weighed, assigned an individual identification tag, and separated into 2 blocks based on BW (light = < 386 kg, heavy = ≥ 386 kg). Steers within a block were randomly assigned to pens by order of processing, and pens were randomly assigned to treatment within each block (8 pens/light block, 4 pens/heavy block). Treatments were no supplemental AA (**CON**) or rumen-protected BCAA top-dressed to a receiving diet (Table 1) that was fed for 28 d after initial processing (d 0). Feed bunks were managed to near *ad libitum* intake using a slick-bunk management system. All pens were fed and managed the same after d 28. Beginning on d 30, steers were transitioned to a finishing diet (Table 1) over 2 wk.

Vaccination and Management of Animals

Each steer was vaccinated (Pyramid 10, Fort Dodge Animal Health, Overland Park, KS; Vision 7 with SPUR, Intervet, Millsboro, DE), treated for internal and external parasites (Cydectin, Fort Dodge Animal Health, Overland Park, KS), and implanted with a growth promoting implant (Revalor S, Intervet, Millsboro, DE) on d 0. Additionally, each steer was inoculated with ovalbumin (**OVA**) by subcutaneous injection on d 0 and 14. The inoculation consisted of 2 mL of a 1:1 solution of commercially prepared aluminum hydroxide adjuvant solution (Anhydrogel, Cat. No. A1090 S; Acurate Chemical Corp., Westbury, NY) and sterile saline containing suspended OVA (Cat. No. A5503; Sigma-Aldrich, St.Louis, MO). This vaccine, which contained 6 mg OVA per mL, was prepared by first dissolving the OVA in sterile saline with gentle stirring to avoid foaming and then adding the adjuvant. The vaccine was stored in sterile bottles and refrigerated (4°C) until used.

Cattle were observed daily for sickness. Cattle with disease symptoms (lethargy, nasal and ocular discharge, labored breathing) were treated with florfenicol (Nuflor; Intervet, Millsboro, DE) for bovine respiratory disease syndrome. Penicillin G (Agricillin, Agrilabs, St. Joseph, MO) was used for treatment of inflammation related to mechanical injury of the penis of cattle with long sheaths.

Collections

Steers were individually weighed upon initial processing (d 0), and on d 14, 28, and 56. Before transport to the abattoir, final BW were taken on a pen-weight basis. Before each BW measurement, any residual feed in feed bunks was collected, weighed, and a sample was saved for DM analysis. Samples of diet were collected weekly, composited, and analyzed by a commercial laboratory (SDK labs, Hutchinson, KS) for DM, CP, ADF, NDF, and mineral concentration.

Blood samples were collected from the jugular vein on d 0, 14, 28, and 56. Whole blood samples in vacuum tubes (Vacutainer K_2EDTA, BD Diagnostics, Franklin Lakes, NJ) from d 0, 14, and 28 were shipped on ice to the New Mexico Veterinary Diagnostic Lab for analysis of total and differential white blood cell (**WBC**) counts. Additional blood samples in vacuum tubes (Corvac serum separator and Monoject Sodium Heparin, Kendall, Ontario, CA), were centrifuged (Beckman Coulter, Brea, CA) at 1,100 × g for 20 min at 5°C to separate serum and plasma from other blood components, decanted into 7 mL polypropylene storage vials (Thermo Fisher, Waltham, MA), and stored at -80°C until analysis. Serum samples were analyzed for OVA-specific antibody concentration using enzyme-linked immunoassay adapted from the procedure described by Rivera et al. (2002). For our procedure, 5 dilutions of each sample were used and each dilution was analyzed once. Data from a 1:800 dilution were used for statistical analysis. Pooled serum samples from all calves on d 14, diluted 1:500 in PBS (Sigma-Aldrich, St. Louis, MO), was used as a positive control. Within and between plate CV for OVA analysis were ≤ 7% and 16%, respectively. Serum samples were also analyzed for insulin using radioimmunoassay as described by Reimers et al. (1982); within and between assay CV were 7 and 11%, respectively. Plasma samples were analyzed for free AA using gas chromatography as described by Gilliam et al (2008); within assay CV was less than 2%.

Statistical Analysis

Data were analyzed using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). Pen was the experimental unit. The model of each response variable included treatment as a fixed effect with block as a random effect. Blood metabolites were analyzed using the same model with the addition of a repeated measures statement with pen (day) being the subject. For insulin, anti-OVA IgG, Ile, and Val, an auto regressive covariance structure was used, and for Leu, total WBC, and differential WBC counts, a compound symmetry covariance structure was used. Significance level was $P \leq 0.05$.

RESULTS

No BCAA × day interactions ($P \geq 0.29$) were detected for serum anti-OVA IgG and insulin, plasma Ile, Leu, and Val, or WBC and differential counts from whole blood. Serum insulin, and plasma Ile, Leu, and Val were not different ($P \geq 0.30$) between treatments (Table 2). Serum OVA-specific antibody concentrations were greater ($P = 0.02$) in BCAA-supplemented steers than CON steers (Table 2). Total WBC were not different ($P = 0.56$) between treatments (Table 2). The proportion of neutrophils out of total WBC was greater ($P = 0.04$) for BCAA steers vs. CON steers. The proportions of lymphocytes, monocytes, eosinophils and basophils were not different ($P \geq 0.23$) between treatments. Morbidity was not different ($P = 0.99$; data not shown), and only 1 steer that received BCAA supplementation was treated once for bovine respiratory syndrome. However, 15 steers with excessive sheath (*bos indicus* influence) were precautionary treated with penicillin for penile inflammation and swelling (data not analyzed statistically).

From d 0 to 14, DMI, ADG, and G:F were not different ($P \geq 0.44$) between treatments (Table 3). From d 15 to 28, DMI was less ($P = 0.03$), and ADG and G:F tended to be greater ($P \leq 0.11$) for steers fed BCAA than CON. From d 29 to 56, DMI was not different ($P = 0.50$), and ADG and G:F were greater ($P < 0.05$) for BCAA than CON steers. From d 57 to finish, DMI, ADG, and G:F of steers were not different between treatments ($P \geq 0.60$). Overall, DMI and ADG were not different ($P \geq 0.25$), and G:F was greater ($P = 0.05$) for steers supplemented with BCAA compared with CON.

DISCUSSION

Immune Response

Production of OVA-specific antibody in response to vaccination indicated that the vaccine effectively stimulated the adaptive immune system of steers. Additionally, a 30% increase in OVA-specific antibody production in steers supplemented with BCAA compared with CON indicated that BCAA supplementation improved the magnitude of the adaptive immune response. These observations are consistent with Calder (2006), who described that a deficiency of some BCAA decreases antibody production in mice. Ozturk and Palsson (1991) also observed that BCAA are consumed at the second highest rate by antibody producing hybridomas.

With increased OVA-specific antibody production, total WBC counts and differential proportions of monocytes and lymphocytes were expected to increase. The lack of change in total WBC production and differential proportions of leukocytes (exception for neutrophils) may indicate that vaccination did not stimulate leukocyte proliferation to the extent of a live pathogen challenge. Alternatively, supplemental BCAA may have increased activity of leukocytes rather than absolute cell populations. Neutrophils rapidly proliferate in the presence of antigen (Abbas and Lichtman, 2006), and may have undergone cellular expansion to a greater extent than other leukocytes, making changes in neutrophil differential proportions observable in BCAA-supplemented steers.

Animal Performance

Improved animal performance and feed efficiency (d 15 to 28, and d 29 to 56) of BCAA-supplemented steers suggests that BCAA are limiting in newly received feedlot steers. These results are consistent with Löest et al. (2001), who demonstrated that BCAA limited N retention of growing steers fed diets low in RUP. Also, Gilliam et al. (2008) demonstrated that calves supplemented with BCAA during a lipopolysacharride challenge exhibited less N loss compared with calves receiving no supplemental BCAA. In this study, the G:F advantage for BCAA-supplemented steers, which lasted through finishing, may be explained by improved efficiency of N retention. Additionally, improved ADG and feed efficiencies may be due in part to the role of BCAA in modulating protein anabolism (Kobayashi et al., 2006). Lower DMI between d 15 and 28 for BCAA-supplemented steers could be explained by the role of BCAA in increasing satiety (Dunshea et al., 2007).

While lack of differences in plasma BCAA concentration might be partially explained by timing of blood sample collection (24 h after feeding), no change in plasma BCAA concentration in conjunction with improved performance among BCAA steers compared with CON steers supports our hypothesis that BCAA are limiting. To our knowledge, this is the first study to measure long term health and performance of cattle supplemented with BCAA.

Conclusion

These results imply that BCAA are limiting in stressed cattle during the transition from ranch to feedlot. Thus, supplementing BCAA during the feedlot receiving phase improves performance and immune response of newly-received feedlot steers.

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Table 1. Composition of diets (DM basis) fed to steers

Item	Receiving	Finishing
Ingredient, %		
Corn, Steamed flaked	59.80	76.67
Hay, Alfalfa	30.56	9.95
Molasses	4.41	4.45
Soybean Meal	2.03	4.46
Vitamin and Mineral Premix[1]	2.17	2.41
Medicated Premix[2]	1.01	1.03
Urea	-	1.03
Nutrient[3]		
NDF, %	15.60	12.59
ADF, %	10.51	7.38
CP, %	12.74	16.55
Ca, %	0.66	1.03
P, %	0.33	0.36
Fe, mg/kg	101.8	121.0
Zn, mg/kg	68.6	117.0
Cu, mg/kg	8.95	22.25
ME[4], Mcal/kg	2.78	2.83
NE_m[5], Mcal/kg	1.84	1.89
NE_g[6], Mcal/kg	1.21	1.25

[1]Supplied (% of supplement DM): limestone (44.8), soybean hulls (20.4), salt (11.8), potassium chloride (8.0), ammonium sulfate (6.6; 21% S), magnesium oxide (3.6; 56% Mg), molasses (1.3), monocalcium phosphate (1.1; 21% P), manganese (0.51) zinc sulfate (0.86; 36% Zn), copper sulfate (0.15; 25% Cu), iron sulfate (0.13), Se (0.02), potassium iodide 68 (0.003), cobalt carbonate (0.002), vitamin E (0.56; 20,000 IU/g), and vitamin A (0.27; 30,000 IU/g).

[2]Supplied 33 mg of monensin and 10 mg of tylosin per kg of dietary DM (Elanco Animal Health, Indianapolis, IN).

[3]Analyzed by a commercial laboratory (SDK labs, Hutchinson, KS).

[4]ME, Mcal/kg = 0.01 × (81.81 - 0.48 × %ADF) × 4.409 × 0.82 (Bull, 1981; NRC, 2000).

[5]NE_m, Mcal/kg = 1.37 × ME - 0.138 × ME^2 + 0.0105 × ME^3 - 1.12 (NRC, 2000).

[6]NE_g, Mcal/kg = 1.42 × ME - 0.174 × ME^2 + 0.0122 × ME^3 - 1.65 (NRC, 2000).

Table 2. Plasma Ile, Leu, and Val, serum insulin and IgG, whole blood total white blood cell (WBC) count, and differential proportions of WBC for beef steers fed no supplemental AA (CON) or supplemental branched-chain AA (BCAA)

	Treatment			*P*-
Item	CON	BCAA[1]	SEM	value
Ile, μ*M*	87.3	89.8	2.64	0.30
Leu, μ*M*	145.1	146.5	3.17	0.74
Val, μ*M*	240.0	242.5	5.41	0.66
Insulin, ng/mL	1.67	1.59	0.12	0.61
IgG[2], OD[3]	0.183	0.238	0.02	0.02
WBC, 1000/μL	9.64	9.17	0.58	0.56
% of WBC				
Neutrophils	36.2	38.8	0.88	0.04
Lymphocytes	56.0	53.7	2.54	0.23
Monocytes	3.38	3.74	0.87	0.56
Eosinophils	4.14	3.55	1.22	0.41
Basophils	0.16	0.21	0.04	0.43

[1]Branched-chain AA were top-dressed to a receiving diet that was fed for 28 d after initial processing.

[2]IgG were specific to ovalbumin. All steers were inoculated with ovalbumin by subcutaneous injection on d 0 and 14 of the study.

[3]OD = optical density.

Table 3. Dry matter intake, ADG and G:F for beef steers fed no supplemental AA (CON) or supplemental branched-chain AA (BCAA)

	Treatment			*P*-
Item	CON	BCAA[1]	SEM	value
d 0 to 14				
DMI	4.84	4.76	0.21	0.72
ADG	0.47	0.23	0.22	0.44
G:F	0.099	0.048	0.05	0.46
d 15 to 28				
DMI	8.71	8.35	0.44	0.03
ADG	1.53	1.87	0.16	0.11
G:F	0.177	0.226	0.02	0.06
d 29 to 56				
DMI	12.27	11.78	0.56	0.50
ADG	1.97	2.22	0.10	0.04
G:F	0.161	0.188	0.01	0.02
d 57 to finish[2]				
DMI	10.98	11.16	0.85	0.60
ADG	1.61	1.62	0.22	0.93
G:F	0.145	0.144	0.01	0.88
d 0 to finish[2]				
DMI	6.44	6.54	0.33	0.66
ADG	1.55	1.63	0.16	0.25
G:F	0.148	0.157	0.01	0.05

[1]Branched-chain AA were top-dressed to a receiving diet that was fed for 28 d after initial processing.

[2]Total days on feed were 116 for heavy BW block and 166 for light BW block.

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EFFECTS OF AMINO ACID SUPPLEMENTATION ON NITROGEN METABOLISM AND IMMUNE RESPONSE OF BOTTLE-FED CALVES EXPOSED TO AN ENDOTOXIN[1]

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ABSTRACT: Stressed calves that are predisposed to infection may have increased demand for AA to support immune function. This study evaluated effects of lipopolysaccharide (LPS) injection and AA supplementation on N metabolism and immune response of 32 bottle-fed Holstein bull calves (28 d of age, 44 ± 0.8 kg BW). The experiment was a randomized complete block with 15-d adaptation, 1-d blood collection, and 5-d fecal and urine collection. Calves were fed milk replacer (0.454 kg/d of 20% CP powder) twice daily and calf starter (18% CP; 0.454 kg/d) once daily. Treatments (2 × 2 factorial) were daily supplementation of 10 essential AA (0 vs 25 g/d dissolved in milk; -AA vs +AA) and subcutaneous injection of LPS (0 vs 4 μg/kg BW; -LPS vs +LPS) on d 16. Rectal temperature (RT) and blood samples were collected at 0, 4, 8, 12, and 24 h after LPS injection. No LPS × AA × h interactions occurred ($P > 0.18$). Cortisol was greater at 4 h only, haptoglobin was greater at 24 h only, and RT was greater at 4, 8, and 12 h for +LPS vs -LPS calves (LPS × h, $P < 0.05$). Insulin was greater (LPS, $P < 0.05$) for +LPS vs -LPS. Plasma Gly, Ser, Asn, Hyp, and Thr of +LPS calves decreased at 4 h and was lower than -LPS calves at 8, 12, and 24 h after LPS injection (LPS × h, $P < 0.05$). Plasma Pro was lower at 8 and 24 h in +LPS vs -LPS calves (LPS × h, $P < 0.05$). Plasma Asp, Glu, Gln, and Met were lower ($P < 0.05$) for +LPS than -LPS calves. Plasma Met, Leu, Ile, His, Phe, Thr, Trp, and Orn were greater for +AA than -AA calves at 0, 4 (except Leu, Ile, His, Trp), 12 (except Ile), and 24 h after LPS injection (AA × h, $P \leq 0.05$). Plasma Gly was lower ($P < 0.05$), and Val was higher ($P < 0.05$) in +AA than -AA calves. No LPS × AA interactions ($P > 0.12$) or LPS effects ($P > 0.19$) were observed for N balance. Calves fed +AA vs -AA had greater ($P < 0.05$) intake and digested N, and tended to have greater urinary N excretion ($P = 0.07$) and N retention ($P = 0.12$). In summary, LPS increased inflammation and decreased plasma AA, but did not alter N retention. Supplementing milk replacer with AA did not significantly improve N retention.

Key words: essential amino acid, lipopolysaccharide, Holstein calves

INTRODUCTION

Exposure to infectious agents stimulates inflammatory processes and results in metabolic changes that alter protein and AA requirements. Absorbed AA are directed away from lean tissue growth during immunological stress due to greater demand for synthesis of proteins and cells related to the immune response (Le Floc'h et al., 2003). During inflammation, AA are used for the synthesis of acute-phase proteins, glucose precursors, plasma proteins, antibodies, free radical scavengers, metabolic cofactors, and hormones (Li et al., 2007). It has been speculated that if there is an imbalance between the composition of AA obtained from tissue protein catabolism and the composition of AA of acute-phase proteins, AA loss via N excretion will increase (Reeds and Jahoor, 2001). Waggoner et al. (2009b) demonstrated that plasma AA concentrations of growing steers decreased in response to lipopolysaccharide (**LPS**), which suggests that providing cattle with these potentially limiting AA may be beneficial during periods of immune system activation and stress.

Hill et al. (2008) reported that supplementation of a 20% CP milk replacer with essential AA, such as Lys and Met, increased performance of young Holstein calves. Based on the above evidence, we hypothesized that AA supplementation will increase the supply of potentially limiting AA for the immune response and thus alleviate decreases in N retention associated with excess body protein catabolism in LPS-challenged calves. The objectives were to evaluate the effects of AA supplementation on N metabolism and health responses of bottle-fed Holstein calves exposed to LPS.

MATERIALS AND METHODS

Animals, Facilities, and Experimental Design

The Institutional Animal Care and Use Committee at New Mexico State University approved all procedures for this study. Thirty-two Holstein bull calves (28 d of age, 44 ± 0.8 kg BW) were obtained from a single-source commercial dairy. Calves had previously received colostrum and were vaccinated with Clostridium Chauvoei-Septicum-Haemolyticum-Novyi-Sordellii-Perfringens types C & D Bacterin-Toxoid (Vision-8 with SPUR; Intervet/ Schering-Plough Animal Health; Millsboro, DE), Moraxella Bovis Bacterin (Piliguard pinkeye triview; Intervet/Schering-Plough Animal Health; Millsboro, DE), and Pyramid 10 Bovine Rhinotracheitis-Virus Diarrhea-Parainfluenza 3-Respiratory Synctial Virus Vaccine (Fort

[1] The authors acknowledge J. L. Gonzalez (Gonzalez Dairy Inc.) for supply of Holstein calves.

Dodge Animal Health; Fort Dodge, IA). The calves received primary and secondary inoculations using the aforementioned vaccines at 25 and 56 d of age, respectively. Calves were immediately weighed upon arrival and placed in individual pens (2 × 3 m) in a metabolism building where they had free access to fresh water. Calves were bottle-fed milk replacer (0.454 kg/d of powder that contained 20% protein and 20% fat; MJ 20/20, Central Supply, Mesquite, NM) in equal portions twice daily, and were fed calf starter (0.454 kg/d; 18% CP; DairyWay Calf Starter; Cargill Incorporation, Minneapolis, MN) once daily.

The experiment was a randomized complete block design with 2 groups (blocks) of 16 calves (blocked because of a limited number of metabolism crates). Each group of calves remained on the experiment for 21 d (from 28 d of age to 49 d of age), which provided 12 d for the calves to adapt to facilities and diet, 3 d for adaptation to the metabolism crates, 1 d for blood collection, and 5 d for fecal and urine collections. During the collection period, calves were housed in metabolism crates in a continuously lit building with evaporative cooling (22.5 ± 2.7°C).

Calves were observed daily for signs of anorexia, decreased activity, abnormal posture, abnormal gait, and diarrhea. Calves that developed diarrhea were supplemented with a commercial oral electrolyte solution (Resorb; Pfizer Animal Health; New York, NY) according to label recommendations, and calves with apparent sickness (based on visual appraisal described above and a rectal temperature > 39.7°C) were treated with florfenicol (Nuflor; Intervet/Schering-Plough; Millsboro, DE) according to label instructions.

Treatments

Treatments, arranged as a 2 × 2 factorial, were milk replacer supplemented with or without 10 essential AA (+**AA** vs **-AA**), and subcutaneous injection of sterile saline with or without LPS (**+LPS** vs **-LPS**). The +AA solution was prepared by dissolving 4 g L-Leu, 2 g L-Ile, 2 g L-Val, 2 g DL-Met, 2 g L-Phe, 2 g L-His, 2 g L-Lys, 2 g L-Thr, 6 g L-Arg, and 1 g L-Trp in 500 g deionized water containing 4 g 6 *M* HCl, and then adjusting the pH to 4.0 with NaOH. The +AA solution (500 g/d per calf) was divided into 2 equal portions, and each portion was thoroughly mixed with milk replacer before each feeding at 0600 and 1800. The suckling reflex from bottle feeding was used as a rumen by-pass mechanism for AA to be supplied to the small intestine for absorption. The +LPS was prepared by dissolving 1 mg LPS (E. coli 055:B5; Sigma Chem. Co., St. Louis, MO) in 10 mL sterile saline. At 3 h after the morning feeding on the first and third day of collection (d 15 and 17), +LPS calves were injected subcutaneously (Ballou et al., 2008) with 4 and 2 µg of LPS per kg of BW, respectively. Simultaneously, -LPS calves were subcutaneously injected with sterile saline.

Collections

From d 15 to 19, total fecal output was collected daily into fecal collection pans and total urinary output was collected into 20-L buckets containing 100 mL of 6 *M* HCl to minimize NH_3 loss. The weight of total fecal and urinary output was recorded daily. All the feces and a representative sample (1%) of urine were stored at -20°C and later composited for each calf for analysis. Also, dietary samples and grain refusals were collected, composited for each calf, and frozen at -20°C.

On d 15, rectal temperatures were measured (Cooper TM99A digital thermometer, Cooper Atkins Corp., Middlefield, CT) and blood samples were collected via jugular venipuncture into vacuum tubes (10 mL Corvac serum separator and 9 mL Monoject Sodium Heparin, Kendall, Ontario, CA) before LPS injection and at 4, 8, 12, and 24 h thereafter. Blood samples obtained for the collection of serum were allowed to coagulate at room temperature for 30 min, whereas samples obtained for plasma were immediately placed on ice. All blood samples were centrifuged (Sorvall RT600B, Thermo Electron Corp., Asheville, NC) at 1,500 × g for 20 min at 10°C. Serum and plasma samples were immediately decanted into 1.5-mL and 7-mL vials and frozen at -70°C for later analysis.

Sample Analysis

Composite samples of grain starter, feed refusals, and feces were dried at 55°C for 72 h in a forced-air oven (Blue M Electric Company, Blue Island, IL), allowed to air-equilibrate for 48 h, weighed to determine moisture loss, and then ground to pass a 2-mm screen (Wiley Model 4, Thomas Scientific, Swedesboro, NJ). Ground samples were analyzed for DM (105°C for 24 h) in a convection oven (Model 845, Precision Scientific Group, Chicago, IL). Concentrations of N in milk, grain, feed refusals, feces, and urine were determined by measuring N_2 from N-combustion products with a thermo-conductivity cell (Leco FP-528, Leco Corp., St. Joseph, MI).

Serum concentrations of cortisol and insulin were determined in duplicate by solid-phase RIA, using components of commercial kits (Siemens Diagnostic, Los Angeles, CA). These kits use antibody-coated tube technology, and assays were performed without prior extraction of the individual hormones from serum. Serum samples were analyzed for haptoglobin by the Kansas State University Veterinary Diagnostic Lab (Manhattan, KS) as described by Smith et al. (1998). Plasma AA concentrations were analyzed by gas chromatography (CP-3800, Varian, Walnut Creek, CA) using a commercially available kit (EZ:FAAST No. KGO-7165, Phenomenex, Torrance, CA) as described by Waggoner et al. (2009a).

Statistical Analysis

All data were analyzed statistically as a randomized complete block design using the MIXED procedure (SAS Inst. Inc., Cary, NC). Because there were only 16 metabolism crates, data collection occurred over 2 periods. Therefore, the experiment was blocked (16 calves in block 1, and 16 calves in block 2) by collection date. Two calves were removed from the experiment because of health concerns, and all data collected from these calves were excluded from the statistical analysis.

The statistical model included the effects of LPS, AA, and LPS × AA interaction for all dietary measures; block and calf were random effects. Rectal temperature and blood metabolites were analyzed as repeated measures (autoregressive order one covariance structure). The model included all possible interactions of LPS, AA, and hour. The experimental unit was calf. Data are presented as least squares means, and differences were considered significant at $P \leq 0.05$.

RESULTS

No LPS × AA × h interactions ($P > 0.18$) were observed for rectal temperature, serum hormones, or plasma AA. Rectal temperature (Figure 1) increased and was greater for +LPS than -LPS calves at 4 (peak), 8, and 12 h, but was not different at 24 h after LPS injection (LPS × h, $P < 0.01$). Serum cortisol increased and was greater in +LPS than -LPS calves at 4 h after LPS injection, but was not different at 8, 12, and 24 h after LPS injection (LPS × h, $P < 0.01$). Serum haptoglobin was not different at 0, 4, 8, and 12 h, but was greater in +LPS than -LPS calves at 24 h after injection (LPS × h, $P < 0.01$). Serum insulin was greater (LPS, $P < 0.05$) for +LPS vs -LPS calves (Table 1).

Plasma Thr, Asn, Gly, Ser, and Hyp (Figure 2) of +LPS calves decreased at 4 h and was lower than -LPS calves at 8, 12, and 24 h after LPS injection (LPS × h, $P < 0.05$). Plasma Pro was lower at 8 and 24 h in +LPS vs -LPS calves (LPS × h, $P < 0.05$). Plasma Asp, Glu, Gln, and Met were lower (LPS, $P < 0.05$) for +LPS than -LPS calves (Table 1). Plasma Met, His, Leu, Ile, Phe, Trp, Thr, and Orn (Figure 3; Thr and Orn not shown) were greater for +AA than -AA calves at 0, 4 (except His, Leu, Ile, Trp), 12 (except Ile), and 24 h after LPS injection (AA × h, $P \leq 0.05$). Plasma Gly was lower ($P < 0.05$), and Val was higher ($P < 0.05$) in +AA than -AA calves (Table 2). Plasma Ala, Tyr, and Lys were not affected ($P \geq 0.09$) by treatment.

No LPS × AA interactions ($P > 0.12$) or LPS effects ($P > 0.19$) were observed for N balance (Table 1). Calves supplemented with AA had greater ($P < 0.05$) intake N and digested N, and tended to have greater urinary N excretion ($P = 0.07$) and N retention ($P = 0.12$; Table 2).

DISCUSSION

Effects of LPS on N Metabolism

Lipopolysaccharide has been used extensively as a non-infectious means of evaluating innate immune function of stressed calves. The use of LPS in cattle has been shown to produce the metabolic effects associated with stress and disease, including increased body temperature, blood cortisol, cytokines, and acute-phase proteins, as well as altered protein and energy metabolism (Steiger et al., 1999). In the current study, symptoms of an inflammatory response to LPS injection were evidenced by increases in rectal temperature, cortisol, insulin, and haptoglobin. Similar increases in serum insulin concentration in response to exposure to LPS were observed by Steiger et al. (1999) and Waggoner et al. (2009a). Spurlock (1997) stated that LPS reduces the apparent rate of glucose uptake by the peripheral tissue which in turn causes insulin resistance. Decreases in plasma concentrations of Thr, Asn, Gly, Ser, Hyp, Pro, Asp, Glu, Gln, and Met in calves exposed to LPS indicate either a shift in AA utilization toward the synthesis of proteins related to the immune response, or increased AA catabolism. These decreases in blood AA concentrations due to LPS exposure are similar to those reported by Gilliam et al. (2008), Waggoner et al. (2009a; 2009b), and Carter et al. (2010).

Previous research (Gilliam et al., 2008; Waggoner et al., 2009a; 2009b; Carter et al., 2010) reported increased urinary N excretion and decreased N retention in cattle exposed to LPS, and the authors attributed these responses to increased tissue protein degradation to support an increased AA demand for the synthesis of immune system protein. However, the lack of such a response in the current study might be attributed to the different route of LPS administration used (subcutaneous vs intravenous), and a higher dose of subcutaneous LPS injection may be needed to obtain similar results.

Effects of AA on N Metabolism

Greater plasma concentrations of Met, Leu, Ile, Val, His, Phe, Thr, Trp, and Orn (a metabolite of Arg) indicated that the 10 essential AA supplemented in milk replacer escaped rumen degradation and were effectively absorbed. No increases in plasma Lys in response to +AA supplementation suggests that the Lys supply was either insufficient to alter plasma Lys concentrations, or that Lys was the most limiting AA in calves fed milk replacer. Hill et al. (2007, 2008) reported that supplementing Lys to a 20% CP milk replacer increased ADG of calves. However, supplementation of 10 essential AA did not significantly increase N retention of calves in the current study. Additionally, the tendency for AA-supplemented calves to have greater urinary N excretion suggests that supplemental AA were not needed to support additional growth. The tendency for AA supplementation to increase the amount of N retained by calves was due to increased N digested, which appeared to be a function of the additional N supplied via the AA supplement.

Conclusions

Increases in rectal temperature, cortisol, insulin, and haptoglobin indicated that LPS activated an immune response, and decreases in plasma AA in calves exposed to LPS indicated an increased need for AA during periods of immunological stress. However, despite these observations, exposure to LPS did not decrease N retention. In calves supplemented with AA, the combined effect of increased plasma AA with no increase in N retention indicates that 20% CP milk replacer is not limiting in essential AA.

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Table 1. Plasma AA and N balance of Holstein calves in response to lipopolysaccharide (LPS)

	Treatment[1]			*P*-
Item	-LPS	+LPS	SEM	value[2]
N	15	15		
Insulin, ng/mL	1.09	2.08	0.25	0.01
Plasma AA, μM				
Asp	4.71	1.73	0.63	<0.01
Glu	117.7	96.6	8.50	0.02
Gln	185.6	155.4	12.4	0.03
Met	46.6	37.8	2.63	0.02
Nitrogen, g/d				
Grain intake	10.06	8.65	1.10	0.30
Milk intake	20.31	20.31	-	-
Total intake	30.22	28.81	1.10	0.30
Fecal	4.71	4.82	0.27	0.77
Digested	25.52	23.98	0.89	0.19
Urinary	12.68	11.65	1.44	0.21
Retained	12.78	12.39	0.89	0.73

[1]Treatments were a 2 × 2 factorial of subcutaneous injection of sterile saline with or without lipopolysaccharide (+LPS vs -LPS), and milk replacer supplemented with or without 10 essential AA (+AA vs -AA).

[2]Observed significance level for the effects of LPS; no LPS × AA interactions were observed ($P > 0.12$).

Table 2. Plasma AA and N balance of Holstein calves in response to AA supplementation

	Treatment[1]			*P*-
Item	-AA	+AA	SEM	value[2]
N	15	15		
Plasma AA, μM				
Gly	439.3	377.6	20.2	0.04
Val	146.6	181.7	7.02	<0.01
Nitrogen, g/d				
Grain intake	9.87	8.84	1.10	0.45
Milk intake	17.97	22.35	-	-
Total intake	27.84	31.19	1.10	0.02
Fecal	4.80	4.73	0.27	0.86
Digested	23.03	26.47	0.89	<0.01
Urinary	11.40	12.92	1.44	0.07
Retained	11.69	13.49	0.89	0.12

[1]Treatments were a 2 × 2 factorial of subcutaneous injection of sterile saline with or without lipopolysaccharide (+LPS vs -LPS), and milk replacer supplemented with or without 10 essential AA (+AA vs -AA).

[2]Observed significance level for the effects of AA; no LPS × AA interactions were observed ($P > 0.12$).

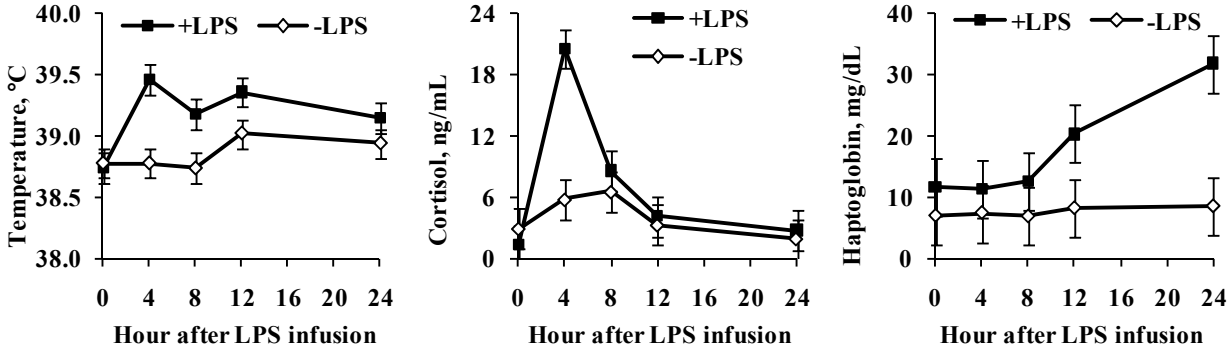

Figure 1. Rectal temperature and serum concentrations of cortisol and haptoglobin in Holstein calves in response to subcutaneous injection of sterile saline with or without lipopolysaccharide (+LPS vs -LPS). Effect of LPS × h ($P < 0.05$).

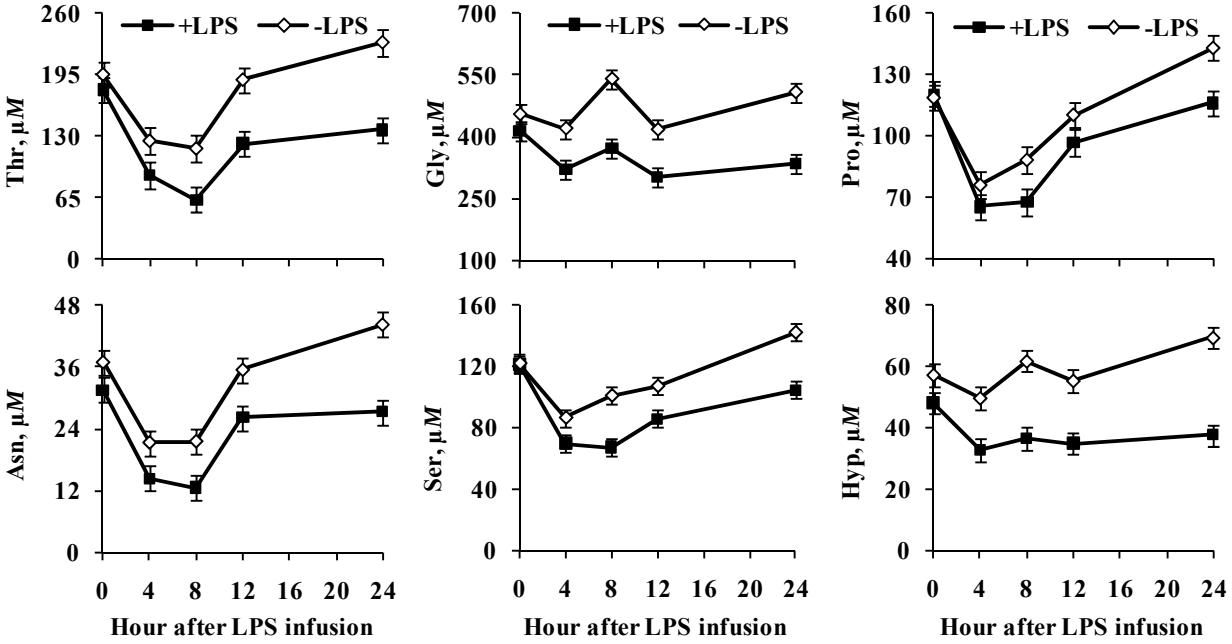

Figure 2. Plasma concentrations of AA in Holstein calves in response to subcutaneous injection of sterile saline with or without lipopolysaccharide (+LPS vs -LPS). Effect of LPS × h ($P < 0.05$).

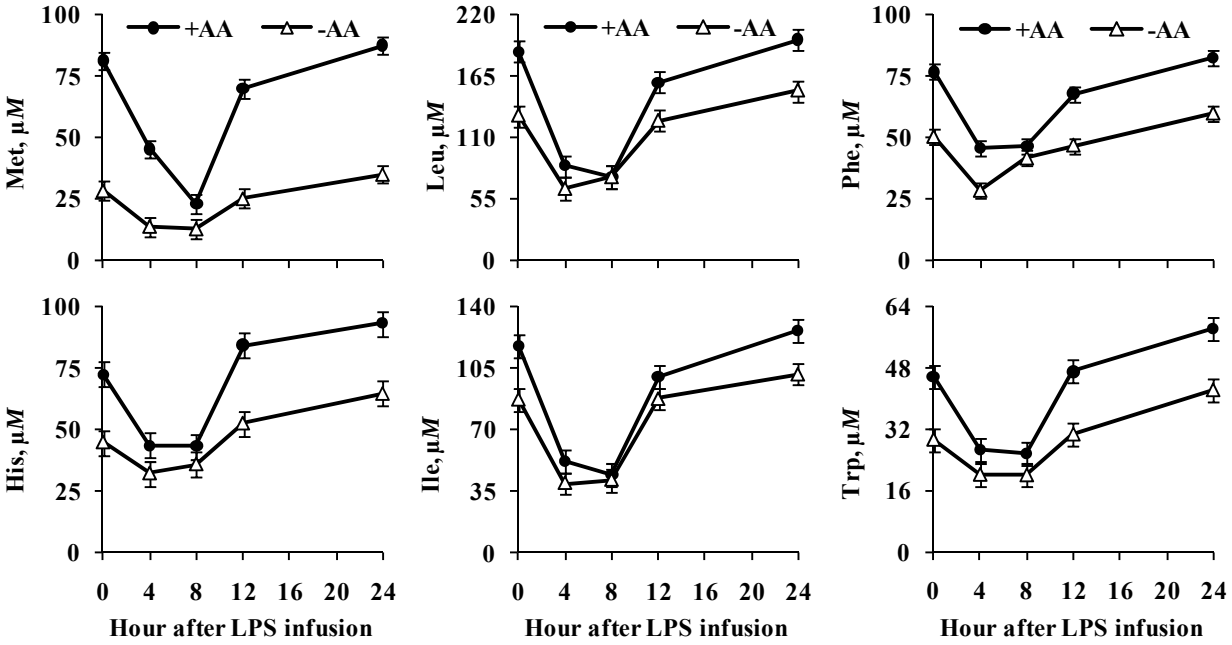

Figure 3. Plasma concentrations of AA in Holstein calves in response to and milk replacer supplementation with or without 10 essential AA (+AA vs -AA). Effect of AA × h ($P < 0.05$).

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WHOLE CORN AND WET DISTILLERS GRAINS SUBSTITUTION IN STEAM-FLAKED CORN DIET ALTERS RUMEN FERMENTATION AND BACTERIAL DYNAMICS

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ABSTRACT: A study evaluated effects of whole shelled corn (WSC) in steam-flaked corn (SFC) finishing diets containing differing amounts of wet distillers grains with solubles (WDGS) on rumen fermentation and shifts in ruminal bacterial populations. A total of 642 heifers (initial BW = 412 ± 18 kg) were blocked by BW and randomly assigned to 36 pens in a 108 d experiment. Treatments were arranged as a 2 × 3 factorial with two amounts of WSC (0 and 20% DM) and three amounts of WDGS (0, 15, and 30% DM) replacing SFC. Ruminal fluid samples, collected from 2 heifers/pen on d 98,were analyzed for VFA, ammonia, pH, and shifts in ruminal bacterial populations using denaturing gradient gel electrophoresis (DGGE) targeting the 16S rDNA gene. Richness and Shannon-Wiener indices evaluated bacterial presence and diversity. Similarity matrix of DGGE banding patterns were constructed using Dice (binary) coefficient. Microbial indices and ruminal samples were evaluated for effects of WSC, WDGS, and interactions using Mixed Procedure of SAS with block as the random variable and pen as experimental unit. Addition of 0 to 15% WDGS to 0% WSC increased total VFA; however, total VFA decreased from 0 to 30% WDGS with 20% WSC (WSC x WDGS interaction, $P = 0.02$). Ammonia was not affected ($P \geq 0.19$). Diets with 0% WSC had 3.9% lower pH ($P < 0.01$), 4.4% less acetate ($P = 0.05$), 7.4% greater propionate ($P = 0.02$), and lower acetate:propionate ($P< = 0.02$). Inclusion of WDGS decreased acetate (quadratic, $P < 0.01$), increased propionate (quadratic, $P = 0.03$), and decreased acetate:propionate (quadratic, $P = 0.03$). Bacterial diversity based on DGGE showed Richness and Shannon-Wiener indices did not differ with inclusion of WSC ($P = 0.63$), but increased with WDGS (linear, $P = 0.01$). All samples were 69.02% similar according to Dice. Microbial population shifts and fermentation characteristics imply use of WSC in SFC based diets may negatively alter rumen use of WDGS.

Key words: cattle, wet distillers grains, rumen bacteria

Introduction

Ethanol in recent years has become a popular biofuel. Distillers grains, a coproduct resulting from the ethanol industry, are used extensively in finishing diets of feedlot cattle. Distillers grains can be fed wet (**WDGS**) or dry (**DDGS**) and usually are included to supply energy and (or) protein in the diet. Most feedlot diets use corn grain as the predominant source of energy. Processing methods of the corn varies, but a survey of feedlot nutritionists showed that steam-flaking is the predominant corn processing method in over 11 states (Vasconcelos and Galyean, 2007). Currently there is little data available showing the value of WDGS in steam-flaked corn (**SFC**) diets. When WDGS replaces high moisture corn (**HMC**) or dry rolled corn (**DRC**), ADG and G:F increase (Klopfenstein et al., 2008). Tracey et al. (2010) showed that use of WDGS in SFC-based diets did not negatively alter rumen fermentation or performance characteristics of feedlot steers.

Though steam-flaking is common, processing of corn grain is an added expense to the feedlot. If whole shelled corn (**WSC**) can replace a portion of the SFC in finishing diets containing WDGS without negatively altering animal performance, it might be possible to reduce grain processing costs. This study evaluated the effect of replacing 20% SFC with WSC while adding up to 30% WDGS to the diet on the ruminal bacterial population and rumen fermentation characteristics. A companion-study by McDaniel et al. (2011) evaluated impacts of these treatments on performance and carcass characteristics. We hypothesize that use of WSC in finishing diets containing WDGS would lead to differences in microbial population and rumen fermentation characteristics due to decreased starch availability over SFC-based diets. Our objective was to evaluate the effects of WSC and WDGS on microbial ecology using denaturing gradient gel electrophoresis (**DGGE**) and to compare these results with ruminal fermentation characteristics.

Materials and Methods

Animals and Experimental Design. All animal procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. A total of 642 heifers (412 ± 18 kg average BW) were blocked by BW and randomly assigned to one of 36 pens (n = 17 to 18 heifers). Each pen was randomly assigned to one of six treatments. The feeding trial was 108 d in duration. On d 98, two animals from each pen (n = 72) were randomly chosen for sampling of ruminal fluid for analysis of rumen characteristics and microbial dynamics.

Dietary treatments and Sampling Dietary treatments were a 2 × 3 factorial arrangement with two amounts of WSC (0 and 20% DM) and 3 amounts of WDGS (0, 15, and

30% DM) replacing SFC. Animals were fed once per day. Diet ingredients and nutrient composition is in Table 1. All heifers were allowed to adapt to a finishing diet prior to the onset of the 108 d experiment. Ruminal fluid samples were collected once on d 98. Samples were collected via oral lavage with suction strainer (Lodge-Ivey et al., 2009) approximately 4 h post feeding. Samples were placed on ice immediately following collection and were later maintained at -20°C until analysis for ruminal pH, ammonia and VFA concentrations, and extraction of community DNA could be performed.

Laboratory analysis. Ruminal ammonia was analyzed using the phenol–hypochlorite procedure of Broderick and Kang (1980), adapted to a microtiter plate (BioTek Instruments, Winooski, VT). Volatile fatty acid concentration was determined by gas chromatography (Goestch and Galyean, 1983; Varian 3400; Varian Inc., Walnut Creek, CA). Community rumen DNA was extracted from ruminal fluid using the repeated bead beating and column method described by Yu and Morrison (2004b). The quality of the DNA was assessed by electrophoresis on 1.0% agarose gel. Extracted DNA was used as template for subsequent PCR-DGGE.

Each PCR reaction was performed in 25 µL and amplified on DNA Engine PTC-200 (MJ Research, Watertown MA) for each PCR-DGGE analysis. The PCR mixture contained 1.25 U Platinum taq (Invitrogen, Carlsbad CA) 60 mM Tris-SO4 (pH 8.9), 18 mM ammonium sulfate,1.75 mM MgCl2, 250 µM of each deoxynucleoside triphosphate, 0.0674%BSA, 0.5 µM each primer and approximately 100 ng of template DNA. The V3 reigion of the *rrs* gene was amplified using primers 357f (5'-CCTACGGGAGGCAGCAG-3') and 519r (5'-ATTACCGCGGCKGCTGG-3'). The 357f primer has a 40-bp GC clamp attached to the 5' end (CGC CCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGG GGG) to prevent dissociation of the DNA strands (Yu and Morrison, 2004a). To reduce the production of spurious PCR products, touchdown PCR was performed. The PCR cycle consisted of an initial denaturing at 94ºC for 4 minutes, 10 cycles of touchdown PCR wherein the starting annealing temperature of 61ºC was decreased 0.5^{o}C per cycle for ten cycles to 56ºC. This was followed by 25 cycles with denaturing step at 94^{o}C for 30 s, annealing at 56ºC 30 s, and a final primer extension at 72º for 30 s. Quality of the PCR products was confirmed visually using a 1.5% agarose gel stained with ethidium bromide.

Using the Bio-Rad D-Code system (BioRad, Hercules, CA), DGGE was performed as described by Simpson et al., 1999. To separate PCR fragments, 30 µL of PCR product was resolved on 7.5 % polyacrylamide gel (37.5:1) containing a 30 to 60% gradient denaturants (100% denaturants consisting of 40% [vol/vol] formamide and 7 M urea). Electrophoresis was performed at 60°C and 82 V for 16 hrs. Additionally, a standard sample was included in each gel to allow for normalization of band migration and gel curvature among different gels (McCracken et al., 2001). After electrophoresis, gels were stained with GelStar (Cambrex, Rockland, ME) according to manufacturer's specifications and the images were captured using Kodak Imaging Systems (New Haven, CT).

Statistical analysis of DGGE banding patterns. Images of DGGE gel patterns were imported as TIFF files into Bionumerics software (version 5.2; Applied Maths, Applied Maths, Inc., Austin, TX). Lanes of each DGGE gel were converted to densiometric curves and an automatic band search was performed on normalized patterns. Gels were normalized using five bands from a 1 kb DNA ladder and a standard sample to ensure the location of bands was consistent across all gels. After visual control of the assigned bands, a band-based analysis was performed using the algorithm for Dice coefficient. To calculate Dice coefficient all bands are divided into classes of common bands and for each band pattern, a particular band class can have two states: present or absent (binary matrix). A 5% band position tolerance was used and is an arithmetic determination of the degree to which banding patterns are alike, (i.e. contain the same bands in similar locations). For cluster analysis similarities were displayed graphically as a dendrogram. The clustering algorithms used to calculate the dendrograms was an unweighted pair group method with average linkages (UPGMA). Clusters (groups) were determined by sequentially comparing patterns and the construction of a related dendrogram reflected relative similarities.

Diversity indices. Richness (*S)* was determined from the number of bands in each lane using the band matching table generated during the Dice analysis of the DGGE profiles. The Shannon-Wiener index (*H'*) of diversity was used to determine the proportional abundances of bacterial taxa present in the rumen samples of cattle fed increasing levels of WDGS. Shannon-Wiener index was calculated for binary (band presence or absence) using the following equation: $H' = -\Sigma P_i \ln P_i$, where P_i is the importance probability of the bands in a lane, calculated from n_i/N where n_i is the peak height of a band and N is the sum of all peak heights in the densitometric curve.

Statistical analysis. Richness, Shannon-Wiener index, ruminal ammonia, VFA concentrations, and pH were analyzed as a randomized complete block design using the MIXED procedure of SAS version 9.2 (SAS Inst. Inc., Cary, NC). Pen was used as the experimental unit, and block was the random variable. Model included the effect of WSC, WDGS, and WSC × WDGS interactions. Satterthwaite was used as the variance structure. Mean comparisons with *P*-values less than or equal to 0.05 were declared significant, and values less than or equal to 0.10 were considered tendencies. Means were calculated using LSMEANS and separated using PDIFF. Polyorthagonal contrasts were used to evaluate linear and quadratic effects of WDGS concentrations.

Results and Discussion

Distillers grain use has increased over the last decade due to increased ethanol production and the rising price of cereal grains. The use of distillers grains has been evaluated

in different feedlot diet systems including DRC, HMC, and SFC. Steam flaking is a corn processing method designed to increase availability of nutrients; particularly starch. However, the high cost of this method inhibits ability for some to use SFC as the main feed source in their operation. In 2006 the cost of steam-flaking for a 5,000 head feedlot was $9.57/t (Metric ton, DM basis; Macken et al., 2006). Though the NE_g value of SFC is 18% higher than that of whole corn (Zinn et al., 2002) the cost may become inhibitory to today's producer. Because of this, the possibility of including WSC in a SFC diet containing WDGS is an important avenue to explore.

The only interaction between WSC and WDGS was seen in total VFA concentration (WSC x WDGS interaction, $P = 0.02$). When no WSC was in the diet total VFA production increased from 0 to 15% WDGS, but decreased with 30% (99.08, 129.41, and 104.13 ± 5.79 mM for 0, 15, and 30%, respectively). However, when 20% WSC was in the diet, total VFA production decreased linearly as WDGS amounts increased (94.19, 91.84, and 90.95 ± 5.79 m*M* for 0, 15, and 30% WDGS, respectively). A study evaluating the use of DDGS in either DRC or SFC diets showed that SFC diets led to increased total VFA production with an increase in the production of the glucogenic precursor, propionate (May et al., 2009). This increase in propionate, likely contributes to the 18% increase in feeding value of the more highly processed grain source found by Zinn et al. (2002).

Effects of inclusion of WSC and WDGS are summarized in Table 2. Ruminal ammonia concentrations were not affected by WSC ($P = 0.19$) or WDGS inclusion ($P = 0.40$). This was by experimental design as the diets were balanced for RDP. Ruminal pH increased 3.9% from 6.4 to 6.6 with inclusion of WSC ($P = 0.01$). Ruminal pH has been shown to be affected by grain processing level with an increased processing causing a decreased pH (de Vargas et al., 2011). Acetate concentrations were higher ($P = 0.05$) and butyrate concentrations tended ($P = 0.08$) to increase in animals consuming 20% WSC compared to those not receiving WSC. Propionate concentrations decreased 7.4% as the inclusion of WSC increased (40.5 vs 37.5 ± 0.73 for 0 and 20% WSC, respectively; $P = 0.01$). Because of the decrease in acetate and increase in propionate in animals consuming 20% WSC, acetate:propionate was lower in the 0% WSC group ($P = 0.02$). Similar to these results, Sharp et al. (1982) showed acetate decreases, propionate increases, and acetate:propionate decreases as a result of increased corn processing. Similarly, when comparing SFC- and DRC-based diets the inclusion of DDGS had effects on ruminal VFA profiles similar to those in the current study with increased acetate, decreased propionate, and increased acetate:propionate in DRC diets over SFC diets (May et al., 2009). Ruminal pH and VFA profiles are interconnected. Ruminal pH levels may alter fermentation endproducts as individual bacterial species are metabolically active at differing environmental pH. In a continuous flow in vitro fermentation study an induced increased pH led to increased acetate and decreased propionate and butyrate levels (Shriver et al., 1986). This may be due to a difference in active bacterial species due to their optimal pH range.

Ruminal pH tended (P = 0.07) to decrease linearly with the increase in dietary WDGS; however, as the values ranged from 6.63 to 6.01 it is likely that this is of no biological significance to the animals. Callaway et al. (2010) showed a decrease in ruminal pH with increased DDGs. Ruminal pH in that study ranged from 6.58 to 7.18 which are higher than one might expect in a finishing diet. These pH values, similar to those in our study, may be a result of distillers coproducts having 80% more NDF than corn grain (distillers grains + solubles vs flaked corn grain; NRC, 2000) Al-Suwaiegh et al., (2002) showed no difference in ruminal pH in dairy cattle consuming 30% WDGS vs a control diet. A study evaluating the feeding of corn DDGS at levels of 0 to 1.2% BW showed no change in ruminal pH values (Leupp et al., 2009b). However, an additional study (Leupp et al. 2009a) which evaluated inclusion of 0 to 60% DDGS in a DRC-based diet noted a linear increase in pH from 6.42 to 6.63.

Amounts of WDGS did not affect butyrate concentrations ($P = 0.37$). Acetate concentrations decreased quadratically with increasing inclusion of WDGS (49.5, 45.5, and 47.3 ± 0.91 for 0 15, and 30% WDGS, respectively; $P = 0.01$). Propionate concentrations increased quadratically ($P = 0.03$), with the 30% WDGS diet having the highest concentration at 40.6 compared to 37.0 and 39.3 ± 0.90for 0 and 15% WDGS, respectively. Accordingly, acetate:propionate decreased quadratically, with the highest ratio at 15% WDGS (1.15 vs 1.36 and 1.23 ± 0.05for 0, 15, and 30% WDGS, respectively). Several studies with lactating dairy cows have reported no significant difference in VFAs resulting from the inclusion of distillers grains (Al-Suwaiegh et al., 2002; Anderson et al., 2006). A previous study of ours also showed no difference in VFA production when up to 60% of a SFC-based finishing diet was WDGS (Tracey et al., 2010). The difference in sampling time and duration may have some role in the differences we see between the two studies. In the previous study repeated samples were taken as the animals were being adapted to the WDGS diets, while in the current studies a single sample was taken toward the end of the study period allowing the animals to be fully adapted to the diets and for optimal fermentation to be seen. Leupp et al. (2009a) showed a linear decrease in acetate, linear increase in propionate, a linear decrease in acetate:propionate, and no difference in butyrate concentration with 0 to 60% DDGS.

Microbial diversity indices analyzed included Richness and Shannon-Wiener. Richness was not affected by amount of WSC ($P = 0.20$), but increased linearly with increased WDGS (23.2, 23.3. and 27.1 ± 1.11 for 0, 15, and 30% WDGS respectively; P 0.01). Similarly, Shannon-Wiener increased linearly as WDGS increased (2.40, 2.42, and 2.81 ± 0.11 for 0, 15, and 30% WDGS, respectively; $P = 0.01$), but did not differ with WSC amounts ($P = 0.20$). When dendrograms were constructed using banding pattern based on band presence or absence all samples were 69.02% similar, implying that differences in microbial diversity are occurring due to inclusion of WSC and WDGS. Interestingly, animals consuming no WSC and no WDGS had the least presence/absence similarity amongst themselves. It is understood that changes in diets alters the

ruminal bacterial population. Inclusion of up to 15% condensed distillers coproduct in a DRC-based diet increased fibrolytic and lactilytic bacteria (Fron et al., 1996). While in the current study the bacterial diversity changed with the inclusion of WDGS, in our previous study there was no change in the overall diversity of bacterial population, although there were differences in the number of individuals in each species present (Tracey et al., 2010). A pyrosequencing study showed a change in the proportions of bacterial species present in the rumen and lower gut in animals consuming different levels of DDGS (up to 50% of diet) in a concentrate diet (Callaway et al., 2010).

Though there were few significant interactions between the amount of WSC and the amount of WDGS, the decrease in favorable fermentation characteristics when WSC is included in SFC-based diets with WDGS implies that ruminal fermentation may be negatively altered, which may ultimately impact performance and carcass characteristics.

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Table 1. Ingredient and chemical composition (DM basis) of experimental diets containing whole shelled corn and wet distillers grains with solubles fed to heifers.

Item	0% Whole Shelled Corn			20% Whole Shelled Corn		
	0% WDGS[1]	15% WDGS	30% WDGS	0% WDGS	15% WDGS	30% WDGS
Ingredient, %						
Steam-flaked corn	78.5	67.6	56.8	58.5	47.7	36.8
Whole shelled corn	-	-	-	20.0	20.0	20.0
WDGS	-	15.0	30.0	-	15.0	30.0
Wheat silage	9.0	9.0	9.0	9.0	9.0	9.0
Soybean meal	7.0	4.0	1.0	7.0	4.0	1.0
Supplement[3]	2.5	2.5	2.5	2.5	2.5	2.5
Tallow	2.0	1.0	-	2.0	1.0	-
Urea	1.0	0.9	0.8	1.0	0.9	0.8
Nutrient, %						
CP	14.5	16.3	18.3	14.2	16.3	18.1
NDF	13.3	16.5	18.9	13.9	16.5	19.6
ADF	7.2	9.7	10.7	7.5	8.8	10.5
EE	4.4	5.1	5.8	4.8	5.2	5.7

[1]WDGS = wet distillers grains with solubles (produced from corn grain, blended with no more than 10% sorghum grain)
[2]Supplement composition (DM): limestone = 75.54%; KCL = 8.01%; salt = 7.71%; MgO = 2.89%; $ZnSO_4$ = 0.86%; selenium premix (0.06% Se) = 0.49%; $MnSO_4$ = 0.42%; $CuSO_4$ = 0.31%; EDDI (4.4%) = 0.04%; vitamin E (500 IU/g, 90% DM) = 0.03%; vitamin A (30,000 IU/g, 90% DM) = 0.03%; $CoSO_4$ = 0.004%; Rumensin (Elanco Animal Health, Indianapolis, IN; 176.4 mg/kg, 90% DM) = 0.72%; Tylan (Elanco Animal Health; 88.2 mg/kg, 90% DM) = 0.48%; mGA 200 (Pfizer Animal Health, New York, NY; 441 mg/kg melengestrolacetate) = 0.39%; and mineral oil = 2.11%
[3]Nutrient composition analyzed by Servi-Tech Laboratories, Amarillo, TX

Table 2. Effect of whole shelled corn (WSC) and wet distillers grains with solubles (WDGS) on ruminal ammonia, pH, VFA production, Richness, and Shannon-Wiener indices in cattle on steam flaked corn diet.

	Treatments							*P* – values			Contrasts[2]	
	WSC			WDGS[1]								
Item	0	20	SE	0	15	30	SE	WSC	WDGS	WSC × WDGS	Lin.	Quad.
Ammonia, m*M*	0.79	1.20	0.32	0.93	0.80	1.39	0.37	0.19	0.40	0.58	0.31	0.37
pH	6.35	6.62	0.07	6.63	6.41	6.43	0.08	0.01	0.09	0.25	0.07	0.19
VFA, mol/100 mol												
Acetate	46.4	48.5	0.74	49.5	45.5	47.3	0.91	0.05	0.01	0.17	0.10	0.01
Propionate	40.5	37.5	0.73	37.0	40.6	39.3	0.90	0.01	0.02	0.14	0.08	0.03
Butyrate	7.92	8.93	0.34	8.04	9.97	8.27	0.48	0.08	0.37	0.63	0.74	0.18
A:P[3]	1.17	1.31	0.04	1.36	1.15	1.23	0.05	0.02	0.03	0.15	0.11	0.03
Richness	23.9	25.3	0.97	23.2	23.3	27.1	1.11	0.20	0.01	0.33	0.01	0.11
Shannon-Wiener	2.47	2.62	0.10	2.41	2.42	2.81	0.11	0.20	0.01	0.33	0.01	0.11

[1]WDGS = wet distillers grains with solubles (corn-based, not exceeding 10% sorghum)
[2]*P*-value for the linear and quadratic effect of increasing WDGS inclusion level
[3]A:P = acetate:propionate

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Access to warm drinking water prevents rumen temperature drop without affecting in situ NDF disappearance in grazing winter range cows[1]

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ABSTRACT: Ingestion of large quantities of cold water or frozen forage may result in changes in temperature of ruminal contents. Rumen microorganisms may be sensitive to temperature changes in the ruminal environment. Therefore, this study was conducted to assess the variability in ruminal temperature and extent of in situ OM and NDF disappearance during winter in grazing range cows supplied with drinking water at either 8.2 ± 0.4˚C (cold) or 31.1 ± 1.3˚C (warm). Two adjacent paddocks (average 320 ha) were grazed from December through February in 2010-2011 by 24 pregnant range cows of which 4 were fitted with rumen cannulas at USDA-ARS Fort Keogh Livestock and Range Research Laboratory in Miles City, MT. Each paddock provided cold or warm stock water delivered in Ritchie waters. Warm water drinkers were heated by a Rheem outdoor tankless propane water heater. The four cannulated cows had Kahne rumen temperature continuous recording boluses (KB1000; recorded temperature at 5 min intervals) for 22 d in January. The recorded data were used to determine the frequency of timed events when rumen contents were below 37.9˚C. Two separate in situ trials were conducted 1 wk apart for approximately 72 h. Three nylon bags containing approximately 5g of winter range forage extrusa collected in November were placed in each rumen at 1400 h and incubated for 72 h for OM and NDF disappearance analysis. Cows in warm water paddocks had less ($P < 0.01$) variability in ruminal temperature than cows in the cold water paddocks. During a 22-d period only 1.5% of the time did ruminal temperature drop below 38˚C while the cows that had access to colder water were below 38˚C 9.4% of the time. In situ NDF and OM disappearances were not influenced by the temperature of water the cow had access to drink ($P \geq 0.64$). Results from this study show that daily rumen temperature is less variable when heated water is provided to cows grazing winter range with no influence on extent of in situ NDF and OM disappearance.

Keywords: Cows, Water Temperature, Water Intake

INTRODUCTION

Ingestion of large quantities of cold water or frozen forage may result in changes in temperature of ruminal contents. Rumen microorganisms may be sensitive to temperature changes in the ruminal environment. Cold shocks may decrease microbial activity, thereby reducing the number of microbes (Hungate, 1966). A decrease in rumen temperature may also impair forage digestion. Rumen microbial attachment to fibrous substrates has been reported to be optimal at 38°C, with lower or higher temperatures markedly reduce adhesion (Roger et al., 1990). In vitro NDF disappearance decreases when water bath temperature is below 39°C (Reil et al., 2011).

Our hypothesis was that cows provided warm drinking water would have increased NDF and OM disappearances and promote a stable temperature in the rumen. Therefore, this study was conducted to assess the variability in ruminal temperature and rate of in situ NDF disappearance during winter in grazing range cows supplied with drinking water at two different temperatures: 8.2 ± 0.4˚C (cold) or 31.1 ± 1.3˚C (warm).

MATERIALS AND METHODS

This study was conducted in at the USDA-ARS Fort Keogh Livestock and Range Research Laboratory near Miles City, MT. Two adjacent paddocks (average 320 ha) were grazed from December through February in 2010-2011 by 24 pregnant range cows of which 4 were fitted with rumen cannulas. One paddock provided 8.2 ± 0.4˚C (cold) water and the other provided 31.1 ± 1.3˚C (warm) stock water delivered in Ritchie waterers (Conrad, IA). Warm water drinkers were heated by a Rheem outdoor tankless propane water heater. The four cannulated cows had Kahne (Auckland, New Zealand) rumen temperature continuous recording boluses (KB1000; recorded temperature at 5 min intervals) for 22 d in January. The recorded data were used to determine the frequency of timed events when rumen contents were below 38˚C.

Two separate in situ trials were conducted 1 wk apart for approximately 72 h. Extrusa samples were collected in November from 4 ruminally canulated cows grazing native range. Ruminal contents were removed from the cows and stored. The ruminal walls were sponged dry to remove any moisture, as described by Lesperance et al. (1960). The cows were allowed to graze in the experimental pastures for 30 min. After the 30-min grazing period, the extrusa was removed from the rumen and thoroughly mixed. An aliquot was saved for analysis and the original ruminal contents were replaced. The

[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 authors and does not imply its approval to the exclusion of other products that may be also suitable.

aliquot was frozen at -20°C, lyophilized, and passed through a 1mm screen. Samples were analyzed for DM, OM (AOAC 1990) and NDF (Goering and Van Soest, 1970). Ground extrusa samples (5 g) were placed in triplicate Dacron bags (10 × 20 cm; pore size = 53 ± 10 µm; Ankom Technology Corp., Fairport, NY). Triplicate bags containing ground extrusa, as well as empty, sealed Dacron bags (i.e., blanks) were placed into 60 × 60-cm zippered laundry bags with an attached cord. Dacron bags (5/cow) containing ground extrusa samples and blank bags (2/cow) were placed into the rumen for 72 h of incubation. The 19-L bucket was filled with cold water to stop fermentation. Upon removal from the rumen, the bags were rinsed by submerging them 3 times in a 19-L bucket. Bags were individually rinsed in cold tap water until the effluent was clear, after which the bags were frozen (−20°C), lyophilized, and weighed. The amount of residue in the blank Dacron bag was subtracted from each sample bag. Residue remaining in the bag was analyzed for DM, OM, and NDF, and NDF disappearance was calculated.

Statistical Analysis. Rumen temperature was analyzed as the percentage of time above 38°C using Proc Freq of SAS (SAS Institute, Cary, NC). Neutral detergent fiber and OM disappearance data were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC). The model for NDF and OM disappearances included pasture, water temperature, and their interaction with cow utilized as a blocking term. Mean separation was conducted with pdiff. Significance was determined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Cows grazing paddocks with access to warm water had less ($P < 0.01$; Table 1) variability in ruminal temperature than cows in the cold water paddocks. During a 22-d period only 1.5% of the time did ruminal temperature drop below 38˚C for cows with access to warm water while the cows that had access to colder water were below 38˚C 9.4% of the time. Cows in cold water paddocks ranged in rumen temperature from 31.6°C – 40.8°C, whereas cow in warm water paddocks ranged from 34.5°C – 40.6°C. Warm water allows the rumen temperature to be more stable while providing a consistent optimal temperature for microbial attachment to forage (Roger et al. 1990). In a corresponding study, Petersen et al. (2011) reported cows drank more warm (27.7 L/d) water than cows on cold (19.5 L/d) water. However, in situ NDF and OM disappearances were not influenced by the temperature of water the cow had access to drink ($P \geq 0.63$; Table 1). In support, Cunningham et al. (1964) reported no effects on digestion occurred when cows consumed either extremely cold (1.1°C) or warm (39.4°C) water. Brod et al. (1982) reported that water temperature (0, 10, 20 or 30°C) did not significantly influence crude fiber digestibility, however, results showed a consistent trend for digestion coefficients to be lowest in the 0°C water treatment. However, Reil et al. (2011) showed that in vitro NDF disappearance decreases from 41 to 14 % when water bath temperature decreases from 39 to 31°C. Thus, time spend below 38°C may not have been sufficient enough to impact NDF degradation.

Results from this study shows that cows grazing range and coping with climatic low winter temperatures are found to have daily rumen temperature above 37°C 98% of the time when heated water is provided to cows grazing winter range with no influence on extent of in situ NDF and OM disappearance.

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Table 1. Effect of drinker water temperature on variability of rumen temperature and extent of in situ NDF and OM disappearance during winter in grazing range cows

	Water Temperature[1]			
Measurement	Warm	Cold	SEM	*P*-value
Freq Above 38°C, %	98.5	90.6	--	< 0.01
NDF disappearance, %	57.5	58.9	2.9	0.77
OM disappearance, %	57.8	59.7	2.4	0.64

[1]Drinker water temperature; Warm = 31.1 ± 1.3˚C; Cold = 8.2 ± 0.4˚C

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GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF BEEF STEERS GRAZING TALL FESCUE WITHOUT OR WITH NITROGEN FERTILIZATION

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ABSTRACT: A grazing study was conducted to determine if growth performance and carcass characteristics of beef steers would be affected by N fertilization on tall fescue (TF) pasture. Eighteen Angus crossbred steers (394 ± 5.5 kg of BW) were grazed on the following two treatments: TF without N fertilizer (TF–NF) and TF with N fertilizer (TF+NF). A total of 168 kg/ha N fertilizer was applied in three split applications of 56 kg/ha to the TF+NF. The treatments were arranged in a randomized complete block design with 3 pasture replicates and 3 steers per pasture. Replicated 0.47-ha paddocks were established during spring 2010 and were grazed with beef steers from May through September 2010 for total of 16 wk. Grazing was for 7 d per paddock on a 28-d rotation interval. After the completion of 16-wk grazing, ultrasound measurements were performed to assess carcass characteristics. Intake of DM averaged 7.51 kg/d throughout grazing, and it did not differ between treatments ($P > 0.26$). In response to N fertilization, BW and ADG of grazing steers also did not differ ($P > 0.16$). With progression of grazing season, ADG and G:F gradually declined regardless of treatments. Ruminal pH averaged 7.27, and it was similar between treatments. Concentration of ruminal ammonia-N was increased due to N fertilization ($P < 0.01$), indicating that there was an imbalance between dietary N and energy on TF+NF during the grazing season. Sizes of rump and rib fat, rib eye area, and intramuscular fat percentage did not differ between grazing treatments. Overall results of this grazing study showed that applying N fertilizer to TF did not influence growth performance and carcass characteristics of grazing beef steers.

Key words: grazing beef steers, tall fescue, N fertilization

Introduction

Tall fescue (**TF**; *Festuca arundinacea*) is a coarse-textured grass that is weakly rhizomatous and is considered a moderately drought tolerant turfgrass because of its deep root system (Christians, 2004). Tall fescue is tolerant of periodic drought, low fertility, and fluctuating seasonal temperatures, and thus it is a popular pasture grass in the Intermountain West. The excellent agronomic performance of this cool-season grass has been well documented (Bacon, 1995; Bowman et al., 2006; Teuton et al., 2007). The amount of N fertilization has significant impacts on DM yield and N concentration of TF (Berg and Sims, 2000; Teuton et al., 2007). Wolf and Boberfeld (2003) reported that application of N fertilizer in two split applications resulted in higher CP concentration of TF, and its effect was cumulative, causing higher rate of CP increase at later N application.

Although N fertilization improves biomass production and N concentration of TF, this process demands energy and leads to lower concentrations of non-fibrous carbohydrates (**NFC**), causing increased fiber concentration and decreased digestibility. For example, Peyraud et al. (1997) reported that N fertilizer treatment increased NDF and ADF from 50 to 53% and 25 to 28%, respectively, while Probasco and Bjugstad (1980) found that IVDMD decreased from 63 to 60% due to N fertilization. Forages containing high CP concentrations are often considered to supply CP required by rapid growing ruminants, but its utilization efficiency depends on several factors such as carbohydrate availability. Berg and Sims (1995) indicated that steer BW gain/ha during the 4 summer grazing seasons increased with N fertilization at rates up to 68 kg N/ha when animals were grazed in Old World bluestem (*Bothriochloa ischaemum*) pasture. The positive response in BW gain to pasture N fertilization may have resulted from increases in forage production and ADG. Berg and Sims (2000) also reported that BW gain of steers responded linearly to N fertilization with an increase of BW gain from 185 to 416 kg/ha due to 68 kg N/ha/yr.

Research on forage quality and animal performance often targets ways of improving yield and N utilization efficiency in pastures. Much is known about fertilizer type, amount, and timing of application for maximizing crop yield. However, major gaps still exist in our knowledge on the relationships between application of N fertilizer and its impacts on the growth performance of beef steers. The objective of this grazing study was to test our overall hypothesis that fertilizing TF with N would improve the growth performance and affect carcass quality of beef steers.

Materials and Methods

Animals and experimental design. A grazing experiment was conducted at the Lewiston Pasture Research Farm at Utah State University, from mid-May through mid-September 2010. Eighteen Angus crossbred steers (394 ± 5.5 kg of BW) were randomly assigned into 6 paddocks where each paddock had 0.47 ha pasture. Two treatments were assigned in a randomized complete block design, resulting in 3 replications per treatment with 3 steers per paddock. The following 2 treatments were tested: TF without N fertilizer (**TF–NF**) and TF with N fertilizer (**TF+NF**). A total of 168 kg/ha N fertilizer was applied in 3 split applications of 56 kg/ha to the TF+NF. Each pasture was divided into 4 equal-size paddocks (51 × 23 m) with a

single strand of polywire electrified by a battery-powered fence charger. Each paddock was grazed for 7 d, and then the same paddock was rested for 21 d until wk 12. However, starting on wk 13, each paddock was grazed for 3 to 5 d, and then it was rested for 9 to 15 d until the end of grazing study due to pasture availability. All animals were rotated to new paddocks between 0900 and 1000 on weekly basis. All steers had *ad libitum* access to fresh water and mineral supplement (Right Now® Emerald, Cargill Animal Nutrition, Minneapolis, MN). All steers were weighed every 4 weeks to determine BW (Table 1).

Table 1. Weeks and dates for pasture sampling in 2010

Wk	Dates
4	May 18 to June 15
8	June 16 to July 13
12	July 14 to August 10
16	August 11 to September 7

Intake of DM, pasture sampling, and analysis. Pasture intakes were measured according to the herbage disappearance method based on the difference between pre- and post-grazing herbage mass (Lantinga et al., 2004). Intake of DM was estimated using rising plate meter (**RPM**; Research Park UMG, Columbia, MO) on weekly basis. Seven measurements were obtained on each paddock at pre- and post-grazing prior to animal rotation to determine the total DMI per paddock. Pasture sampling for DM yield calibration was done every 4 wk on each paddock (Table 1). The total DM yield (DM_{yield}) per paddock was calculated by using the equation: $DM_{yield} = RPM_{average} \times DM_{cal} \times Area_{paddock}$, where $RPM_{average}$ = average of RPM height (cm), DM_{cal} = DM yield calibration per quadrat (g/cm), and $Area_{paddock}$ = area of paddock on each week (1,165 m^2). Sampling of herbage mass (pre- and post-grazing) occurred weekly by clipping 6 0.102-m^2 quadrats in each plot before and immediately after the grazing period. Clippings were taken to ground level with the aid of battery-powered portable mower (SSC 1000, Black & Decker, Inc., Towson, MD), and care was taken to avoid soil contamination. Clippings were made at the same time of the day, approximately 0900 on one day. Samples were placed in paper bags and immediately transported to the laboratory. Samples were weighed, dried at 60°C for 48 h, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Philadelphia, PA), and stored for subsequent analyses. Analytical DM concentration of samples was determined by oven drying at 135°C for 3 h; OM was determined by ashing, and N concentration was determined using an elemental analyzer (LECO TruSpec N, St. Joseph, MI) (AOAC, 2000). The NDF and ADF concentrations were sequentially determined using an ANKOM$^{200/220}$ Fiber Analyzer (ANKOM Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination and pre-treatment with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO).

Analysis of ruminal fluid and carcass characteristics. Ruminal fluid samples were obtained using Geishauser probe on wk 4, 12, and 16. The pH of the ruminal fluid was measured within 5 min of collecting the samples using a portable pH meter (Oakton pH 6; Oakton Instruments, Vernon Hills, IL). Five milliliters of the ruminal fluid were mixed with 1 mL of 1% sulfuric acid and stored frozen (-40°C) for ammonia-N (**NH_3-N**) analysis. Concentration of NH_3-N in the ruminal contents was determined as described by Rhine et al. (1998), using a plate reader (MRX^e, Dynex Technologies, Chantilly, VA).

Table 2. Nutrient concentration of tall fescue grass

	Treatment[1]			
Item	TF–NF	TF+NF	SE	*P*
DM, %				
Wk 4	20.5	19.1	0.73	0.26
Wk 8	22.4	22.8	0.77	0.73
Wk 12	26.3	24.0	0.73	0.09
Wk 16	30.3	30.8	0.57	0.63
OM, % DM				
Wk 4	85.7	85.5	0.27	0.67
Wk 8	84.9	85.1	0.19	0.64
Wk 12	84.7	85.3	0.22	0.12
Wk 16	86.4	86.7	0.38	0.57
CP, % DM				
Wk 4	12.5	13.0	0.74	0.64
Wk 8	9.1	10.2	0.81	0.40
Wk 12	10.9	12.1	0.81	0.32
Wk 16	7.3	9.8	0.81	0.32
NDF, % DM				
Wk 4	57.3	59.3	0.54	0.06
Wk 8	62.2	63.2	0.63	0.31
Wk 12	65.3	67.0	0.51	0.08
Wk 16	63.9	64.1	0.30	0.68
ADF, % DM				
Wk 4	30.1	31.3	0.38	0.09
Wk 8	33.4	34.1	0.48	0.39
Wk 12	35.6	36.7	0.40	0.13
Wk 16	33.4	33.7	0.37	0.68

[1]TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer.

At the end of the grazing period, steers were scanned using ultrasound (Aloka SSD-500V, Wallingford, CT) to determine the carcass characteristics.

Statistical analysis. All data in this study were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC) with paddock as an experimental unit. The model included treatment as a fixed effect. Least squares means were generated and separated using the PDIFF option of SAS for the main effect. Significant effects of the treatment were declared if $P < 0.05$, and trends were accepted if $0.05 < P < 0.15$.

Results

The DM of TF showed an increasing trend throughout grazing regardless of N fertilization (averaged from 19.8 to 30.6%), with a tendency to decrease with N fertilization on

wk 12 ($P = 0.09$; Table 2). Concentration of CP was similar between treatments throughout the grazing season, but the difference between the treatments seems to be gradually increased because of N fertilization, as grazing was progressed: 0.5, 1.1, 1.2, and 2.5 percentage units on wk 4, 8, 12, and 16, respectively. On wk 4 and 12, NDF and ADF concentrations tended ($P < 0.13$) to increase in response to applying N fertilizer.

Table 3. Effect of N fertilization of tall fescue on growth performance of grazing beef steers

	Treatment[1]			
Item	TF–NF	TF+NF	SE	*P*
DMI, kg/d				
4	7.19	6.70	0.263	0.26
8	8.69	8.59	0.155	0.70
12	7.68	7.96	0.260	0.50
16	6.56	6.74	0.303	0.70
BW, kg				
0	405	400	6.2	0.61
4	442	433	4.7	0.26
8	476	459	6.7	0.16
12	481	485	7.1	0.69
16	493	502	7.9	0.43
ADG, kg/d				
4	1.06	0.95	0.055	0.23
8	1.42	1.24	0.106	0.30
12	0.85	0.91	0.108	0.70
16	0.63	0.68	0.122	0.79
G:F				
4	0.149	0.142	0.0116	0.72
8	0.164	0.144	0.0142	0.38
12	0.111	0.115	0.0131	0.87
16	0.097	0.101	0.0209	0.91

[1]TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer.

Table 4. Effect of N fertilization of tall fescue on ruminal pH and ammonia-N (NH_3-N) concentration of grazing beef steers

	Treatment[1]			
Item	TF–NF	TF+NF	SE	*P*
Ruminal pH				
Wk 4	7.41	7.32	0.060	0.35
Wk 12	7.32	7.38	0.074	0.62
Wk 16	7.08	7.11	0.070	0.73
NH_3-N, mg/dL				
Wk 4	5.64	8.47	0.306	<0.01
Wk 12	9.46	12.29	0.391	<0.01
Wk 16	12.3	19.0	0.29	<0.01

[1]TF–NF= tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer.

Intake of DM averaged 6.95, 8.64, 7.82, and 6.65 kg/d on wk 4, 8, 12, and 16, respectively, and N fertilization on TF did not affect DMI throughout grazing (Table 3). In addition, applying N fertilizer on TF did not influence BW, ADG, and G:F. The ADG and G:F reached their maximum on wk 8 regardless of treatment (1.33 and 0.154 on average, respectively). Starting on wk 12, DMI, ADG, and G:F gradually decreased until the completion of this grazing study.

Ruminal pH averaged 7.27 throughout grazing, and it was not affected by N fertilization (Table 4). Progression of grazing gradually increased ruminal NH_3-N concentration regardless of N fertilization, and steers grazed TF+NF had higher NH_3-N concentration compared with those grazed TF–NF. However, a difference on the NH_3-N concentration due to N fertilization was greater with progression of grazing: 2.83 and 6.70 mg/dL on wk 12 and 16, respectively. Application of N fertilizer on TF did not influence carcass characteristics (back fat, rib fat, rib eye area, and intramuscular fat) of the beef steers (Table 5).

Table 5. Effect of N fertilization of tall fescue on carcass characteristics of grazing beef steers

	Treatment[1]			
Item	TF–NF	TF+NF	SE	*P*
Back fat, cm	0.21	0.23	0.011	0.45
Rib fat, cm	0.21	0.23	0.007	0.39
Rib eye area, cm²	10.6	9.87	0.361	0.35
Intramuscular fat, %	4.19	4.25	0.032	0.88

[1]TF–NF = tall fescue without N fertilizer; TF+NF = tall fescue with N fertilizer.

Discussion

Although we did not observe effect of applying N fertilizer to TF on CP concentration, a difference of CP concentration between TF–NF vs. TF+NF increased from wk 4 to 16. This data indicate that by applying 168 kg N/ha in 3 different times on TF contributed to increasing CP concentration of TF in a cumulative manner. Similarly, Wolf and Boberfeld (2003) observed cumulative effect of N fertilization on CP concentration of TF when 50 kg N/ha were applied at two different times, but the authors reported that when more than 100 kg N/ha were applied, there was no cumulative effects of CP concentration on TF. The contradictory result between the two studies may be related to time of N fertilization and its application frequency (twice vs. three times).

In our study, fiber concentrations were in peak on wk 12, whereas NFC concentration (100 – CP – NDF – crude fat – ash) was the lowest on wk 12 (5.89 and 3.38% to TF–NF and TF+NF, respectively). Rapid growth of plants demands high energy input, which typically leads to less NFC retention in the plant tissue (Lechtenburg et al., 1972).

Intake of DM in both treatments was not different, and it reached in peak on wk 8, and then decreased until the end of grazing study. The decreasing trend after wk 8 is likely induced by the increasing CP and decreasing NFC concentration of TF during the grazing study. Peyraud et al. (1997) noticed that DMI decreased when CP concentration increased, but soluble carbohydrate decreased. Progression of grazing season resulted in gradually decreased ADG due to increased energy demand of rapid growing animals. Wolf and Boberfeld (2003) reported that the energy concentration of TF decreased throughout grazing season. The NFC concentration in our study decreased from 10.9 to 4.64% from wk 4 to wk 16.

Applying N fertilizer on TF influenced dietary N utilization in the rumen as indicated by increased NH_3-N concentration throughout grazing in the current study. Increased difference on NH_3-N between TF–NF and TF+NF with advance on grazing implies that dietary N utilization was less efficient in response to N fertilization. Satter and Slyter (1974) suggested 5 mg/dL of NH_3-N as the minimum required for optimal microbial growth. In our study, NH_3-N concentration was maintained over the minimum requirement regardless of grazing treatment, indicating that the NH_3-N concentration would not be a limiting factor for microbial growth. Increased ruminal NH_3-N concentration in TF+NF may reflect reduced ruminal capture of the NH_3-N for microbial protein synthesis. In order to optimize dietary N utilization in ruminants, protein degradation in the rumen should be decreased, whereas N use by ruminal microbes must be increased (Hoover and Stokes, 1991). It is believed that energy is the most limiting factor in microbial growth (Bach et al., 2005), and consequently decreased NFC as a proportion of carbohydrate may contribute to increased difference on NH_3-N concentration between TF–NF and TF+NF.

The carcass characteristics support the performance data of grazing steers, with no difference between treatments. All steers achieved similar feeding endpoints, and it appears that N fertilization of TF did not have any impact on the carcass characteristics when N fertilizer was applied at 168 kg/ha.

Implications

No beneficial effect on growth performance of grazing beef steers in response to N fertilization on TF pasture in this first year study is likely to be caused by residual N in the soil, which would be often the case for the seedling year. This condition may dilute the overall effects of applying N fertilizer on TF pasture. High fertilizer costs and emphasis on environmental stewardship have required better utilization of dietary N by ruminants. Increased ruminal NH_3-N concentration by fertilizing N on TF found in this study indicates that there is a need to supplement NFC in diets to improve utilization of increased dietary CP due to N fertilization for optimizing microbial protein synthesis.

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THE EFFECTS OF AGE AT WEANING AND POST-WEANING MANAGEMENT ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF BEEF STEERS

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ABSTRACT: To assess impacts of weaning date and post-weaning management on carcass quality and feedlot performance, eighty steer calves were randomly assigned to be weaned at 120 (EW) or 205 (NW) days of age. Early weaned calves were immediately acclimated to a typical corn-based high concentrate diet targeting an ADG of 1.4 kg. At 205 d of age, EW steers were randomly assigned to either remain on a high concentrate diet until finish (n=20; EWF), or a low input management system grazing corn crop residue for 60 d and then returned to a high concentrate corn based diet until finish (n=20; EWC). Normal weaned steers were randomly assigned to either a typical corn based feedlot ration (n=20; NWF) or a low input management system where they were allowed to graze corn crop residue for 60 d then returned to a high concentrate corn based diet until finish (n=20; NWC). All steers remained on study until a 12th rib fat thickness of 1.3 cm was achieved. Individual feedlot performance was collected for 90 days during the finishing period utilizing Growsafe technology. Dry matter intake during the Growsafe period tended (P=0.09) to be greater for calves that were maintained in the feedlot rather than grazed corn stalks, while G:F tended (P=0.10) to be greater for calves that had grazed corn stalks. Total number of days on feed was greater ($P < 0.01$) for the EW calves compared to the NW calves, as well as calves that were maintained in the feedlot compared to those allowed to graze cornstalks. Calves that were weaned early and kept in the feedlot (EWF) tended to have heavier (P=0.09) HCW compared to NWC and EWC calves. However, no differences were detected for ADG (P=0.41), 12th rib fat depth (P=0.72), LM area (P= 0.54), YG (P=0.77), marbling score (P=0.45), or quality grade (P=0.50) due to weaning date or nutritional strategy. This data suggest that placing weaned beef calves on a low plane of nutrition for 60 d prior to feedlot entry will not negatively affect feedlot performance or carcass characteristics.

Key words: Early wean, low input, carcass characteristics

Introduction

The recent surge in grain prices has placed a heavy financial burden on feedlot operators. Current feeder calf production systems rely heavily on feeding high levels of corn to increase carcass quality. While this system has been effective in producing quality carcasses (high marbling) at an acceptable body weight for over a decade, high input costs and grain prices will force a philosophical change in the industry towards low input systems that produce high quality cattle grown to equivalent market weights. Forage-based development programs will be necessary if the U.S. is to remain a leader in world markets. Since early weaning from 120 days until about 205 days provide an excellent window for nutritional management to improve marbling, we believe feeding corn based diets to early weaned calves followed by a period of slow growth that allows for "compensatory" skeletal growth will create equivalent sized market cattle with higher quality grades at lower input costs. Utilizing existing low cost feed resources such as crop residues and standing winter forage can result in a savings of approximately $60 per steer depending on feeding period length and feed costs. The savings anticipated with such a shift in management practices could prove to be the difference between negative and profitable feeding margins. Research has suggested that marbling score correlates more closely to the number of adipocytes/gram of tissue than to the diameter of the adipocytes (Cianzio 1985). Other research has reported that grain-fed cattle have smaller but more numerous adipocytes than forage-fed cattle (Prior 1983). The primary substrate for intramuscular fat deposition is glucose, which differs from the use of acetate to synthesize subcutaneous fat. Wertz et al. (2002) surmised that feeding grain to younger cattle may stimulate the onset of marbling and their research proved that early weaning heifers and finishing them in an accelerated program resulted in i.m. fat deposition with more efficient gains than cattle grown on pasture and finished as 2-year-olds. Early weaned cattle have

consistently proved to have greater gains, less intake, greater efficiency, and reduced slaughter age along with comparable carcass characteristics to cattle grown on grass and finished on corn (Myers 2005, 1999 a,b,c). Therefore, our hypothesis was that the beneficial effects of high grain diets on adipocyte development could be maintained regardless of nutritional plane later in life.

Materials and Methods

Steers and Diets. Eighty Angus based steer calves raised at the University of Wyoming were assigned to two weaning strategies. Calves were born between March 1 and April 30, 2009and all pairs were managed as a common group prior to the initiation of the study. Calves assigned to the early weaned (EW) strategy (n=40) were weaned on July 17, 2009 at an average of 120 days of age. The steer calves in the EW were transported to the University of Wyoming Sustainable Agriculture Research and Extension Center (SAREC) in Lingle, Wyoming and placed in the feedlot. The EW steer calves were acclimated to a typical corn-based high concentrate diet targeting an ADG of 1.4 kg for 90 days. At 205 d of age, the EW steers were reassigned to one of two nutritional management programs in a 2X2 factorial designed study. Twenty steers remained on the high-concentrate diet from the initial EW phase until they achieved a 12th rib fat thickness of 1.3 cm (EWF) while the remaining 20 were placed on a low input management system where they were allowed to graze corn crop residues for 60 days (EWC). Due to the cold, wet conditions steers were supplemented for the last 28 days of residue grazing with alfalfa hay fed three times per week to achieve average daily supplemental hay intakes of 4 lb/hd for the initial 14 days, followed by 10 lb/hd during the final 14 days. At 275 d of age the EWC steers were returned to the feedlot and fed a high-concentrate corn based diet until they achieved a 12th rib fat thickness of 1.3 cm. The second half of the EW steers

The forty calves assigned to the normal wean (NW) strategy were weaned October 22, 2009 at an average of 205 d of age. Similar to the EW group, twenty of the NW steers were placed on a backgrounding diet for 45 d to acclimate to a corn-based high concentrate finishing diet (85% corn) and fed until they achieved a 12th rib fat thickness of 1.3 cm (NWF). The remaining 20 NW steers were placed on a corn-based ration in the feedlot until the corn crop residues were available for grazing and then were allowed to graze the residue for 60 d before returning to the feedlot (EWC), where they were acclimated to a high concentrate diet and fed until they reached a 12th rib fat thickness of 1.3 cm.

Individual feedlot performance was collected from steers for a sixty day period utilizing Growsafe (Airdrie, Alberta, Canada) technology. Steers were ultrasounded to assess accretion of marbling, backfat, and 12th rib muscle area. Daily feed intake, event feed intake, ADG, net feed efficiency, and RFI were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Differences between steer treatment groups were tested using an LSD.

Collection of Ultrasound Data. Real-time ultrasound was used throughout the growing and finishing phase to monitor longisimus dorsi (Ribeye) area, intramuscular and subcutaneous fat deposition over the course of the study. Images were collected by a certified technician, and were interpreted using Beef Image Analysis chuteside software. Ultrasound measurements were recorded at 5 times during the feeding period for EW and NW calves. Ultrasound measurements for the EW steers were recorded at 56, 222, 284 (EWC only), 334 and 404 d in the feedlot and actual carcass measurements were recorded at slaughter. Ultrasound measurements for the NW steers were recorded at 33, 124, 186 (NWC only), 236 and 306 d in the feedlot and actual carcass measurements were recorded at slaughter.

Collection of Carcass Data. All steer calves were shipped to a commercial packing plant (Cargill Beef, Ft. Morgan, CO.) for harvest and collection of individual carcass data.

Statistical Analysis. Data were analyzed as a 2 x 2 factorial designed experiment using the MIXED procedure of SAS. Factors were weaning date (early and normal) and plane of nutrition after weaning (feedlot or corn stalks). Statistical significance was declared at $P < 0.05$ with a tendency towards significance at $P < 0.10$.

Results and Discussion

Dry matter intake (Table 1) during the Growsafe period tended (P=0.09) to be greater for calves that were maintained in the feedlot rather than grazed corn stalks, while G:F tended (P=0.10) to be greater for calves that had grazed corn stalks. Schoonmaker et al. (2002) reported that early-weaned calves had the greatest efficiency, followed by calves that entered the feedlot at 202 d of age, and yearlings had the lowest feed efficiency ($P < 0.01$), which was in agreement with Myers et al. (1999a). Barker-Neef et al. (2001) reported an 18% gain:feed ratio for the early-weaned steers in their study for the finishing period; from d 0 to d 100 of the finishing period the EW steers were 28% more efficient than the NW steers.

Total number of days on feed was greater ($P < 0.01$) for the EW calves compared to the NW calves, as well as calves that were maintained in the feedlot compared to those allowed to graze cornstalks. Myers et al. (1999) reported that steers fed high concentrate diets from weaning until slaughter were 37 d younger than steers allowed to graze pasture after weaning prior to entering the feedlot. Contrarily, Schoonmaker et al. (2002) reported that early-weaned cattle spent the greatest amount of time in the feedlot, followed by cattle that entered at 202 d of age and yearlings spent the least amount of time to achieve a similar fat thickness. However, ADG was greatest for early-weaned calves, intermediate for calves placed into feedlot at 202 d of age and lowest for yearlings. Barker-Neef et al. (2001) reported that early-weaned steers had greater ADG from time of early weaning to normal weaning and were 18% heavier at time of normal weaning than normally weaned calves. They reported that the normally weaned calves tended to have greater ADG for the finishing period (weaning to harvest) than the early weaned calves; this tendency was attributed to the fact that the normally weaned steers had faster BW gains early in the finishing period.

Calves that were weaned early and kept in the feedlot (EWF) tended to have heavier (P=0.09; Table 2) HCW compared to NWC and EWC calves. However, no differences were detected for ADG (P=0.41), 12th rib fat depth (P=0.72), LM area (P= 0.54), YG (P=0.77), marbling score (P=0.45), or quality grade (P=0.50) due to weaning date or nutritional strategy. Myers et al. (1999) reported no differences between growing treatments for carcass weight; fat thickness; LM area; yield grade; or marbling score.

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Table 1. Feedlot performance of steers

	Early Weaned[a]		Normal Weaned[b]			
Item	EWF[c]	EWC[d]	NWF[e]	NWC[f]	SE	P value
Initial Wt, lbs	328	326	521	521	14.4	>.01
Final Wt., lbs	1411	1363	1378	1335	38.3	.61
DMI[g], lbs	22.73	24.01	21.18	23.61	0.584	.09
ADG, lbs	2.68	2.57	2.80	2.67	0.085	.41
G:F[h]	.12	.11	.13	.11	.098	.12
Days on Feed	419	347	319	254	1.82	<.01

[a] Weaned at an average of 120 d of age.
[b] Weaned at an average of 205 d of age.
[c] At 205 d of age EWC steers were allowed to graze corn crop residues for 60 d and then returned to a corn-based high concentrate finishing diet (85% corn) until a 12th rib fat thickness of 1.3 cm was achieved.
[d] EWF steers remained on the high concentrate diet from the initial EW phase until they achieved a 12th rib fat thickness of 1.3 cm.
[e] NWF steers were placed on a back grounding diet for 45 d to acclimate to a corn-based high concentrate finishing diet (85% corn) and fed until a 12th rib fat thickness of 1.3 cm was achieved.
[f] NWC were allowed to graze corn crop residues for 60 d and then were placed in the feedlot on the corn-based high concentrate finishing diet (85% corn) until a 12th rib fat thickness of 1.3 cm was achieved.
[g] DMI was calculated for period that steers were in GrowSafe feeding system (3/9/2010-6/9/2010).
[h] G:F ratio was calculated for period 3/9/2010 to 6/9/2010 while in GrowSafe feeding system.

Table 2. Carcass Characteristics of Steers

	Early Weaned[a]		Normal Weaned[b]			
Item	EWF[c]	EWC[d]	NWF[e]	NWC[f]	SE	P value
HCW, lbs	874	821	842	827	18.22	.088
12th rib fat depth, in	.50	.51	.53	.48	.031	.715
LM area, in^2	12.3	12.84	12.45	12.34	.333	.537
YG	3.5	3.2	3.4	3.3	.077	.768
Marbling score	667.0	628.9	639.4	599.3	32.21	.446
Quality Grade	18.3	17.8	17.9	17.5	.379	.504

[a] Weaned at an average of 120 d of age.
[b] Weaned at an average of 205 d of age.
[c] At 205 d of age EWC steers were allowed to graze corn crop residues for 60 d and then returned to a corn-based high concentrate finishing diet (85% corn) until a 12th rib fat thickness of 1.3 cm was achieved.
[d] EWF steers remained on the high concentrate diet from the initial EW phase until they achieved a 12th rib fat thickness of 1.3 cm.
[e] NWF steers were placed on a back grounding diet for 45 d to acclimate to a corn-based high concentrate finishing diet (85% corn) and fed until a 12th rib fat thickness of 1.3 cm was achieved.
[f] NWC were allowed to graze corn crop residues for 60 d and then were placed in the feedlot on the corn-based high concentrate finishing diet (85% corn) until a 12th rib fat thickness of 1.3 cm was achieved.
[g] Marbling score: 400=Slight 0, 450=Slight 50, 500=Small 0, etc.
[h] Quality Grade: 15=Select, 16=Select+, 17=Choice-, 18=Choice0, 19=Choice+, etc.

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EFFECT OF LEVEL OF DRY DISTILLERS GRAINS PLUS SOLUBLE AND SUPLEMENTATION OF ORGANIC COPPER ON FATTY ACID COMPOSITION IN FEEDLOT LAMBS

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ABSTRACT: The objective was to evaluate the effect of corn dry distillers grains plus solubles (DDGS) and chelated Cu on fatty acids in muscle and adipose tissue of feedlot lambs. Thirty Rambolliet lambs (27.8 ± 1.47 initial BW, and 60 d of age approx.) were used in a 63-d finishing experiment. 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, and 32% DDGS plus 1.008 mg of Cu (32Cu). Fatty acids were determined in semitendinosus (ST) and longissimus dorsi muscles, Liver (L), adipose tissue from the tail head (THAT), adjacent to the 12th rib (AT), and KPH. Data were analyzed in a completely randomized design. Orthogonal contrast was used to test for linear, quadratic and cubic effects of increasing DDGS amounts. Also, orthogonal contrast was used to compare 32 vs 32Cu. Longgisimus dorsi and AT fatty acids profiles were not affected ($P \geq 0.05$) with increasing DDGS level. In ST, C16:1 increased (cubic, $P < 0.05$) and C18:2n6 and polyunsaturated fatty acids (PUFA) increased (linear, $P < 0.05$) with increasing DDGS amount. For L, CLAc9t11 increased ($P = 0.04$) quadratically and C20:5n3 increased ($P = 0.02$) linearly with increasing DDGS amount. Data for KPH showed that CLAt10c12 increased linearly ($P = 0.02$) and C20:5n3 increased quadratically ($P = 0.01$) with increasing DDGS amount. For THAT C18:2n6, C20:4n6, PUFA, and total unsaturated fatty acids increased linearly ($P \leq 0.03$), and C14:0, C16:0 and C18:3n3 increased quadratically ($P \leq 0.02$) with increasing DDGS amount. Also, concentration of C16:0and 22:5n6 were greater ($P \leq 0.04$) for 32 than 32Cu. Feeding lamb with DDGS during this productive stage resulted in increasing PUFA content in fatty acid profile which is considered a favorable response for human diets.
Key Words: fatty acids, DDGS, lambs

INTRODUCTION

The current availability and price of dried distillers grains (DDG) and their abundant energy and protein content (NRC, 2007) has increased the interest in using this product to decrease the cost of BW gain (Whitney and Lupton, 2010). Also, DDGS are competitively priced compared with other protein and energy sources. Protein and fat content of DDGS are 3-fold greater than that of corn (Stock et al., 2000). Because sulfuric acid is used in ethanol production, diets containing distillers grains may contain high concentration of S. Sulfur reduces Cu absorption because of the formation of copper sulfide in the gut (Suttle, 1974). Therefore, Cu might become deficient when high-S diets are fed. Copper is essential component of a number of enzymes including lysyl oxidize, cytochrome oxidize, superoxide dismutase, ceruloplasmin, and tyrosinase (McDowell, 1992).

Replacing dry rolled corn with DDGS in lambs feedlot diets can potentially modify energy density of the diet and increase the supply of unsaturated fatty acid, which in turn might alter the fatty acid composition of muscle and adipose tissue in lambs fed DDGS-containing diets. Previous studies demonstrate that dietary regimens can influence fatty acid composition (Rule et al., 1994; Mandell et al., 1997) and sheep (Boles et al., 2005; Kim et al., 2007). Fatty acid profile of beef cattle had been altered by feeding WDG (Kinman et al., 2011), and DDG (Gill et al., 2008). However, limited data exist on the impact of feeding DDGS and of Cu supplementation of DDGS-containing diets on tissue fatty acid profile of lambs fed feedlot finishing diets. Therefore, the objective of this experiment was to determine the impact of DDGS level and of Cu supplementation to a DDGS-containing diet on fatty acid profile of muscle, adipose tissue, and liver of lambs consuming an 80% concentrate corn-based diet.

MATERIALS AND METHODS

Animals were managed in accordance to an approved New Mexico State University Institutional Animal Care and Use Committee protocol. Thirty Rambouliet lambs (27.8 ± 1.47 kg initial BW, and 60 d of age approx.) were used in a 63-d finishing experiment. Lambs were castrated and were vaccinated against tetanus and enterotoxemia at 28 and 60 d of age. Lambs were maintained outdoors in individual pens (2 × 4 m), and had free access to water and their experimental diets. Treatments consisted of 4 levels (8, 16, 24, and 32% of diet DM) of DDGS replacing dry rolled corn, and 32% DDGS plus 1.008 mg of Cu (32Cu). All diets were composed of 15% sudangrass and 5% alfalfa hay as 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 D3, and 2.2 IU/g vitamin E).

Lambs were slaughtered and tissues were collected. Collected tissue samples included semitendinosus (ST) and longissimus dorsi muscles, liver (L), adipose tissue from the tail head (THAT), adjacent to the 12th rib (AT), and KPH.

Duplicate 100 mg muscle samples and 20 mg adipose tissue samples were subjected to direct saponification by incubating in 2.0 mL of 0.2 M methanoic plus 1 mL of 33% KOH in 16 mm × 125 mm screw-cap tubes at 50ºC for 30 min. Tubes were vortex-mixed two to three times/min until muscle was dissolved. Nonsaponified materials were extracted in hexane and discarded, and then 1.0 mL of 12.1 *N* HCl was added to neutralize the fatty acids for extraction in hexane. Fatty acids were extracted in 2.0 mL of hexane, transferred to clean tubes, and dried under N_2, and fatty acids methyl esters were prepared by incubating fatty acids in 2.0 mL of 1.09 *M* HCl in methanol at 70ºC for 1 h. Use of methanolic HCl for fatty acid methyl ester preparation was verified for analysis of CLA in muscle lipids by comparison with sodium methoxide in methanol.

Fatty acid methyl esters were transfer to GLC vials that contained a 1.0 mm bed of anhydrous sodium sulfate. Tridecanoic acid (13:0) was used as internal standard. Separation of fatty acid methyl esters was achieved by GLC (Model 5890 series II, Hewlett Packard; Avondale, PA) with a 100 m capillary column (SP-2560, Supelco, Bellefonte,PA) and He as a carrier gas at 0.5 mL/min. Oven temperature was maintained at 175ºC for 40 min and then ramped to 240ºC at 10º/min to 240ºC. Injector and detector temperatures were 250ºC. Identification peaks was accomplished using purified standards (Nu-Chek Prep, Elysian, MN; Matreya, Pleasant, PA).

Data were analyzed as a completely randomized design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Orthogonal contrast was used to test for linear, quadratic and cubic effects of increasing DDGS amounts. Also, orthogonal contrast was used to compare 32 vs 32Cu.

RESULTS AND DISCUSSION

Increasing level of DDGS had no effect on Longgisimus dorsi and AT fatty acids profiles ($P \geq 0.05$). For L, CLAc9t11 increased ($P = 0.04$) quadratically and C20:5n3 increased ($P = 0.02$) linearly with increasing DDGS amount. Data for KPH showed that CLAt10c12 increased linearly ($P = 0.02$) and C20:5n3 increased quadratically ($P = 0.01$) with increasing DDGS amount. In ST, C16:1 increased (cubic, $P < 0.05$) and C18:2n6 and polyunsaturated fatty acids (PUFA) increased (linear, $P < 0.05$) with increasing DDGS amount. For THAT C18:2n6, C20:4n6, PUFA, and total unsaturated fatty acids (TUFA) increased linearly ($P \leq 0.03$), and C14:0, C16:0 and C18:3n3 increased quadratically ($P \leq 0.02$) with increasing DDGS amount.

Increasing saturated fatty acids (SFA) in meat is undesirable because of negative effects on human health (i. e. increased blood cholesterol and incidence coronary health disease). In this study, SFA only increased for THAT (C14:0 and C16:0) by inclusion of DDGS in the diets. In agreement with other studies, Koger et al., (2010) observed an increase only in C18:0 and a decrease in 17:0 with increasing distiller grains concentration of finishing diets for steers. While, no effects of feeding distillers grains to beef cattle on SFA were reported by de Mello et al., (2008); and Gill et al., (2008). Even though ruminal microorganisms are responsible for CLA production, feeding distillers grains can increase concentration of CLA production. Distillers grains may favorably alter rumen environment for *Butyrivibrio fibrisolvens* or *Megasphaera elsdenii* (Kim et al., 2002). Although in the present study CLA concentration only increased in liver and KPH, other studies have reported increases in CLA concentration of tissue of cattle consuming distillers grains (Koger et al., 2010; de Mello et al., 2008).

Ruminants fatty acid profile is typically high in SFA and low in unsaturated fatty acids. Most of the fatty acids deposited on ruminants tissue are saturated by the hydrogenetion of unsaturated fatty acids by rumen bacteria. Prevention and treatment of coronary heart disease in humans have been linked with consumption of PUFA (Wijendran and Hayes, 2004). In the present study, feeding DDGS increased the concentration of some unsaturated fatty acids in most of the tissues analyzed. It is desirable to increase unsaturated fatty acids on tissue most consumed by humans as is the case of longissimus dorsi, and ST. However, in the present study, fatty acid profile of longgisimus dorsi was not affected by DDGS in the diet.

IMPLICATIONS

These results imply that feeding lamb with DDGS during this productive stage resulted in increasing PUFA content in fatty acid profile which is considered a favorable response for human diets. Therefore levels up 32% of DDGS in the diet are recommendable on finishing fed lambs.

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Table 1. Fatty acids profiles in different tissues of lambs fed with different levels of DDGS

Items	Treatments[1]					SEM[3]	*P*-value[4]	Contrast[2]			
	8	16	24	32	32Cu			LIN	QUAD	CUB	32 vs 32Cu
Adipose tissue[5]											
SFA	220.5	203	195.5	184.6	-	37.8	0.92	0.5	0.86	0.95	-
MUFA	75.8	54.9	47.2	61	-	9.9	0.22	0.09	0.74	0.19	-
PUFA	22.6	21.7	31.8	26.5	-	9.4	0.78	0.56	0.78	0.44	-
TUFA	58.4	76.7	78.9	87.5	-	17.4	0.61	0.2	0.74	0.72	-
Longgisimus dorsi[5]											
SFA	14.2	10.7	22	17.6	-	5.5	0.48	0.36	0.94	0.21	-
MUFA	1.6	1.6	5.2	4.6	-	1.7	0.27	0.09	0.85	0.29	-
PUFA	3.3	2.9	4.6	4.2	-	0.864	0.44	0.23	0.93	0.27	-
TUFA	4.8	5	9.9	8.8	-	2.5	0.28	0.11	0.87	0.26	-
Semitendinosus[5]											
C16:1	0.795	0.502	1.1	0.635	0.679	0.266	0.34	0.88	0.65	0.05	0.9
C18:2n6	0.51	0.553	1.3	1	0.903	0.273	0.10	0.03	0.48	0.1	0.7
SFA	6.7	5.4	12.1	8.6	7.1	2.86	0.34	0.25	0.64	0.09	0.7
MUFA	2.4	3	7.7	4.4	4.2	1.81	0.14	0.12	0.20	0.08	0.94
PUFA	0.875	0.934	1.9	1.5	1.4	0.34	0.09	0.03	0.45	0.09	0.76
TUFA	3.3	3.9	9.6	5.9	5.6	2.10	0.12	0.09	0.22	0.07	0.91
Liver[5]											
CLAc9t11	0.049	0.071	0.049	0.033	0.023	0.009	0.02	0.06	0.04	0.23	0.43
C20:5n3	0.056	0.051	0.039	0.034	0.036	0.008	0.16	0.02	0.98	0.62	0.86
SFA	6.1	7.7	6.9	5	4.6	1.04	0.17	0.33	0.07	0.77	0.74
MUFA	0.509	0.395	0.204	0.501	0.04	0.34	0.78	0.87	0.79	0.68	0.30
PUFA	3.2	3.7	3.7	3	3.3	0.539	0.72	0.79	0.19	0.91	0.6
TUFA	5.7	4.1	3.9	3.5	3.4	0.593	0.88	0.74	0.42	0.89	0.9
KPH[5]											
CLAt10c12	0.007	0.007	0.009	0.014	0.016	0.003	0.03	0.02	0.26	0.75	0.51
C20:5n3	0.014	0.004	0.005	0.009	0.006	0.003	0.03	0.18	0.01	0.4	0.5
SFA	35.4	32.1	33	32.3	27.4	4.09	0.64	0.54	0.68	0.68	0.36
MUFA	13.9	16.9	21	19.1	21.6	3.7	0.33	0.12	0.4	0.58	0.6
PUFA	3.1	3	3.5	3.7	3.3	0.463	0.64	0.15	0.73	0.61	0.51
TUFA	17	19.9	24.5	22.8	24.9	4.06	0.35	0.12	0.45	0.57	0.69
Tail Head[5]											
C14:0	0.871	1.5	1.3	1.1	0.706	0.162	0.01	0.46	0.01	0.33	0.11
C16:0	10.9	14.5	14.3	13.1	9.9	1.1	0.01	0.15	0.01	0.49	0.04
C18:2n6	1.3	2.2	2.2	2.3	1.7	0.258	0.03	0.01	0.08	0.33	0.13
C18:3n3	0.184	0.359	0.303	0.252	0.139	0.047	0.01	0.42	0.01	0.21	0.09
C20:4n6	0.128	0.155	0.168	0.178	0.151	0.019	0.25	0.03	0.61	0.87	0.29
22:5n6	0.15	0.241	0.21	0.216	0.131	0.027	0.02	0.11	0.08	0.14	0.03
SFA	17	23.8	22.6	22	16.1	2.18	0.03	0.11	0.06	0.31	0.06
MUFA	6.6	13.9	12.3	13.3	8.9	2.3	0.08	0.05	0.13	0.21	0.18
PUFA	1.8	2	2.9	2.9	2.2	0.285	0.01	0.01	0.03	0.22	0.06
TUFA	8.4	16.9	15.2	16.2	11.1	2.5	0.04	0.03	0.08	0.18	0.13

[1] Treatments consisted of 4 levels (8, 16, 24, and 32% of diet DM) of DDGS replacing dry rolled corn, and 32% DDGS plus 1.008 mg of Cu.

[2] Probabilities for contrasts: linear (L), quadratic (Q), cubic (C), and for the preplanned contrast of 32 vs 32Cu (32 vs 32Cu).

[3] n = 6.

[4] Probability value for the F-test of overall treatment.

[5] SFA (Saturated fatty acids); MUFA (Monounsaturated fatty acids): Sapienic, and T-vaccenic; PUFA (Poliunsaturated fatty acids): Linoleic, Linolenic, Arachidonic, Eicosapentanic, TTFn2, and TTFn3; TUFA (Total unsaturated fatty acids): MUFA plus PUFA.

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EFFECTS OF A LONG ACTING TRACE MINERAL RUMEN BOLUS UPON RANGE COW PRODUCTIVITY [1]

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ABSTRACT: The objectives were to determine if strategic supplementation of range cows in central Arizona with either 2 or 4 long acting (six mo) trace mineral rumen boluses containing Cu, Se, and Co would: (1) decrease yearly calving interval; (2) increase cow body condition, milk production, or calf adjusted weaning weights; and (3) to see if any of the above traits varied by cow breed. There were 194 Hereford (H) and 132 Composite (CGC; 50% Red Angus, 25% Tarentaise, 25% Charolais) control cows, 173 H and 125 CGC 1X treated (2 boluses in late winter) cows, and 183 H and 117 CGC 2X treated (2 boluses in autumn and 2 in late winter) cows used over the four year period. Cows were weighed and scored for body condition (1 to 9, 9 = fattest) in February, May, and September of each year. Milk production was determined by weigh-suckle-weigh on a subset of cows (n = 169) at 50 d lactation. The outcomes were analyzed using a restricted maximum likelihood-based mixed-effects model that included the categorical, fixed effects of breed, bolus, and year with the interactions of breed x bolus, and breed x year. For adjusted weaning wt, year x bolus was added. The random effect of cow was also included. Calving interval had only the breed x bolus interaction. Age of dam was added as a covariate to all models. Milk production used the same model as calving interval with the added covariate of post partum interval. Cow body condition score and calf adjusted weaning weights differed by breed and treatment ($P < 0.05$) with weaning weights being greater ($P < 0.05$) for calves from 2X cows than for control calves. Milk production differed by year ($P < 0.0001$) but did not differ by either breed or treatment ($P > 0.05$). Calving interval was 389 ± 2.7, 382 ± 3.2, and 378 ± 3.2 d for control, 1X, and 2X treatments, respectively and calving interval declined ($P < 0.05$) from the control to the 2X treatment group. Strategic supplementation via a long acting trace mineral bolus was successful in decreasing calving interval and increasing calf weaning weights from cattle grazed in an extensive rangeland environment.

Key Words: Calving interval, Cattle, Copper, Minerals, Selenium

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Introduction

A long acting (6 mo) rumen trace mineral bolus containing Cu, Se, and Co has been developed in the United Kingdom (Cosecure®, Telsol Ltd., Leeds, United Kingdom) and has shown promise for helping alleviate trace mineral deficiencies (Sprinkle et al., 2006). One advantage of the long acting rumen boluses is the capability to provide a trace mineral supplement on rugged topography rangelands that are inaccessible by motor vehicles. Sprinkle et al. (2006) found that use of the Cosecure® bolus caused increased weight loss ($P = 0.02$) from late gestation to early lactation, but milk production was not determined in that study. Also, the same study did not report on the effect of the long acting rumen boluses upon yearly calving interval.

The objectives of this study were to examine the effects of the Cosecure® boluses supplemented either once or twice per year upon BCS, body weights, yearly calving interval, and milk production as well as calf weaning weights; and to determine whether breed response for the above traits differed.

Materials and Methods

Range site. The study site for this experiment was the 32,161 ha V-V Ranch operated by the University of Arizona and located near Camp Verde, Arizona. The ranch ranges in elevation from approximately 975 m to 2195 m. Average yearly precipitation ranges from 33 cm at the lower elevations to 68 cm at the upper elevations. However, annual precipitation during the course of this trial was quite variable (Arizona State Climate Office, 2011): with more winter moisture in 2005 before the study commenced; substantially less annual precipitation in 2006; about the same (lower elevations) and slightly less (upper elevations) annual precipitation in 2007; an El Niño winter occurred in 2008 at all elevations with more annual precipitation at upper elevations (80 cm) and similar precipitation (31 cm) at lower elevations; and substantially less precipitation at all elevations (18 cm for lower elevations, 46 cm for upper) in 2009, particularly during the midsummer and early fall growing season for warm season grasses.

Forage Sampling. Forage was sampled by hand clipping four times a year (January, April, June or August, and September) from four different locations on the ranch as described by Sprinkle et al. (2006). Prior to mineral analysis, forage samples were air dried at ambient temperatures for one year, then shipped to the Oscar E. Olson Biochemistry Analytical Services Laboratory in Brookings, SD where they were ground to pass through a 1 mm screen using a Tecator Cyclotec cyclone pulverizing mill (AOAC, 2005). Samples

were then mixed and moisture determined (on a subsample) at 105° C for 3 h in a mechanical convection oven (Method 2.1.4, NFTA, 2006), then analyzed fluorometrically for Se following digestion in percholoric and nitric acids and reduction with 0.1 M HCl and complexation with diaminonapthalene (AOAC Official Method 996.16, AOAC, 2005). Following these analyses, the samples were shipped to Dairy One Lab in Ithaca, NY and analyzed for Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, Co, and S using inductively coupled, plasma emission spectroscopy as described by Sirios et al. (1991).

Animals. The trial commenced in October 2005 and concluded in September 2009. Treatment and control cattle were randomly allocated at the onset and remained in each treatment group throughout the three-yr trial. There were 194 Hereford (H) and 132 Composite (CGC; 50% Red Angus, 25% Tarentaise, 25% Charolais) control cows, 173 H and 125 CGC 1X treated (2 boluses in late winter) cows, and 183 H and 117 CGC 2X treated (2 boluses in autumn and 2 in late winter) cows used over the four year period. Cows ranged in age from 2 to 12 and 2 to 10 yr for H and CGC, respectively.

In September or October and February or March of each yr, cows in the 2X treatment groups were orally dosed with two 100 gram Cosecure® boluses consisting of 0.30% (wt/wt) selenium as sodium selenate, 13.4% (wt/wt) copper, and 0.5% (wt/wt) cobalt. The 1X treatment group only received boluses in February or March. According to company literature validated with rumen fistulated cattle on a silage and concentrate ration, boluses dissolved in 175 days and released 156, 5.9, and 3.4 mg/d of Cu, Co, and Se, respectively.

A subset of mature cattle (5 to 10-yr-old) from each treatment group was sampled for milk production near expected time for peak lactation (50 d) in 2006, 2008, and 2009 using the weigh-suckle-weigh technique described by Williams et al. (1979). There were 26 CGC Control, 31 CGC 1X, 28 CGC 2X, 28 H Control, 26 H 1X, and 30 H 2X cows used over all three years of milk production data collection. Cattle were not sampled in 2007 due to a lack of an adequate sample size at peak lactation.

Cattle remained in a common herd and moved through 37 upland pastures from low and mid-elevation in winter and spring to high elevation in late summer and fall in a modified Holistic Range Management grazing plan. Cattle did not receive any type of oral trace mineral supplement for the four years of the trial except for free choice white iodized salt blocks and the incidental minerals contained in protein blocks used from April to June in 2006 and from March to May 15 in 2008 (27% crude protein, 17 ppm Cu, 0.301 ppm Se, Eagle Milling Co., Inc., Casa Grande, AZ). At an average daily protein supplement intake of 0.916 kg in 2006 and 0.395 kg in 2008, it was estimated that cattle received an additional 16 mg/d of Cu and 0.28 mg/d Se in 2006 and 7 mg/d of Cu and 0.12 mg/d of Se in 2008.

The majority (74%) of calves born in this trial were sired by Hereford bulls via artificial insemination or pasture exposure. Other sire breeds represented were Waguli (13%), Tuli (4%), Wagyu (3%), Red Angus (3%), Angus (2%), and miscellaneous (1%). Breeding seasons extended from May 20 to September 6 in 2005, May 18 to August 30 in 2006, May 15 to September 20 in 2007, May 23 to September 4 in 2008, and from May 22 to August 5 in 2009. Cows were artificially inseminated once following estrus synchronization using Easi-Breed CIDRs (Pharmacia & Upjohn Co., Kalamazoo, MI), then pasture exposed to bulls.

In September to October and February to March, cows were checked for pregnancy by rectal palpation. Cattle were weighed and scored for BCS (1 to 9; 9 = fattest) in February or March, May or June, and September or October. Birth and weaning weights were collected on all calves. The majority of the calves were weaned in October at approximately 182 d and weaning weights were adjusted to 205 days of age and for age of dam according to BIF (1990) guidelines.

Statistical Analyses. Data were analyzed using a restricted maximum likelihood-based mixed effects model appropriate for repeated measures (SAS Inst., Inc., Cary, NC) with the categorical, fixed effects of breed, bolus, and year with the interactions of breed x bolus, and breed x year. For adjusted weaning weight, year x bolus was added. Cow within breed by bolus was included as a random effect. Calving interval had only the breed x bolus interaction added. Age of dam was added as a covariate to all models. Milk production used the same model as calving interval with the added covariate of post partum interval. The denominator degrees of freedom for treatment *F*-statistics were approximated using the Kenward-Roger's method. For all models except calving interval, a heterogeneous autoregressive structure was used as a covariance structure to model the relationships between repeated observations. In order for calving interval to properly converge with this iterative methodology, a simplified compound symmetry covariance structure was used. Treatment means for all statistical models were separated using the PDIFF function in SAS (SAS Inst., Inc., Cary, NC).

Results

Overall Forage Trace Mineral Concentrations. Concentrations of Cu in forage were adequate (10 ppm; NRC, 1996) in 2006 (12.1 ± 1.70 ppm), nearly adequate in 2007 (8.9 ± 1.21 ppm), adequate in 2008 (10.2 ± 0.88 ppm), and nearly adequate in 2009 (7.5 ± 0.38 ppm). The concentrations of Se were always deficient in forage (< 0.1 ppm; NRC, 1996), being 0.078 ± 0.010 ppm in 2006, 0.057 ± 0.007 ppm in 2007, 0.071 ± 0.005 ppm in 2008, and 0.066 ± 0.007 ppm in 2009. The other trace mineral provided by the boluses, cobalt, was not a concern for this ranch, always being well above the NRC (1996) minimum requirement (0.10 ppm), averaging 1.2 ± 0.13 ppm over all the years of the trial.

We did not detect any antagonistic relationships for either Mo of S in the forage but Fe concentrations in the forage exceeded 600 ppm in 2006, 2008 and 2009 and was 398 ppm in 2007. Corah and Dargatz (1996) reported that Fe levels exceeding 400 ppm reduces Cu absorption.

Cow Performance Data. Cows bolused with the 2X Cosecure® bolus treatment had less body condition in the spring than did either control cows ($P = 0.0447$; Table 1) or 1X treated cows ($P = 0.0109$; Table 1), though the actual difference was small. However, a loss of body condition is

verified between control and 2X treated H cows by spring cow weights ($P = 0.0369$; 2X H = 435 ± 4.9 vs. 448 ± 4.8 kg for control H; Table1). There was also a tendency (P = 0.0535; Table 1) for 1X treated H cows to weigh more than 2X H cows. This trend continued into the fall for H cows, with the 2X cows having less BCS than did 1X cows ($P = 0.0220$). Interestingly, an opposite effect appeared to be in place for CGC cows for fall weight, with 2X cows weighing more than 1X cows ($P = 0.0488$; Table 1). In the study reported by Sprinkle et al. (2006), cows bolused 1X with Cosecure® boluses lost more weight from late gestation to early lactation than did control cows. ($P = 0.020$). The authors hypothesized that this may have been due to increased milk production for treated cows. In this study, we did not find any differences (Table 2) for either breed ($P = 0.1691$) or treatment ($P = 0.9509$) for milk production at 50 d estimated by weigh-suckle-weigh (Williams et al., 1979). We speculate that environmental variation may have overwhelmed treatment differences. Indeed, the only significant difference detected for milk production in this study was for year ($P < 0.0001$) with greater milk production following a wet El Niño year in 2008 (7.1 ± 0.30 kg/24 h) compared to 2006 (5.2 ± 0.31 kg/24 h) and 2009 (5.8 ± 0.30 kg/24 h). Year effects were important ($P < 0.0002$) in this study for all variables measured except for calf birth wt ($P = 0.9236$). Breed effects were detected for differences in weight change from spring to fall ($P = 0.0012$; Table 1) and for fall weight ($P = 0.0371$; Table 1).

Calf Performance Data and Calving Interval. Calf birth weights tended to differ by bolus treatment ($P = 0.0852$), being smaller ($P = 0.0273$) for control cattle than for 1X treated cattle (Table 2).

Breed effects were detected for differences in adjusted weaning weight ($P < 0.0001$; Table 2), and calving interval ($P = 0.0002$; Table 2). It was expected that breed differences could occur with some of these production characteristics considering we were comparing crossbred vs. purebred cattle. The CGC composite cattle had shorter ($P = 0.0002$) calving interval periods than did H cattle (376 ± 2.2 vs. 390 ± 2.9 d) and weaned heavier ($P < 0.0001$) calves (205 ± 1.8 vs. 182 ± 1.4 kg).

The most striking results from this trial were the effects of increasing trace mineral supply via the boluses upon weaning weight and calving interval. There was a linear increase for weaning weight and linear decrease for calving interval with increased trace mineral supply, being significant at the 2X level for both weaning weight ($P = 0.0421$; Table 2) and calving interval ($P = 0.0090$; Table 2) when compared to control cattle. Calves from the 2X treatment weighed 6 kg more than did calves from control cattle and cows on the 2X treatment had yearly calving intervals 11 d shorter (Table 2). For H cattle, cows on the 2X treatment had calving intervals 14 d smaller ($P = 0.0332$) that did control cows.

Other research has reported variable results for added Cu, increasing ADG during finishing trials (Ward and Spears, 1997) and decreasing gain for growing dairy heifers (Lopez-Guisa and Satter, 1992). Awadeh et al. (1998) and Gunter et al. (2003) failed to demonstrate any added growth performance for calves nursing Se supplemented cows while Nelson and Miller (1987) reported that weaning weights for calves nursing Se supplemented cows increased by 20 kg.

It appears that any added weight gains for calves nursing cows supplemented with either Cu or Se are dependent upon several factors, chief of which are the dietary Cu or Se concentrations for cows in the study and the presence or absence of any antagonistic trace minerals in the diet such as Mo, Fe, and S. Our pasture concentrations for Cu were adequate to mostly adequate but with a possible negative absorption influence due to high dietary Fe. Villar et al. (2002) reported that positive growth responses appear to occur when dietary Se in the forage base is less than 0.05 ppm DM. The pasture forage Se reported by Gunter et al. (2003) was 0.11 ppm and 0.07 ppm by Awadeh et al. (1998). Our pasture Se concentrations ranged from 0.057 to 0.078 ppm.

Implications

Strategic supplementation via a long acting trace mineral bolus was successful in decreasing calving interval and increasing calf weaning weights from cattle grazed in an extensive rangeland environment. At current 2011 calf prices, the value added from increased weaning weights to cow gross income by the 2X over the control Cosecure® treatment through supplementations would be $21.74 (6 kg = 13.23 lbs. x $1.6438/lb, NM prices, LMIC, 2011). Added to this gross profit would be the advantages of a reduced yearly calving interval.

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Table 1. Effects of a long acting trace mineral bolus upon range cow weight and body condition score[1]

	Treatment (TRT)						
Item	n	Control	n	1X	n	2X	TRT *P*-value
Winter BCS[2]							
All cows and all yr	244	4.9 ± 0.04	219	4.9 ± 0.04	227	4.9 ± 0.04	0.9483
CGC cows, over all yr[3]	97	4.8 ± 0.06	93	4.9 ± 0.07	89	5.0 ± 0.06	0.3423
H cows, over all yr[c,3]	147	5.0 ± 0.06	126	4.9 ± 0.06	138	4.9 ± 0.06	0.3423
Spring BCS[2]							
All cows and all yr	218	4.6 ± 0.05[a]	203	4.6 ± 0.04[a]	207	4.5 ± 0.04[p]	0.0264
CGC cows, over all yr[3]	92	4.7 ± 0.08[a]	90	4.6 ± 0.06[ap]	82	4.5 ± 0.06[p]	0.5157
H cows, over all yr[3]	126	4.5 ± 0.06	113	4.6 ± 0.06	125	4.4 ± 0.06	0.5157
Fall BCS[2]							
All cows and all yr	313	5.2 ± 0.05	289	5.3 ± 0.05	293	5.2 ± 0.04	0.8505
CGC cows, over all yr[3]	132	5.2 ± 0.09	121	5.2 ± 0.07	115	5.3 ± 0.07	0.0210
H cows, over all yr[3]	181	5.3 ± 0.06[ap]	168	5.3 ± 0.06[a]	178	5.1 ± 0.06[p]	0.0210
Winter wt, kg[2]							
All cows and all yr	244	479 ± 4.4	219	480 ± 3.6	228	486 ± 3.6	0.4116
CGC cows, over all yr[3]	97	477 ± 7.8	93	480 ± 5.5	89	491 ± 5.4	0.3522
H cows, over all yr[3]	147	480 ± 4.6	126	481 ± 4.9	139	480 ± 4.9	0.3522
Spring wt, kg[2]							
All cows and all yr	199	447 ± 4.5	181	445 ± 3.6	185	443 ± 3.7	0.8095
CGC cows, over all yr[3]	83	445 ± 7.7	82	444 ± 5.4	76	451 ± 5.5	0.1583
H cows, over all yr[3]	116	448 ± 4.8[a]	99	445 ± 5.1[ap]	109	435 ± 4.9[p]	0.1583
Fall wt, kg[2]							
All cows and all yr	313	459 ± 4.4	289	457 ± 3.5	293	461 ± 3.5	0.7232
CGC cows, over all yr[3]	132	449 ± 7.7[ap]	121	448 ± 5.4[a]	115	463 ± 5.3[p]	0.0485
H cows, over all yr[3]	181	468 ± 4.6	168	465 ± 4.8	178	459 ± 4.8	0.0485
Change in wt Winter to Spring, kg[2]							
All cows and all yr	194	40 ± 2.8	175	41 ± 2.7	181	47 ± 2.7	0.1518
CGC cows, over all yr[3]	79	40 ± 4.5	79	41 ± 4.0	75	45 ± 4.1	0.9141
H cows, over all yr[3]	115	40 ± 3.5	96	42 ± 3.7	106	49 ± 3.7	0.9141
Change in wt Spring to Fall, kg[2]							
All cows and all yr	192	16 ± 2.7	177	15 ± 2.6	182	21 ± 2.6	0.2983
CGC cows, over all yr[3]	83	10 ± 4.3	79	8 ± 3.8	75	17 ± 3.8	0.6521
H cows, over all yr[3]	109	22 ± 3.5	98	22 ± 3.5	107	24 ± 3.5	0.6521

[a,b]Means within a row without a common superscript differ ($P < 0.05$).

[1]Cosecure® trace mineral boluses had an expected life of approximately 175 d and provided approximately 156 mg/d Cu, 5.9 mg/d Co, and 3.4 mg/d Se. Boluses were provided either at 0, 1X (February or March), or 2X interval (February or March and Sepetember or October).

[2]BCS (1 to 9, 9 = fattest); Winter = February or March; Spring = May or June; Fall = September or October.

[3] Breeds: CGC = Composite (50% Red Angus, 25% Tarentaise, and 25% Charolais); H = Hereford. Significant main effects for breed were detected for change in wt from spring to fall ($P = 0.0012$) and fall wt ($P = 0.0371$). Year was significant for all dependent variables ($P < 0.0001$).

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Table 2. Effects of a long acting trace mineral bolus upon peak 24 h milk production, adjusted weaning wt, yearly calving interval, and calf birth wt[1]

	Treatment (TRT)						
Item	n	Control	n	1X	n	2X	TRT *P*-value
24 h milk production, kg[2]							
All cows and all yr	54	6.0 ± 0.30	57	6.1 ± 0.29	58	6.1 ± 0.29	0.9509
CGC cows, over all yr[3]	26	6.2 ± 0.45	31	6.5 ± 0.40	28	6.2 ± 0.43	0.7781
H cows, over all yr[c,3]	28	5.7 ± 0.42	26	5.7 ± 0.44	30	6.0 ± 0.40	0.7781
Adjusted calf weaning wt, kg[2]							
All cows and all yr	202	190 ± 2.1[a]	184	194 ± 1.8[ab]	184	196 ± 1.8[b]	0.1248
CGC cows, over all yr[3]	88	203 ± 3.5	81	203 ± 2.7	79	207 ± 2.7	0.5034
H cows, over all yr[3]	114	178 ± 2.3[a]	103	184 ± 2.4[b]	105	185 ± 2.5[b]	0.5034
Yearly calving interval, d[2]							
All cows and all yr	94	389 ± 2.7[a]	82	382 ± 3.2[ab]	87	378 ± 3.2[b]	0.0251
CGC cows, over all yr[3]	52	380 ± 2.9	44	377 ± 4.3	51	372 ± 3.9	0.5370
H cows, over all yr[3]	42	399 ± 4.6[a]	38	387 ± 4.7[ab]	36	385 ± 4.9[b]	0.5370
Calf birth wt, kg[2]							
All cows and all yr	220	33.0 ± 0.28[a]	195	33.9 ± 0.28[b]	194	33.5 ± 0.28[ab]	0.0852
CGC cows, over all yr[3]	90	32.8 ± 0.45	85	33.7 ± 0.43	82	33.4 ± 0.43	0.9926
H cows, over all yr[3]	130	33.2 ± 0.36	110	34.1 ± 0.38	112	33.7 ± 0.38	0.9926

[a,b]Means within a row without a common superscript differ ($P < 0.05$).

[1]Cosecure® trace mineral boluses had an expected life of approximately 175 d and provided approximately 156 mg/d Cu, 5.9 mg/d Co, and 3.4 mg/d Se. Boluses were provided either at 0, 1X (February or March), or 2X interval (February or March and Sepetember or October).

[2]Milk production determined by weigh-suckle-weigh at 50 d lactation; milk production not determined in 2007 due to a lack of sufficient sample size at peak lactation. Weaning weights adjusted according to Beef Improvement Federation guidelines (BIF, 1990).

[3] Breeds: CGC = Composite (50% Red Angus, 25% Tarentaise, and 25% Charolais); H = Hereford. Significant main effects for breed were detected for adjusted weaning wt ($P < 0.0001$) and calving interval ($P = 0.0002$). Year was significant ($P < 0.0002$) for all dependent variables except calf birth wt.

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EFFECTS OF WET DISTILLER'S GRAIN INCLUSION ON FINISHING PERFORMANCE AND CARCASS CHARACTERISTICS OF BEEF STEERS FED A SORGHUM-BASED FINISHING DIET

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ABSTRACT: A large portion of the grain sorghum produced in the U.S. is used as livestock feed; however, there is limited information regarding the effects of wet distiller′s grain (**WDG**) in sorghum-based finishing rations on finishing cattle performance and carcass characteristics. Crossbred steers (n = 464; initial BW = 468 ± 38 kg) were utilized in a finishing study to evaluate the effects of WDG inclusion. Steers were stratified by BW and ultrasonically-measured 12th-rib fat thickness and LM characteristics and assigned randomly to 1 of 4 ration treatments (4 pen replicates per treatment). Ration treatments were: 1) soybean meal protein supplement (**CON**); 2) 15% (diet DM basis) wet distiller's grain plus solubles (**15WDG**); 3) 30% (diet DM basis) wet distiller's grain plus solubles (**30WDG**); and 4) 40% (diet DM basis) wet distiller's grain plus solubles (**40WDG**). Steers were adapted to treatment rations for 14 d and fed for 95 d until harvest. Final weight tended ($P = 0.10$) to be greater for steers fed 40WDG (621 kg) than for steers fed 15WDG or 30WDG (607 kg). Steers fed 40WDG had greater ($P < 0.01$; 1.67 kg/d) ADG than CON (1.55 kg/d), 15WDG (1.53 kg/d), or 30WDG (1.55 kg/d) but feed efficiency was not influenced by ration ($P = 0.22$). Steers fed 40WDG produced heavier carcasses than steers fed 15WDG (390 vs. 379 kg, respectively; $P < 0.05$) and tended ($P = 0.09$) to produce heavier carcasses than CON but LM area was not different ($P = 0.23$) between steers fed 15WDG and 40WDG. Marbling score and 12th-rib fat thickness were not influenced by ration ($P > 0.05$) as cattle were managed to achieve a common endpoint of 11.5 mm of subcutaneous fat over the 12th-rib. These results suggest that the inclusion of WDGS in a sorghum-based finishing ration improved ADG but had no effect on feed efficiency. Further research is needed to determine the optimum inclusion level of WDGS in sorghum-based finishing rations.

Key Words: Beef Cattle, Distillers Grain, Sorghum

Introduction

The optimum inclusion level of wet distillers grain plus solubles (**WDG**) in feedlot rations is likely influenced by a number of factors, including the grain processing method used. Vander Pol et al. (2006) reported that ADG and G:F were optimized when 30 to 40% WDG (DM basis) was fed in a dry-rolled corn based diet; whereas the optimum concentration of WDG inclusion in steam-flaked corn diets ranges from 15 (Daubert et al. 2005) to 25%; (Depenbusch, et al., 2009) of the diet (DM basis). A majority of the grain sorghum produced in the United States is utilized as livestock feed or in the production of ethanol (National Sorghum Producers, 2010); however, there is currently limited information regarding the effects of WDG inclusion in sorghum-based rations on fed cattle performance and carcass characteristics. Jaeger et al. (2010) observed an improvement in ADG when 15% WDG was fed in a ground sorghum-based finishing ration. Therefore we hypothesized that greater inclusion levels of WDG in a sorghum-based diet would result in further improvements in fed cattle performance.

The objective of this study was to evaluate the effects of 0, 15, 30 or 40% WDG inclusion (DM basis) in a ground sorghum-based finishing diet on cattle performance and carcass characteristics.

Materials and Methods

Animals, Facilities and Diets. All procedures for this study were approved by the Kansas State University Institutional Animal Care and Use Committee. Crossbred steers (n= 464; initial BW = 468 ± 38 kg) were used for this experiment. Steers originated from three Kansas livestock markets and were maintained in 1033 m^2 earth-floor pens (29 or 30 hd/pen) with 24 cm of linear bunk allocated per head for the duration of the study.

Steers were received and processed at the KSU Agricultural Research Center–Hays (KSU-ARCH) feedlot. At processing steers were weighed, implanted with a Component TE-S with Tylan implant (120 mg trembolone acetate, 24 mg estradiol, 29 mg tylosin tartrate; Vetlife, Overland Park, KS) and measured with ultrasound to determine 12th-rib fat thickness, LM depth and marbling. Steers were stratified by BW and ultrasonically-measured carcass characteristics and assigned randomly to 1 of 4 ration treatments (4 pen replicates per treatment). Ration treatments (Table 1) were: 1) soybean meal protein supplement (**CON**); 2) 15% (diet DM basis) wet distiller's grain plus solubles (**15WDG**); 3) 30% (diet DM basis) wet distiller's grain plus solubles (**30WDG**); and 4) 40% (diet DM basis) wet distiller's grain plus solubles (**40WDG**). All cattle were fed a common receiving diet for 7 d, then adapted to the treatment diets (Table 1) for 14 d. Cattle were then fed for 95 d until harvest. Cattle were fed once daily, using a slick-bunk management method and feed

calls were made each morning at 0630 before feed delivery. Cattle were evaluated daily by KSU-ARCH feedlot personnel for clinical signs of morbidity.

Data Collection. Diet samples were collected weekly and frozen for later analysis. Diet samples were composited at the conclusion of the study and submitted to a commercial laboratory (SDK Labs, Hutchinson, KS) for analysis of DM, NDF, ADF, CP, Ca, P, and S content. Steers were weighed approximately every 28 d during the finishing period and 4 d prior to the scheduled harvest date. Carcass characteristics (12^{th}-rib fat thickness, LM depth and marbling score) were measured ultrasonically on d -22, 55, and 91 of the finishing period using an Aloka 500V (Aloka Co., Ltd., Wllingford CT) B-mode instrument equipped with a 3.5-MHz general purpose transducer array (UST 5021-12mm window). Ultrasound images were collected with Cattle Performance Enhancement Company (**CPEC**, Oakley, KS) software. Backfat thickness, LM depth and marbling score were estimated with procedures that incorporated image analysis software (Brethour, 1994) that are an integral component of the CPEC software. Harvest date was determined by the d 55 scan to meet an average carcass endpoint of 11.5 mm of fat depth over the 12^{th} rib.

On the scheduled harvest date cattle were transported approximately 3 h to a commercial abattoir (National Beef Packing Company, Dodge City, KS). Carcass characteristics were measured by camera and automated software and included 12^{th}-rib fat thickness, 12^{th}-rib LM area, kidney-pelvic-heart fat, USDA maturity grade, USDA yield grade, USDA quality grade, and marbling score (USDA, 1997).

Statistical Analysis. Data were subjected to ANOVA using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included effects for treatment and pen. Treatment within pen was considered the experimental unit. F-tests were constructed using the type-3 error mean squares. The chi-squared analysis in the FREQ procedure of SAS (SAS Inst. Inc., Cary, NC) was utilized to evaluate the categorical distribution of USDA quality and yield grade. All steers that exhibited clinical signs of morbidity or expired during the course of the studied were removed from the analysis. Least Squares Means are presented and differences were considered significant at $P \leq 0.05$.

Results and Discussion

Cattle performance (Table 2) during the 95 d finishing period was influenced by inclusion level of WDG ($P < 0.05$). Final weight tended ($P = 0.10$) to be greater for steers fed 40WDG (621 kg) than for steers fed 15WDG or 30WDG (607 kg) but was not different from Steers fed CON (613 kg). Steers fed 40WDG had greater ($P < 0.01$; 1.67 kg/d) ADG than CON (1.55 kg/d), 15WDG (1.53 kg/d), or 30WDG (1.55 kg/d) but feed efficiency was similar across all ration treatments ($P = 0.23$). Jaeger et al. (2010) evaluated the inclusion of 15% WDG in a sorghum-based ration with and without the inclusion of a direct fed microbial and reported a 16% improvement in ADG and 23 kg increase in final BW of steers fed 15% WDG. In contrast, the same magnitude of increase in ADG or final BW among steers fed the CON and 15WDG was not observed in the present study. Final BW and ADG remained significant ($P < 0.05$) when evaluated on a carcass-adjusted basis. However, on a carcass-adjusted basis ADG of steers fed CON and 15WDG were similar ($P > 0.05$) and 30WDG and 40WDG were also not different ($P > 0.05$), but were greater than CON and 15WDG ($P < 0.05$). A numerical increase in carcass-adjusted ADG was observed among 15WDG, 30WDG and 40WDG (1.40, 1.55, and 1.59 kg/d respectively).

Corrigan et al. (2009) reported a linear increase in HCW in dry rolled corn diets in response to increasing inclusion levels of WDG (0, 15, 27.5, and 40%). In the present study, HCW among steers fed 30WDG and 40WDG were not different ($P > 0.10$), steers fed 40WDG produced heavier carcasses than steers fed 15WDG (390 vs. 379 kg, respectively; $P < 0.05$) and tended ($P = 0.10$) to produce heavier carcasses than CON.

Longissimus muscle area was not different among steers fed CON, 15WDG and 40WDG ($P > 0.05$), and was greatest for 30WDG and lowest in 15WDG. Calculated yield grade was greater ($P < 0.05$) for steers fed 15WDG and 40WDG (3.18 and 3.16, respectively) than for steers fed CON (2.93) and 30WDG (2.97). A linear increase in yield grade in response to increasing dietary concentrations of WDG in steam-flaked corn diets was reported by Daubert et al. (2005). A meta-analysis by Klopfenstein et al. (2008) also reported an increase in yield grade in dry-rolled and high moisture corn diets as WDG inclusion increased. Although we did not observe a linear increase in yield grade; inclusion of WDG in the present study did result in numerically greater calculated yield grades. There was a tendency for ($P = 0.12$) for USDA quality grade distribution to differ among WDG inclusion levels, but there was no difference in distribution of USDA yield grades ($P > 0.05$; Table 3).

Marbling score and 12^{th}-rib fat thickness were not influenced by WDG inclusion level ($P > 0.05$) as cattle were managed to achieve a common endpoint of 11.5 mm of subcutaneous fat over the 12^{th}-rib. In addition, the change in ultrasonically measured 12^{th} rib fat thickness ($P = 0.78$) LM depth ($P = 0.38$) and marbling score ($P = 0.77$) from d -22 to 91 were also not influenced by WDG inclusion level ($P > 0.05$).

Steers fed 40WDG were 15 kg heavier at harvest, gained 0.19 kg/d more on a carcass-adjusted basis than steers fed the soybean meal-based protein supplement diet (CON) and produced carcasses that graded 57.4% USDA choice, and 92% USDA yield grade 1, 2, or 3.

Implications

The results of this study imply that inclusion of 40% WDG (DM basis) in ground sorghum-based finishing rations may improve finishing period ADG, and result in greater final BW without detrimentally affecting feed efficiency or carcass merit. Further research is necessary to determine the optimum level of WDG inclusion in ground sorghum-based finishing rations.

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Table 1. Ingredient and nutrient composition of ground sorghum based finishing diets.

	Treatment[1]			
Item,	CON	15WDG	30WDG	40WDG
Ingredient, % of Diet DM				
Sorghum, Ground	75.1	70.3	54.8	44.5
Sorghum Sudan Hay	12.0	12.0	12.0	12.0
Wet Distiller's Grain	-	15.0	30.0	40.0
Soybean Meal	10.6	-	-	-
Calcium Carbonate	1.5	1.9	2.4	2.7
Ammonium Sulfate	0.2	0.2	0.2	0.2
Salt	0.2	0.2	0.2	0.2
Vitamin and Mineral Premix[2]	0.4	0.4	0.4	0.4
Analyzed Nutrient composition(DM basis)[3]				
ADF,%	9.0	11.0	11.8	12.9
NDF, %	13.4	16.6	16.9	17.7
CP, %	12.71	12.54	15.40	17.66
Ca, %	0.51	0.91	1.02	1.16
P, %	0.32	0.36	0.41	0.47
S, %	0.17	0.26	0.34	0.41
NEm, Mcal/kg	1.96	1.91	1.89	1.87
NEg, Mcal/kg	1.23	1.19	1.19	1.17

[1]Treatments were: CON = soybean meal protein supplement; 15WDG= 15% (diet DM basis) wet distiller's grain plus solubles; 30WDG = 30% (diet DM basis) wet distiller's grain plus solubles; 40WDG = 40% (diet DM basis) wet distiller's grain plus solubles.

[2]Supplied 299.2 mg rumensin per hd.

[3]Analyzed by SDK Laboratories, Hutchinson, KS.

Table 2. Finishing period performance and carcass characteristics of steers fed sorghum-based finishing diets containing soybean meal (CON), 15% (15WDG), 30% (30WDG) or 40% (40WDG) wet distiller's grain (diet DM basis).

	Treatment[1]					*P* -value
Item	CON	15WDG	30WDG	40WDG	SEM	WDG[2]
Performance[3]						
Initial BW., kg	471	468	466	469	3.5	0.72
Final BW, kg	613	607	607	621	4.2	0.07
ADG, kg/d	1.55[a]	1.53[a]	1.55[a]	1.67[b]	0.02	< 0.01
Gain to Feed, g/kg	120	110	100	120	7.0	0.22
Carcass-adjusted performance						
Final BW[4], kg	604[ab]	601[ab]	613 [bc]	619[c]	4.6	< 0.05
ADG, kg/d	1.40[a]	1.40[a]	1.55[b]	1.59[b]	0.03	< 0.01
Gain:Feed, g/kg	110	100	100	110	7.0	0.56
Carcass characteristics						
HCW, kg	381[ab]	379[a]	386[ab]	390[b]	2.9	< 0.05
LM area, cm^2	92.13[ac]	88.84[b]	93.23[ac]	91.23[bc]	0.90	< 0.05
12th Rib fat, mm	9.9	10.8	10.1	10.6	0.4	0.24
Marbling score[5]	628.4	637.2	615.3	629.0	7.6	0.23
Calculated yield grade	2.93[ae]	3.18[bd]	2.97[acde]	3.16[bc]	0.06	< 0.05

[1]Treatments were: CON = soybean meal protein supplement; 15WDG= 15% (diet DM basis) wet distiller's grain plus solubles; 30WDG = 30% (diet DM basis) wet distiller's grain plus solubles; 40WDG = 40% (diet DM basis) wet distiller's grain plus solubles.

[2]Effect of WDG inclusion level.

[3]91 Days on feed.

[4]Carcass-adjusted final BW calculated by dividing HCW by a common dressing yield of 63%.

[5]Marbling score small 00 = 500.

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

Table 3. Distribution of USDA quality and yield grade of steers fed sorghum-based finishing diets containing soybean meal (CON), 15% (15WDG), 30% (30WDG) or 40% (40WDG) wet distiller's grain (diet DM basis).

	Treatment[1]				*P* -value
Item	CON	15WDG	30WDG	40WDG	WDG[2]
USDA Quality Grade, %					0.12
Prime,	-	0.86	0.86	-	
Choice	52.6	56.0	45.7	57.4	
Select	37.9	37.1	39.7	40.0	
Standard	9.5	6.0	13.8	2.6	
USDA Yield Grade, %					0.50
1	15.5	8.6	15.5	10.4	
2	55.2	51.7	56.0	55.7	
3	26.7	37.9	27.6	26.7	
4	2.6	1.7	0.86	2.6	
5	-	-	0.87	-	

[1]Treatments were: CON = soybean meal protein supplement; 15WDG= 15% (diet DM basis) wet distiller's grain plus solubles; 30WDG = 30% (diet DM basis) wet distiller's grain plus solubles; 40WDG = 40% (diet DM basis) wet distiller's grain plus solubles.

[2]Effect of WDG inclusion level.

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EVALUATION OF WHOLE CORN SUBSTITUTION IN STEAM-FLAKED CORN-BASED DIETS CONTAINING DIFFERENT CONCENTRATIONS OF WET DISTILLER′S GRAINS

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ABSTRACT: Substituting steam-flaked corn (**SFC**) with whole shelled corn (**WSC**) in finishing diets containing wet distiller′s grains with solubles (**WDGS**) could reduce grain processing costs without affecting feedlot cattle performance, feed conversion, and carcass characteristics. This study used 642 Angus-cross heifers (412 ± 18 kg initial BW) assigned to 36 pens in a randomized complete block design (3 blocks based on initial BW). Treatments (2 × 3 factorial) were 6 finishing diets based on SFC with 0 or 20% WSC replacing SFC, and 0, 15, or 30% WDGS replacing SFC (DM basis). Diets were formulated to contain equal concentrations of RDP and fat, and were fed to heifers for 108 d. No WSC × WDGS interactions ($P \geq 0.08$) occurred for DMI, ADG, G:F, and carcass characteristics. Heifers fed diets containing 20 vs 0% WSC had greater ($P < 0.01$) DMI, but final BW, ADG, and G:F were not affected ($P \geq 0.11$). The percentage of carcasses grading USDA choice or better tended to be lower ($P = 0.07$), and the percentage grading USDA select were higher ($P = 0.03$) for cattle fed diets with 20 vs 0% WSC. Other carcass characteristics, morbidity, and mortality were not affected ($P \geq 0.16$) by WSC. Increasing WDGS in SFC diets decreased final BW (linear, $P < 0.01$), tended to decrease ADG (linear, $P = 0.10$), tended to increase DMI (linear, $P = 0.08$), and decreased G:F (linear, $P = 0.01$). Addition of WDGS to SFC diets tended to decrease HCW (linear, $P = 0.09$), but other carcass characteristics, morbidity, and mortality were not affected ($P \geq 0.18$). In summary, substituting SFC with 20% WSC in finishing diets did not affect animal performance and feed conversion, but decreased carcass quality. In contrast, substituting SFC in finishing diets with increasing amounts of WDGS decreased animal performance and feed conversion, but did not affect carcass characteristics. Limited responses to the substitution of 20% WSC could in part explain the lack of WSC × WDGS interactions. Thus, it is not clear if grain processing could be reduced in finishing diets containing WDGS without affecting feedlot cattle performance and feed conversion.

Key words: corn processing, distiller′s grains, heifers

INTRODUCTION

Steam flaking corn for feedlot finishing diets is a grain processing method that can increase the corn's energy value by 18% (Zinn et al., 2002), and feed conversion is improved by 10% or more when cattle are fed diets based on steam-flaked corn (**SFC**) compared with dry-rolled corn (**DRC**; Owens et al., 1997). However, processing costs are greater for steam flaking corn than for other grain processing methods (Macken et al., 2006).

According to DiLorenzo and Galyean (2010), the use of co-products in feedlot diets has increased over the last 2 decades due to the rapid growth of wet and dry corn milling for ethanol. In review of the literature, DiLorenzo and Galyean (2010) also demonstrated that the inclusion level of distiller's grains in corn-based finishing diets for optimal performance and feed conversion depends on various factors, such as nature of distiller's grains co-product (corn-based vs sorghum-based) and processing method of the grain (SFC vs DRC).

Previous research (Corrigan et al., 2009; Depenbusch et al., 2009; Vander Pol et al., 2008) has demonstrated that there are interactions between grain processing method and inclusion amount of wet distiller's grains plus solubles (**WDGS**) in the diet. For example, Corrigan et al. (2009) reported that G:F was 11.6% greater for cattle fed SFC vs DRC-based diets containing no WDGS, but that G:F was not different between SFC-based and DRC-based diets containing ≥ 27.5% WDGS. Therefore, it is possible that grain processing costs associated with steam flaking can be partly alleviated without compromising cattle performance by substituting a portion of the SFC with WSC in SFC-based diets containing WDGS. The objectives of this study were to evaluate the effects of WSC substitution in SFC-based diets containing different amounts of WDGS on feedlot cattle performance, feed conversion, and carcass characteristics.

MATERIALS AND METHODS

Animals and Experimental Design

All procedures were approved by the Institutional Animal Care and Use Committee at New Mexico State University. The experiment was conducted at New Mexico State University's Clayton Livestock Research Center and used Angus-crossed heifers (n = 642, average BW = 412 ± 18 kg) in a randomized complete block design (3 blocks based on initial BW). Heifers were assigned to 36 soil-surfaced pens (12 × 35 m) equipped with fenceline bunks and a water source.

Treatments

Treatments (Table 1) were arranged in a 2 × 3 factorial, and consisted of 6 finishing diets based on SFC with 0 or 20% WSC replacing SFC, and 0, 15, or 30% WDGS replacing SFC (DM basis). Within each weight block,

dietary treatments were randomly assigned to 12 pens per block. Diets were formulated to contain approximately 8% RDP, and were not isonitrogenous. Due to the high concentration of fat in WDGS, tallow was used to decrease the variability in ether extract among dietary treatments. The WSC was conditioned, but not flaked. The WDGS contained a blend of corn grain with no more than 10% sorghum grain. The WDGS were procured from an ethanol plant in Hereford, TX, transported as needed to the research center, and stored in a commodity barn.

Dietary treatments were mixed in an overhead mixer, and delivered to pens of cattle once a day. A slick-bunk management protocol was implemented throughout the experiment, in which bunks were managed to allow only trace amounts of feed in the bunks before feeding each morning.

Collections and Sample Analysis

Pens of heifers were weighed using a pen scale on d 0, 56, and 108. Before weighing, residual feed was removed to be analyzed for DM content. Cattle were shipped approximately 216 km to a local commercial abattoir in Amarillo, TX. All heifers were slaughtered after 108 days on feed.

Weekly samples of feedstuffs and complete diets were obtained to calculate dietary DM. Samples were composited and were analyzed by a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX). Orts were collected, weighed, and sampled for DM to calculate DMI. The DM delivered to each pen was calculated by subtracting dried orts from total DM delivered to the pen.

Carcass data were collected by trained personnel from Cattlemen's Carcass Data Service (West Texas A & M University, Canyon, TX). The data collected included HCW, LM area, 12th rib backfat, KPH, and the calculated USDA yield grade. Livers were evaluated for presence and severity of abscesses and were either not condemned or scored (A-, A, A+) using a scale similar to Brown et al. (1975). Cattle which were railed out with excessive trim were not used in analyzing HCW and dressing percent data. Carcasses were evaluated for USDA quality grade, marbling score, and LM area following a 24-h chill.

Statistical Analysis

Performance and carcass data were analyzed as a randomized complete block design using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Treatments included a 2 × 3 factorial arrangement with pen as the experimental unit. The effects of WSC, WDGS, and the WSC × WDGS interaction were included in the model, and the random effect was block. Contrasts included the linear and quadratic effects of increasing concentrations of WDGS.

Due to an abattoir error, 15% of the heifers were slaughtered before an antemortem inspection, which caused these carcasses to be condemned. Therefore, only 542 carcasses were available for statistical analyses, and carcass adjusted performance measures were not calculated. Carcass quality grades and liver abscesses were analyzed as binomial proportions using the GLIMMIX procedure of SAS. Treatment proportions and standard errors were calculated using the ILINK option. Health data were analyzed using the Freq and Chi-Square procedure of SAS using Fisher's exact test. All analyses had an α of ≤ 0.05, and *P*-values ranging from 0.05 and ≤ 0.10 were considered tendencies.

RESULTS

No WSC × WDGS interactions ($P \geq 0.08$) occurred for DMI, ADG, G:F, and carcass characteristics. Therefore, means for the main effects of WSC and WDGS are presented separately.

Heifers fed SFC-based diets containing 20 vs 0% WSC had greater ($P < 0.01$) DMI, but final BW, ADG, and G:F were not affected ($P \geq 0.11$; Table 2). Replacing 20% of the SFC with WSC did not affect ($P \geq 0.16$) HCW, LM area, 12th rib fat thickness, KPH, calculated USDA yield grade, marbling score, or liver abscesses. The percentage of carcasses grading USDA choice or better tended to be lower ($P = 0.07$), and the percentage of carcasses grading USDA select were higher ($P = 0.03$) for cattle fed SFC-based diets with 20 vs 0% WSC. Morbidity and mortality of heifers were not affected ($P \geq 0.60$) by replacing 20% of SFC with WSC (data not presented).

Increasing concentrations of WDGS in SFC-based diets decreased ($P < 0.01$) final BW linearly, tended to decrease ($P = 0.10$) ADG linearly, tended to increase ($P = 0.08$) DMI linearly, and decreased ($P = 0.01$) G:F linearly (Table 3). Addition of increasing amounts of WDGS to SFC-based diets tended to decrease ($P = 0.09$) HCW linearly, but did not affect ($P \geq 0.18$) other carcass characteristics. Morbidity and mortality of heifers were not affected ($P \geq 0.16$) by the addition of WDGS in SFC-based diets (data not presented).

DISCUSSION

Adding WSC to SFC-based Diets

Greater DMI of heifers fed SFC-based diets containing 20 vs 0% WSC was likely due to altered ruminal fermentation. High concentrate diets reduce DMI, fiber digestion, and change the concentrations of VFA within the rumen (Calsamiglia et al., 2008). Replacing 20% of the SFC with WSC may have decreased the concentration of highly fermentable starch, which could have resulted in differences in ruminal pH and fermentation end products. In confirmation of our theory, Tracey et al. (2011) reported a 3.9% lower pH, a 4.4% lower acetate concentration, a 7.4% greater propionate concentration, and a lower acetate:propionate within the rumens of heifers consuming the 0% WSC diets compared to the 20% WSC diets. The reduction in the percentage of carcasses grading USDA choice or better, and the higher percentage of carcasses grading USDA select caused by replacing 20% of the SFC with WSC is not congruent with the thoughts of Owens and Gardner (2007). The authors determined that feeding cattle SFC-based diets has negative implications on quality grades compared to less extensively processed corn.

Adding WDGS to SFC-based Diets

The fact that DMI tended to increase linearly with the addition of a higher amount of WDGS does not align with current research where DMI was either not affected (Depenbusch et al., 2008; Leibovich et al., 2009; Vander Pol et al., 2009) or it decreased (Drouillard et al., 2005) with inclusion of WDGS in finishing diets The tendency for ADG to decrease as the amount of WDGS increased is supported by a similar response observed by Corrigan et al. (2009) with increasing concentrations of WDGS in SFC-based diets. The combined effects of DMI and ADG with increasing amounts of WDGS in SFC-based diets resulted in a linear decrease in feed conversion as the concentration of WDGS rose.

Conclusion

The results of this study demonstrate that substituting SFC with 20% WSC in finishing diets did not affect animal performance and feed conversion, but decreased carcass quality. In contrast, substituting SFC in finishing diets with increasing amounts of WDGS decreased animal performance and feed conversion, but did not affect carcass characteristics. Limited responses to the substitution of 20% WSC could in part explain the lack of WSC × WDGS interactions. Thus, it is not clear if grain processing could be reduced in finishing diets containing WDGS without affecting feedlot cattle performance and feed conversion.

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Table 1. Composition of dietary treatments (2 × 3 factorial arrangement) with whole shelled corn (WSC) and wet distiller's grains plus solubles (WDGS) replacing steam-flaked corn

	0% WSC			20% WSC		
Item	0% WDGS	15% WDGS	30% WDGS	0% WDGS	15% WDGS	30% WDGS
Ingredient, % of DM						
Steam-flaked corn	78.5	67.7	56.8	58.5	47.7	36.8
Whole shelled corn	-	-	-	20.0	20.0	20.0
WDGS	-	15.0	30.0	-	15.0	30.0
Wheat silage	9.0	9.0	9.0	9.0	9.0	9.0
Soybean meal	7.0	4.0	1.0	7.0	4.0	1.0
Supplement[1]	2.5	2.5	2.5	2.5	2.5	2.5
Tallow	2.0	1.0	-	2.0	1.0	-
Urea	1.00	0.85	0.75	1.00	0.85	0.75
Nutrient[2], % of DM						
CP	14.5	16.3	18.3	14.2	16.3	18.1
NDF	13.3	16.5	18.9	13.9	16.5	19.6
ADF	7.2	9.7	10.7	7.5	8.8	10.5
Ether extract	4.4	5.1	5.8	4.8	5.2	5.7
K	0.74	0.78	0.80	0.73	0.78	0.78
Ca	0.70	0.70	0.69	0.67	0.72	0.68
P	0.25	0.32	0.39	0.24	0.32	0.37
S	0.13	0.21	0.29	0.13	0.21	0.27

[1]Supplement composition: 75.5% limestone; 8.0% potassium chloride; 7.7% salt; 2.9% magnesium oxide; 0.86% zinc sulfate; 0.49% selenium premix (0.06% Se); 0.42% manganese sulfate; 0.31% copper sulfate; 0.036% ethylenediamine dihydroiodide (4.4%); 0.034% vitamin E (500 IU/g); 0.028% vitamin A (30,000 IU/g); 0.004% cobalt sulfate; 0.72% Rumensin (176 g/kg monensin; Elanco Animal Health, Indianapolis, IN); 0.48% Tylan (88 g/kg tylosin; Elanco Animal Health); 0.39% MGA-200 (0.44 g/kg melengestrol acetate; Pfizer Animal Health, New York, NY); 2.1% mineral oil.
[2]Nutrient composition analyzed by Servi-Tech Laboratories, Amarillo, TX.

Table 2. Effects of whole shelled corn (WSC) substitution in steam-flaked corn-based finishing diets on performance and carcass characteristics of feedlot heifers[1]

	WSC, % of DM			
Item	0	20	SEM	*P*-value
No of pens	18	18		
Days on feed	108	108		
Initial BW, kg	413.0	410.4	17.96	0.25
Final BW, kg	541.4	545.5	16.76	0.11
DMI, kg/d	7.83	8.18	0.27	<0.01
ADG, kg/d	1.17	1.21	0.018	0.17
G:F	0.150	0.148	0.005	0.56
HCW, kg	332.8	334.9	11.71	0.30
LM area, cm	83.20	85.07	1.81	0.20
12th rib fat, cm	1.48	1.50	0.06	0.67
KPH, %	3.16	3.22	0.04	0.16
Yield grade	3.02	2.94	0.09	0.41
Marbling score[2]	482	474	12.67	0.40
Choice or better, %	88.91	83.09	0.02	0.07
Select, %	9.75	16.49	0.02	0.03
Abscessed livers, %	3.40	3.58	0.017	0.90

[1]Treatments were a 2 × 3 factorial arrangement of whole shelled corn (WSC) and wet distiller's grains plus solubles (WDGS) replacing steam-flaked corn.
[2]400 = Small[0].

Table 3. Effects of wet distiller's grain plus solubles (WDGS) substitution in steam-flaked corn-based finishing diets on performance and carcass characteristics of feedlot heifers[1]

	WDGS, % of DM				*P*-value	
Item	0	15	30	SEM	Linear	Quadratic
No of pens	12	12	12			
Days on feed	108	108	108			
Initial BW, kg	414.9	410.6	409.6	17.99	0.06	0.50
Final BW, kg	550.4	542.6	537.3	16.81	<0.01	0.66
DMI, kg/d	7.90	8.05	8.07	0.27	0.08	0.40
ADG, kg/d	1.21	1.20	1.16	0.02	0.10	0.55
G:F	0.153	0.149	0.144	0.005	0.01	0.81
HCW, kg	335.4	335.0	331.1	11.76	0.09	0.42
LM area, cm	83.76	84.83	83.82	1.95	0.97	0.49
12th rib fat, cm	1.53	1.50	1.44	0.06	0.18	0.70
KPH, %	3.17	3.16	3.23	0.04	0.24	0.43
Yield grade	3.04	2.97	2.94	0.10	0.35	0.81
Marbling score[2]	477	483	474	13.46	0.78	0.46
Choice or better, %	87.49	88.14	82.64	0.02	0.23	0.37
Select, %	12.51	10.06	16.31	0.02	0.33	0.18
Abscessed livers, %	4.06	3.66	2.85	0.02	0.51	0.87

[1]Treatments were a 2 × 3 factorial arrangement of whole shelled corn (WSC) and wet distiller's grains plus solubles (WDGS) replacing steam-flaked corn.
[2]400 = Small[0].

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EFFECTS OF CONTINUOUS AND STEP-UP RACTOPAMINE HYDROCHLORIDE SUPPLEMENTATION PROTOCOLS ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISITICS OF FINISHING STEERS

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ABSTRACT: Thirty-six Angus-Simmental cross steers (510 ± 4.99 kg initial BW) fed identical finishing diets were randomly assigned to receive one of three ractopamine hydrochloride (RH) treatments during the last 42 d of the finishing period to evaluate the effects of continuous (CNT) or step-up (STEP) RH supplementation on feedlot performance and carcass characteristics of beef steers. Treatments were 0 (CON) or 200 (CNT) mg RH from d 0 to d 42, or daily supplementation of 100 mg RH from d 0 to d 21, no RH from d 21 to 28, and daily supplementation of 300 mg RH from d 28 to d 42 (STEP). Steers were individually fed ad libitum and weights were taken at 14 d intervals. Carcass characteristics were collected following a 24-h chill. Dry matter intake (P= 0.61), total BW gain (P = 0.52), G:F (P=0.36), and final BW (P=0.41) were not different due to RH supplementation during the last 42 d of the finishing period. Treatment did not affect ($P \geq 0.26$) initial BW, DMI, ADG, G:F, or total BW gain throughout the feeding period. Likewise, those carcass measurements closely associated with live weight gain such as HCW, dressing percent, 12th rib fat thickness, LM area, % KPH, or yield grade did not differ ($P \geq 0.19$) across treatments. However, CON had greater ($P = 0.04$) marbling scores than CNT, with STEP being intermediate to both treatments. This effect likely contributed to the trend ($P = 0.08$) in quality grade differences between CON and CNT, with STEP again being intermediate. These data suggest that supplementation of 200 mg per day RH continuously, or feeding a step up protocol, did not improve feedlot performance or final BW, nor did it decrease marbling score.

Introduction

Supplementing a ß-adrenergic agonist (BAA), to livestock diets such as ractopamine hydrochloride (RH), has been shown to improve feeding efficiency by partitioning nutrients for protein synthesis rather than fat accretion (Baker et al., 1984; Watkins et al., 1990). Marketed for beef cattle by Elanco Animal Health as Optaflexx™, RH is approved for feeding during the last 28 to 42 d prior to harvest at concentrations ranging from 70 to 430 mg/hd/d to increase average daily gains (ADG) and improve feed efficiency (G:F).

Numerous studies of varying feeding durations have reported increases in ADG and G:F (Anderson et al., 1989; Carroll et al., 1990; and Preston et al., 1990), however, few experiments have been conducted to define the optimal concentration level and feeding duration of RH to beef cattle to maximize production efficiency. Maximal ADG has been reported at feeding levels of 200mg/h/d for 35 d (Abney et al., 2007) and maximal efficiency reported at 42 days with 100 mg/h/d). Desensitization of ß-adrenergic receptors (BAR) due to over stimulation by BAA may have contributed to the results reported by Abney et al. (2007) with 35 d being the rate limiting feeding duration of RAC at 200 mg/hd/d, and 42 d for steers fed 100 mg/hd/d for optimal ADG and G:F.

Recently, chronic stimulation studies have been conducted to determine the effects of RAC on BAR mRNA abundance and mixed results have been reported. Sissom et al. (2007) and Winterholler et al. (2007) reported that feeding RAC for 28 d had no effect on β1- and β3-AR mRNA, but tended to increase the presence of β2-AR mRNA ($P = 0.09$) in the semimebranosus muscle in heifers and steers, respectively. Contrastingly, Winterholler et al. (2008), reported β1-AR mRNA tended to be increased in LM of beef steers, while there was no effect on β2- and β3-AR mRNA. In dairy steers, a decrease in β1- and β2-AR mRNA in LM tissue following RH administration was reported (Walker et al., 2007).

With many inconsistencies reported in the literature dealing with the effects of RH supplementation and chronic BAA stimulation on finishing beef cattle performance and carcass characteristics, it is necessary to determine the most effective RH feeding regimen to ensure production efficiency is maximized for the benefit of beef producers and consumers alike. Therefore, our hypothesis was that by interrupted feeding of a BAA would result in greater feedlot performance without affecting carcass quality. However, over 1/3 of all carcasses reported in the 2000 National Beef Quality Audit received a marbling score of Small with over 63% of those carcasses having a marbling score of less than Small50. Therefore, a 50 point decrease in marbling score, similar to that associated with the CNT treatment in the current study, would have reduced the percent of carcasses grading USDA Choice or better in the 2000 National Beef Quality Audit from nearly 53% to only

approximately 32%. Consequently, the negative economic impact for producers feeding finishing steers diets containing CNT could be severe based solely on carcass merit if the cattle represent the industry average for marbling and respond to CNT similarly to cattle in the current study.

Materials and Methods

Animals and Treatments. All procedures involving animals during this study were approved by the Purdue Animal Care and Use Committee. Thirty-six Angus-Simmental cross steers (510 ± 4.99 kg initial BW) were used in this experiment. Upon arrival, steers were fed a grower ration and transitioned to the finishing diet (Table 1) used in the trial. Initial and final steer BW were the average of two weights taken on consecutive days at the beginning and ending of the trial. Steers were blocked by initial BW, and randomly assigned to be individually fed one of three treatments; 1) control (no dietary RH: CON), 2) daily supplementation of 200 mg RH from d 0 to d 42 (200 mg; CNT) and 3), daily supplementation of 100 mg RH from d 0 to d 21, no RH from d 21 to 28, and daily supplementation of 300 mg RH from d 28 to d 42 (STEP). Bunks were evaluated daily at 0600 to and cattle were fed ad libitum at 0700 daily. Steers were housed individually in a barn with concrete slatted floors in 1.4-m x 3.4-m pens and had free access to an automated watering system.

Performance and Carcass Characteristics Data Collection. Weights were taken at 14 d intervals, and final BW were collected on two consecutive days prior to harvest to measure feedlot performance. Steers were fed ad libitum and feed refusals were weighed to calculate DMI and feed efficiency. Hot carcass weight measurements were taken post-exsanguination, and following a 24-h chill, trained personnel collected dressing percent, 12th rib fat thickness, LM area, KPH %, preliminary yield grade, marbling score and quality grade. Final yield grades were determined according to the formula established by Aberle et al. (2001).

Statistical Analyses. Growth performance and carcass characteristics were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) for a randomized complete block design, and individual steer was used as the experimental unit. Difference in means were considered significant when the p-value was ≤ 0.05, with a p-value ≤ 0.10 considered as a tendency approaching significance.

Results

Feedlot Performance. Initial BW were similar ($P = 0.53$) across all treatments (505.30, 513.26, 510.04 ± 4.99; CON, OPT, STEP, respectively; Table 2), and no BW differences were apparent at d 14 ($P = 0.48$), d 28 ($P = 0.26$), or at the conclusion of the feeding period ($P = 0.41$). In agreement with this, daily gains were also similar throughout the feeding period d 0–d 14 ($P = 0.20$), d 14-d 28 ($P = 0.41$), d 28-d 42 ($P = 0.95$), d 0-d 28 ($P = 0.27$), and overall daily gain ($P = 0.52$). Accordingly, there were no changes in total BW gain ($P = 0.52$), DMI ($P = 0.61$) G:F ($P = 0.36$), or F:G ($P = 0.40$) due to dietary treatment.

Final Carcass Characteristics. The lack of performance differences was reflected in several carcass measurements often impacted by live weight gain with no differences across treatments being detected for HCW ($P = 0.31$; Table 3), dressing percent ($P = 0.80$), 12th rib fat thickness ($P = 0.35$), LM area ($P = 0.19$), KPH % ($P = 0.97$), and yield grade ($P = 0.38$). However, CON fed cattle had greater marbling scores ($P = 0.04$) than CNT fed steers, with STEP fed steers being intermediate to both treatments, this effect likely contributed to the trend in quality grade differences ($P = 0.08$) between CON and CNT supplemented groups with the STEP treatment again being intermediate.

Discussion

Production variables that have a significant impact on the financial viability of beef finishing operations such as ADG, DMI, and feed efficiency ratios have repeatedly been improved through RH supplementation both in classical and contemporary feeding trials (Anderson et al., 1989; Carroll et al., 1990; Preston et al., 1990; Avendaño-Reyes et al., 2006; and Abney et al., 2007). In beef cattle, there is evidence of an optimal duration of feeding threshold of 33-35 d (Avendaño-Reyes et al., 2006; and Abney et al., 2007, respectively) when RH is supplemented at a minimum level of 200/mg/hd/d prior to desensitization and subsequent downregulation of the BAR which can result in decreased animal performance. However, the optimization of ADG at a level of 100 mg/hd/d has been reported to be at 42 d (Abney et al., 2007). The results of the present trial corroborates that a 42 d feeding period of RH is not beneficial on feeding performance when levels exceed 100 mg/hd/d at any time of the feeding period.

The current trial protocol was designed to test the theory of desensitization, and its impact on performance in feedlot steers. There is little data published investigating the effects of chronic stimulation on the performance effects of RH supplementation in feedlot cattle. Quinn et al. (2008), however, tested the effects of chronic stimulation in heifers with an intricate study designed to determine the effects of RH administration based on duration and dosage level which included a step-up protocol where the level of RH was increased from 100 mg/hd/d to 300 mg/hd/d each 14 d over a period of 42 d. In the same report, the step-up group was intermediate in all observed performance areas; however, the RH fed cattle had an increase in carcass gain, which can be directly attributed to live performance. No differences in performance due to the RH feeding were reported in the present study, as was the case in the lone publicized trial evaluating a step-up regimen (Quinn et al., 2008). In the present study, a challenge exists when evaluating the results of the STEP feeding protocol. Although the maximal benefit of feeding RH at a level of 100 mg/hd/d has been previously thought to be d 42 (Abney et al., 2007), the BAR may have been desensitized by trial d 21 when RH was removed from the diet, and therefore, the reinitiating of

RH to the diet 7 d later, despite the 200 mg/hd/d increase, would have less of a positive impact than predicted. As stated previously, increases in performance in beef cattle have been reported up to 35 d following the inclusion of RH at a level of 200 mg/hd/d, however, in swine d 22 has been noted as the point of inhibition for increased animal performance (Williams et al., 2004), and corresponding desensitization. Accordingly, had our STEP protocol been configured for 21 d of 300 mg/hd/d immediately following 14 d of 100 mg/hd/d and a 7 d ceasing of administration, instead of 21 d at 100 mg/hd/d followed by a 7 d removal of RH from the diet, and finishing with 14 d at the 300 mg/hd/d level, the secondary administration may have been more effective on feeding performance. The absence of negative effects on carcass characteristics due to the STEP regimen, in contrast to the effects of the CNT feeding protocol, suggests that chronic stimulation may in fact have a negative effect on carcass quality.

There are many large numerical differences presented in the current data, which point out the inherent flaw of limited experimental units. These differences are presented in final BW, total BW gain, ADG, HCW, LM, and YG between the CNT and CON treatments, with final BW, total BW gain, and ADG, showing a broad numerical change between CNT and STEP. Therefore, it can be hypothesized that an increase in steers/treatment may have resulted in statistical changes that would be more consistent with previous reports. However, in the present study, the numeric differences were inconclusive. Results from the present study still contradict previous reports regarding carcass characteristic differences due to RH supplementation, and in fact the decrease in marbling score, and concomitant trend to decrease quality grade as a result of constant administration is the first negative report of its kind. Due to the genetic consistency of the steers on the present study, and the extended transitioning period to the finishing period prior to treatment initiation, it is likely the decrease in intramuscular fat observed was associated with chronic stimulation. However, the decrease in marbling was not great enough to decrease potential carcass value in the present study since the numerical mean marbling and quality grade scores for each treatment still resulted in carcasses grading USDA Choice.

Implications

Many of the results of the present study conflict with that of some early investigations of RH supplementation, yet provides insight into pinpointing the most appropriate method of integrating RH into feeding protocols. Feedlot performance was not altered due to RH supplementation at either a constant level or a step-up protocol over a period of 42 days. Carcass quality was not improved, and chronic stimulation due to constant RH administration may have a negative effect on marbling, and potentially final quality grades of beef steers. The phenomenon of chronic stimulation and its effect on desensitization in beef steers is still uncertain, and further research should be conducted to elucidate the most appropriate level of RH inclusion and feeding duration to maximize beef production efficiency.

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Table 1. Ingredients and chemical composition of diets fed to finishing steers.

	Treatments		
Ingredient, % of diet DM	CON	CNT	STEP
Corn silage	12.0	12.0	12.0
Cracked corn	80.0	80.0	80.0
Soybean meal	8.0	8.0	8.0
Supplement	2.5	2.5	2.5
Urea	5.69	5.69	5.69
Ackey Mineral[1]	4.49	4.49	4.49
Calcium Carbonate	79.09	79.09	79.09
Salt	10.09	10.09	10.09
Rumensin, 80 mg/d[2]	0.52	0.52	0.52
Tylan 100[3]	0.126	0.126	0.126
Nutrients			
DM	68.1	68.1	68.1
CP	15.9	15.9	15.9
NEg[4]	1.43	1.43	1.43
Ractopamine hydrochloride, mg/hd/d			
Trial d 0-21	---	200.0	100.0
Trial d 21-28	---	200.0	---
Trial d 28-42	---	200.0	300.0

[1]Ackey Mineral, Dayton OH.
[2]80 g Monensin per lb.
[3]100 g Tylosin per lb.
[4]Expressed as Mcal/kg

Table 2. Effects of continuous and step-up ractopamine hydrochloride supplementation protocols on finishing performance in finishing steers

	Treatment[1]				
Item	CON	CNT	STEP	SEM[2]	*P*-value
Initial BW, kg	505.30	513.26	510.04	4.99	0.53
d 14 BW, kg	534.66	544.89	539.20	5.92	0.48
d 28 BW, kg	557.39	571.97	560.99	6.38	0.26
Final BW, kg	582.10	597.44	587.22	8.12	0.41
DMI, kg/d	8.66	8.26	8.48	0.29	0.61
Daily gain, kg (d 0-14)	2.10	2.26	2.09	0.80	0.20
Daily gain, kg (d 14-28)	1.62	1.94	1.56	0.21	0.41
Daily gain, kg (d 28-42)	1.77	1.82	1.87	0.24	0.95
Daily gain, kg (d 0-28)	1.86	2.10	1.82	0.13	0.27
Daily gain, kg (overall)	1.83	2.01	1.84	0.12	0.52
Total BW gain, kg	76.80	84.19	77.18	5.08	0.52
Gain/feed, kg/kg	0.16	0.18	0.17	0.01	0.36
Feed/Gain, kg/kg	6.79	5.68	6.16	0.57	0.40

[1]CON = no RH supplementation; OPT = 200 mg/hd/d RH supplementation from d 0-42; STEP = 100 mg/hd/d RH supplementation from d 0-21, no RH supplementation from d 21-28, 300 mg/hd/d RH supplementation from d 28-42

[2]The greatest SEM was presented (n = 12/treatment)

Table 3. Effects of continuous and step-up ractopamine hydrochloride supplementation protocols on carcass characteristics in finishing steers

	Treatment[1]				
Item	CON	CNT	STEP	SEM[2]	*P*-value
Hot carcass weight, kg	349.73	360.19	354.51	4.76	0.31
Dressing percent	60.17	60.33	60.50	0.003	0.80
12th rib fat thickness, cm	1.08	0.93	1.14	0.10	0.35
LM area, cm^2	94.95	102.31	99.45	2.79	0.19
KPH, %	2.17	2.13	2.17	0.15	0.97
Yield grade	2.21	1.85	2.09	0.18	0.38
Marbling score[3]	585.00^{a}	530.83^{b}	553.33^{a,b}	14.32	0.04
Quality grade[4]	17.50^{c}	17.00^{d}	17.25^{c,d}	0.15	0.08

[1]CON = no RH supplementation; OPT = 200 mg/hd/d RH supplementation from d 0-42; STEP = 100 mg/hd/d RH supplementation from d 0-21, no RH supplementation from d 21-28, 300 mg/hd/d RH supplementation from d 28-42

[2]The greatest SEM was presented (n = 12/treatment)

[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]Means within a row lacking a common superscript differ ($P \leq 0.05$)

[c,d] Means within a row lacking a common superscript differ ($P \leq 0.10$)

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EFFECTS OF RUMEN-PROTECTED ARGININE SUPPLEMENTATION ON SERUM AMINO ACID CONCENTRATIONS IN FORAGE-FED STEERS

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ABSTRACT: Four ruminally cannulated steers were used in a 4 x 4 Latin square with the following treatments (2x/d): ad libitum grass hay (7.2% CP and 67.6% NDF; CON), hay and 27 mg Arg/kg BW injected intravenously (Arg-INJ), hay with 90 mg rumen-protected Arg/kg BW (Arg-180), and hay with 180 mg rumen-protected Arg/kg BW (Arg-360). Arginine in Arg-180 was estimated to be equal to Arg-INJ. Each period consisted of a 7-d adaptation then 14 d of treatment. Immediately prior to feeding, rumen-protected Arg was dosed ruminally and Arg-HCl or saline was injected in the jugular vein. Daily blood samples were taken from the contralateral jugular vein 2 h post-feeding from d -1 to 14 of treatment. On d 8 of treatment, blood samples were taken at -0.03, 0, 0.5, 1, 2, 4, 6, 8, 10, and 12 h relative to feeding to estimate 12-h circulating AA. Data were analyzed with treatment as a fixed effect and steer and period as random effects. As expected, daily and 12-h serum Arg area under the curve (AUC) was greater ($P < 0.08$) in Arg-INJ than all other treatments. Additionally, Arg was greater ($P < 0.1$) in Arg-360 than CON, with Arg-180 intermediate. Although daily and 12-h total AA were unaffected by treatment ($P > 0.16$), total daily essential AA were greater ($P < 0.01$) for Arg-INJ than Arg-180. Daily citrulline (Cit) AUC was greater ($P \leq 0.05$) for Arg-360 and Arg-INJ than CON, greater ($P = 0.09$) for Arg-360 than Arg-180, and greater ($P < 0.1$) for Arg-180 than CON. Steers fed Arg-360 also had the greatest ($P < 0.06$) 12-h Cit AUC, and CON had less ($P < 0.03$) Cit than Arg-180 and Arg-INJ. Daily and 12-h ornithine (Orn) AUC was greatest for Arg-INJ ($P < 0.01$) and was greater ($P = 0.03$) for Arg-360 than CON. Daily Lys AUC was greatest ($P < 0.03$) in CON, and Arg-180 had greater ($P = 0.07$) Lys than Arg-360. There was no effect ($P = 0.17$) of treatment on 12-h Lys AUC. Steers fed Arg-360 had greater ($P < 0.05$) daily and 12-h Gln AUC than CON and Arg-INJ, whereas Pro and Glu were unaffected ($P > 0.34$) by treatment. Results indicate that circulating Arg, Arg metabolites (Cit and Orn), and associated AA can be altered with rumen-protected Arg supplementation.

Key words: amino acids, arginine, supplementation

Introduction

Feed represents the greatest fixed cost for beef cow-calf producers (Miller et al., 2001), thus development of targeted supplementation schemes may be able to improve both productivity and profitability. Arginine is a potential supplement, especially in the reproducing female, because of its many functions in the body including synthesis of nitric oxide (**NO**), polyamines, creatine, and glutamate (Wu and Morris, 1998). Nitric oxide increases blood flow by causing vasodilation, stimulating angiogenesis via vascular endothelial growth factor, and increasing vascular permeability (Martin et al., 2001; Roy et al., 2006). Increased blood flow can have many positive effects systematically, including nutrient uptake, ovarian function, embryonic and fetal development, and lactation.

Dietary Arg supplementation in non-ruminants has increased embryonic and fetal survival (Mateo et al., 2007; Zeng et al., 2008), prevented fetal growth restriction of hypoxia (Vosatka et al., 1998), and positively impacted neonatal intestinal growth and development (Zhan et al., 2008; Yao et al., 2011). Despite this, supplemental Arg research in ruminants has been limited because Arg must escape degradation by ruminal microbes, making intravenous injection or post-ruminal infusion necessary. Using these methods, supplemental Arg has increased milk production and circulating hormones in dairy cows (Chew et al., 1984), increased GH in sheep and cattle (Davenport et al., 1990a; Davenport et al., 1990b), stimulated LH production in prepubertal ewes (Recabarren et al., 1996), and increased plasma flow in the portal and hepatic veins (Maltby et al., 2005). Recently, injected Arg-HCl has decreased embryonic loss in ewes (Luther et al., 2008), increased lamb birth weight in gestationally nutrient restricted ewes (Lassala et al., 2010), and improved fetal lamb survival to term in prolific ewes (Lassala et al., 2011).

Because multiple intravenous injections or post-ruminal infusions are not practical for Arg supplementation in a production setting, investigation of alternatives such as rumen-protected Arg is necessary. We hypothesize that injecting L-Arg-HCl will increase circulating levels of Arg and its metabolites and increase systemic blood flow through its role in NO production. Additionally, we hypothesize that feeding rumen-protected L-Arg will produce a less immediate but more sustained increase in circulating Arg that will elicit similar blood flow responses in a dose-dependent manner.

Materials and Methods

Animals and Diets. All procedures were approved by the North Dakota State University Animal Care and Use Committee. Four ruminally, duodenally, and ileally cannulated steers were used in a 4 x 4 Latin square design. Steers were allocated to receive 1 of 4 treatment diets in each experimental sampling period: 1) ad libitum grass hay (7.2% CP, 67.6% NDF, 43.0% ADF, 9.5% ash, 0.55% Ca,

0.22% P; **CON**) 2) CON hay with 27 mg L-Arg-HCl/kg BW injected twice daily (54 mg/kg BW daily; **Arg-INJ**); 3) CON hay with 90 mg rumen-protected L-Arg/kg BW supplementation twice daily (180 mg/kg BW daily; **Arg-180**); and 4) CON hay with 180 mg rumen-protected L-Arg/kg BW supplementation twice daily (360 mg/kg BW daily; **Arg-360**). The Arg-180 treatments was estimated to deliver the same Arg to circulation as Arg-INJ, based on the assumptions that 50% of rumen-protected Arg fed is degraded by rumen microbes and 40% of Arg reaching the small intestine is catabolized in this tissue (Wu, 1998). Serum AA data presented here are part of a larger study to also determine the effects of rumen-protected Arg on systemic blood flow, intestinal appearance of AA, site and extent of digestion, and ruminal fermentation.

Steers were fed hay only for a 7-d adaptation period (d -7 to -1), followed by a 14-d collection period (d 1 to 14) when steers also received their respective Arg treatments. Steers were housed in a climate-controlled facility in individual pens (3.0 x 3.7 m) from d -7 to 7 of each sampling period and in individual tie-stall stanchions (1.0 x 2.2 m) from d 8 to 14 of each sampling period, with ad libitum access to fresh water (every day) and trace-mineralized salt (d -7 to 7).

Steers were fed ad libitum chopped grass hay (10.2 cm) at 0700 and 1900 daily in approximately equal amounts. During the collection period, rumen-protected Arg was dosed intraruminally via the ruminal cannulae and intravenous jugular injections of either Arg-HCl (Arg-INJ treatment) or saline (all other treatments, based mL/kg BW dose of Arg-HCl) were given immediately before feeding hay. Daily hay allowance was based on 110% of the average hay intake during the previous week to ensure ad libitum intake. Two-day BW were taken at the beginning of each sampling period to determine necessary dosage of each Arg treatment.

Sample Collections and Analyses. Daily blood samples for serum AA analysis were collected via jugular venipuncture (contralateral to injection site) at 2 h after feeding and treatment dosing from d -1 to 14 of treatment. Prior to d 8, a jugular catheter was placed in the contralateral jugular vein to that used for injection of Arg-HCl or saline. Intensive blood samples were taken via the jugular catheter at -0.03, 0, 0.5, 1, 2, 4, 6, 8, 10, and 12 h relative to treatment on d 8.

Blood samples (9 mL) were collected using Corvac serum separator tubes with thrombin (Tyco Healthcare Group LP, Mansfield, MA). Whole blood was refrigerated for at least 1 h after collection, and then centrifuged at 1,500 x *g* for 20 min at 4˚C to obtain serum. Serum was pipetted into 2-mL screw-cap vials and stored at -20˚C until analysis.

Serum AA concentrations were determined by ultra performance liquid chromatography (UPLC) using an Aquity UPLC System with TUV Detector with a MassTrak AAA Column (2.1 x 150 mm, 1.7 μm) and Solution Kit (Waters Corporation, Milford, MA).

Calculations and Statistical Analysis. Amino acids were expressed as concentration and area under the curve (AUC) of AA concentration over time. Sigmaplot 12.0 (Systat Software, Inc., San Jose, CA) was used to calculate AUC.

Data were analyzed as a 4 x 4 Latin square (Cochran and Cox, 1957) using the mixed model procedure of SAS version 9.1 (SAS Institute, Inc., Cary, NC). The general model included random effects of steer and sampling period and the fixed effect of treatment. Serum AA over time was analyzed first as repeated measures within the 4 x 4 Latin square, with sampling time as the repeated effect and steer within treatment as the subject. Because of the prevalence of treatment x time interactions, AUC was calculated for all AA concentrations. Means were separated using least significant difference and considered significant if $P \leq 0.10$.

Results and Discussion

Daily blood samples. Serum Arg, citrulline (Cit), ornithine (Orn), and Gln concentrations, measured at 2 h post-treatment, were affected by the interaction of time × treatment ($P < 0.05$). When expressed as AUC (Table 1), serum Arg was greater ($P = 0.08$) for Arg-INJ than all other treatments. In addition, Arg AUC was greater ($P < 0.10$) for steers fed Arg-360 than CON, with Arg-180 intermediate.

Serum Orn AUC followed Arg AUC (Table 1). Arginine is converted to Orn by arginase, which is not only an important component of the urea cycle, but also occurs in other tissues independent of the urea cycle (Wu and Morris, 1998). Polyamines, critical for growth and development, are synthesized from Orn, therefore an increase in Orn gives the potential for increased polyamine synthesis.

Daily Cit AUC (Table 1) was decreased ($P < 0.10$) in CON compared with all other treatments and was greater ($P = 0.09$) for Arg-360 than Arg-180. Nitric oxide synthase converts Arg to NO and Cit. Thus, serum Cit may be indicative of vascular endothelial NO production. Alternatively, Cit is converted to Arg by many tissues (Wu and Morris, 1998), and therefore provides possible additional Arg.

Although there was no effect ($P > 0.34$ of treatment on daily Pro or Glu AUC, Gln AUC (Table 1) was greater ($P < 0.05$) in steers fed Arg-360 compared with CON and Arg-INJ, with Arg-180 intermediate. Arginine can be synthesized from or can be used to synthesis Pro, Glu, or Gln (Wu and Morris, 1998). In this study, it appears that either Gln synthesis increased or Gln was spared from Arg synthesis with rumen-protected Arg supplementation.

Daily serum Lys AUC (Table 1) was greater ($P < 0.01$) for CON compared with all other treatments. In addition, steers fed Arg-180 had greater ($P = 0.07$) Lys AUC than Arg-360. Lysine absorption may have been reduced in steers fed rumen-protected Arg in a dose-dependent manner because Lys competes with Arg for intestinal transport, as previously reported by Zhan et al. (2008).

Although daily total AA AUC were unaffected by treatment ($P = 0.33$), total essential AA AUC was greater ($P < 0.07$) for Arg-INJ than Arg-180 and Arg-360, while CON was also greater ($P < 0.04$) than Arg-180.

Intensive 12-h blood samples. Serum Arg, Cit, and Orn concentrations were influenced by the interaction of time × treatment ($P < 0.08$; Arg shown in Figure 1). As expected, serum Arg was elevated dramatically immediately after

injection in Arg-INJ. Serum Arg of steers injected with Arg-HCl showed an uncharacteristic increase in Arg at 1 h post-injection, however, which is difficult to explain.

Circulating AA 12-h AUC were similar to daily AA AUC determined at 2 h post-treatment. Serum Arg (Trt $P = 0.04$) and Orn (Trt $P = 0.003$) 12-h AUC (Table 2) followed daily Arg and Orn AUC. Serum Cit 12-h AUC (Table 2) was greatest ($P < 0.06$) for Arg-360, and CON had less ($P < 0.03$) Cit than Arg-180 and Arg-INJ.

Twelve-hour Pro, Glu, and Lys AUC (Table 2) were unaffected by treatment ($P > 0.16$), although 12-h Gln AUC (Table 2) followed daily Gln AUC. There was no effect of treatment on total AA or essential AA 12-h AUC ($P > 0.44$).

Conclusions. Circulating Arg was increased by supplementation with 360 mg rumen-protected Arg/kg BW, but not with 180 mg/kg BW. Conversely, steers fed 180 mg/kg BW had improved vascular hemodynamics in the carotid and caudal arteries in this study (Meyer et al., 2011). This indicates that elevated jugular Arg concentration may not be necessary to alter NO production and vascular hemodynamics, as was previously suggested in ewes (Saevre et al., 2010). Jugular vein Arg concentrations may not be representative of Arg available to tissues including endothelial cells, however.

In summary, rumen-protected Arg supplementation may increase circulating concentration of Arg and its metabolites in forage-fed steers. This may alter systemic blood flow and elicit other Arg-related physiological responses, although more research is necessary to determine the appropriate dosage and timing for targeted supplementation.

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Table 1. Effects of injected L-Arg-HCl or rumen-protected L-Arg supplementation on daily serum amino acid area under the curve[1] in forage-fed steers

	Treatments					
Item	CON	Arg-INJ	Arg-180	Arg-360	SEM	*P*-value
Arg	3,941[a]	5,860[c]	4,256[ab]	4,633[b]	206	0.002
Cit	1,078[a]	1,295[bc]	1,256[b]	1,441[c]	117	0.04
Orn	1,178[a]	2,355[c]	1,286[ab]	1,535[b]	87	<0.001
Pro	1,645	1,665	1,607	1,658	46	0.35
Glu	2,003	1,868	1,831	1,854	98	0.37
Gln	7,186[a]	7,058[a]	7,469[ab]	7,875[b]	390	0.08
Lys	2,646[c]	2,368[ab]	2,447[b]	2,289[a]	179	0.02

[a,b,c] Within a row, means with different superscripts differ ($P < 0.10$)

[1]Area under the curve expressed in μM × d of serum samples obtained 2 h post-treatment from d -1 to 14 of Arg supplementation.

Table 2. Effects of injected L-Arg-HCl or rumen-protected L-Arg supplementation on 12-h serum amino acid area under the curve[1] in forage-fed steers

	Treatments					
Item	CON	Arg-INJ	Arg-180	Arg-360	SEM	*P*-value
Arg	2,814[a]	3,972[c]	3,226[ab]	3,365[b]	199	0.04
Cit	900[a]	1,110[b]	1,100[b]	1,278[c]	97	0.01
Orn	927[a]	1,698[c]	1,089[ab]	1,230[b]	74	0.003
Pro	1,365	1,366	1,329	1,378	51	0.82
Glu	1,687	1,604	1,577	1,591	82	0.59
Gln	5,697[a]	5,552[a]	5,904[ab]	6,471[b]	358	0.09
Lys	2,131	1,974	2,170	1,957	156	0.17

[a,b,c] Within a row, means with different superscripts differ ($P < 0.10$)

[1]Area under the curve expressed in μM × h of serum samples obtained at -0.03, 0, 0.5, 1, 2, 4, 6, 8, 10, and 12 h relative to Arg supplementation on d 8 of treatment.

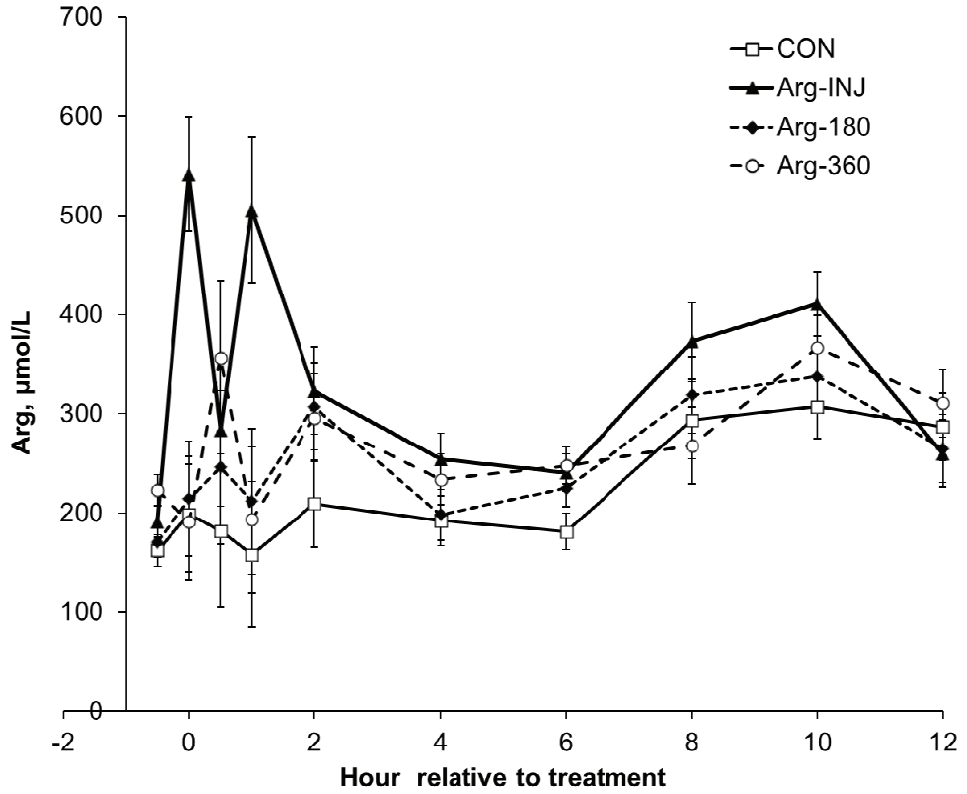

Figure 1. Effect of injected L-Arg-HCl or rumen-protected L-Arg supplementation on 12-h serum Arg concentration of forage-fed steers on d 8 of treatment. Treatment × time $P = 0.003$.

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Effects of summer supplementation on long yearling steers grazing native range

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ABSTRACT: A 3-yr study was designed to evaluate the effects of supplementing modified wet distillers grains with solubles (**MDGS**) on long yearling steer performance grazing native range and subsequent feedlot performance. Annually, steers (n=240; initial BW = 271 ± 46 kg) were backgrounded on cornstalks from late fall to mid-spring (144 d). While grazing corn residue calves were supplemented 2.27 kg/steer daily of Sweet Bran. Following backgrounding steers were allowed to graze smooth bromegrass pastures for 23 d. Before grazing smooth brome, calves were weighed, stratified by BW, assigned randomly to summer grazing treatments to be applied on Sandhills range (cool and warm season grasses). Summer grazing treatments included: grazing native range with no supplement (**CON**); and grazing native range with MDGS supplementation equivalent to 0.6% BW (**SUPP**). Modified wet distillers grains with solubles were fed daily on the ground. Steers were allowed to graze Sandhills range for the remainder of the summer grazing period (136 d) before entering the feedlot in early fall. At the time of summer treatment assignment, BW was not different (P = 0.92) between SUPP and CON steers. However at feedlot entry, SUPP steers were 49 kg heavier (P < 0.01) than CON steers. Therefore, SUPP steers had 0.30 kg/d greater (P < 0.01) ADG than CON steers during summer grazing. Feedlot harvest date was targeted to equal fat thicknesses (1.27 cm) between CON and SUPP steers; thus, rib fat thickness was not different (P = 0.57) between the two treatments. As a result of the harvest goal, final BW was not different (P = 0.92) between CON and SUPP steers; however, it required 24 fewer days in the feedlot (P <0.01) for SUPP steers to reach this point. Feedlot ADG tended to be greater (P = 0.07) for CON steers than SUPP steers, but feed efficiency and DMI were not different (P > 0.16). Supplemental MDGS can be fed, increase cattle gains during summer grazing, and decrease days on feed in the feedlot.

Key words: Beef Cattle, Supplementation, Distiller Grains

Introduction

Yearling production systems capitalize on use of the animal to harvest forage; as opposed to more intensive systems, that require harvested forages and longer grain feeding. Yearling production systems are further segregated into two groups: 1) short (summer) yearlings, which are received in the fall, backgrounded during the winter, then enter the feedlot in the spring; and 2) long (fall) yearlings, which are received in the fall and backgrounded during the winter then graze summer pasture before entering the feedlot. Co-products of the corn dry milling industry fit well into forage feeding programs because distillers grains provide a highly fermentable fiber source that does not negatively impact forage digestion (Loy et al., 2008), and also supply additional undegradable intake protein to meet metabolizable protein deficiencies common to lighter weight cattle grazing forage (NRC, 2000).

The objective of the current study was to determine effects of supplementing MDGS on the ground to long yearling steers while grazing native Sandhills range and subsequent feedlot performance and carcass characteristics.

Materials and Methods

Winter Phase. All procedures and facilities utilized were under the approval of the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Each year of a 3 yr study, 240 crossbred steers (initial BW = 226 ± 14 kg) were backgrounded as a common group on cornstalk residue at the University of Nebraska research feedlot facilities (near Mead, NE) from late fall to mid-spring (145 d). While grazing cornstalks, calves were supplemented the equivalent of 2.27 kg DM·hd^{-1}·d^{-1} of Sweet Bran (Cargill, Blair, NE). After cornstalk backgrounding, steers were limit fed at 1.8% BW (DM basis) for 5 d. Initial BW for summer grazing was the mean of weights taken on two consecutive days.

Summer Phase. Approximately April 15 each year, calves were implanted with Revalor G (40 mg trenbolone acetate and 8 mg extradiol; Intervet Inc., Millsboro, DE), weighed, stratified by BW, and assigned randomly to one of two summer grazing treatments. First, they were relocated on the University facilities and allowed to graze smooth bromegrass pastures for approximately 23 d. After grazing brome, steers were transported to the University of Nebraska Barta Brothers Ranch (near Rose, NE) to graze native Sandhills range where summer grazing treatments were applied. Summer grazing treatments included: grazing native range with no supplementation; and grazing native range with MDGS supplementation at 0.6% BW. Weights were projected using ADG for determination of summer grazing supplementation. Modified wet distillers grains with soluble was procured from one source prior to cattle arrival and stored on the ground in a plastic silo bag. Modified wet distillers grains with solubles was fed daily on the ground with a tractor and feed wagon, allowing steers to be distributed to different locations within each pasture at the time of feeding. Steers grazed Sandhills

range an average of 136 d before entering the feedlot in late September each year.

Feedlot Phase. At the end of summer grazing, steers were transported back to University of Nebraska research feedlot facilities (near Mead, NE), reimplanted with Revalor S (120 mg trenbolone acetate and 24 mg estradiol; Intervet Inc., Millsboro, DE), and final summer grazing BW was collected (same procedure as above). Upon re-entry in the feedlot, steers were targeted to harvest at a constant 12th rib fat thickness depth (1.27 cm). Steers were adapted (27 d) to a common finishing diet of 50% high moisture corn, 40% Sweet Bran (Cargill, Blaire, NE), 5% wheat straw or alfalfa, and 5% dry supplement.

Statistical Analyses. Data were analyzed using the GLIMMIX Procedure of SAS (SAS Inst. Inc., Cary, NC) as a completely randomized design with feedlot pen as the experimental unit. Summer grazing treatment was considered a fixed effect. Random effects were animal nested within summer grazing treatment and year.

Results and Discussion

Data collected in winter, summer, and feedlot phases are summarized in Table 1. By trial design, initial BW, ending BW and ADG during the winter phase were not different ($P > 0.71$) between SUPP and CON steers. At feedlot entry SUPP steers were 49 kg heavier ($P < 0.01$) than CON steers. Therefore, SUPP steers had 0.30 kg/d greater ($P < 0.01$) ADG than CON steers during summer grazing. Because feedlot harvest date was targeted to equal fat thickness between CON and SUPP steers, 12th rib fat thickness was not different ($P = 0.57$) between the two treatments. Also as a result of the harvest goal, final BW was not different ($P = 0.92$) between CON and SUPP steers; however, it required 24 fewer days in the feedlot ($P <0.01$) for SUPP steers to reach this point. Feedlot ADG tended to be greater ($P = 0.07$) for CON steers than SUPP steers, but G:F and DMI were not different ($P > 0.16$).

Longissimus muscle area was greater ($P = 0.01$) for SUPP steers. Protein analyses of diet samples collected from nearby summer pastures where the yearlings were maintained, indicated CON steers were deficient in ruminally degradable protein in August and September. Because MDGS was fed in excess of metabolizable protein requirements, urea recycling supplied sufficient ruminally degradable protein to SUPP steers. Unsupplemented steers had greater ($P < 0.01$) numerical marbling score, likely due to the longer time spent on feed in the feedlot phase. Calculated yield grade was also greater ($P < 0.01$) for CON steers than SUPP steers.

Using summer performance data, *in vitro* dry matter digestibility of the native Sandhills range from the two previous years, and NRC energy equations, it was determined that 0.62 kg grass was saved for every 1.0 kg MDGS fed (DM basis). Based on previous research (Musgrave et al., 2010), loss of MDGS fed on the ground was estimated at 15%, which was accounted for when estimating forage replacement. Also, based on visual appraisal, feeding MDGS on the ground did not have a negative impact on native range.

Supplementing MDGS at 0.6% BW increased summer cattle gains on pasture when fed on the ground. Cattle were heavier entering the feedlot when supplemented on pasture, thus requiring less days in the feedlot to reach a similar finish endpoint.

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Table 1. Effects of summer supplementation on grass on growth, subsequent feedlot performance, and carcass characteristics of yearling steers

Item	CON[1]	SUPP[2]	SEM	*P*-value
Winter phase				
Initial BW, kg	226	226	3	0.71
Ending BW, kg	317	317	2	0.92
ADG, kg	0.65	0.65	0.03	0.74
Summer phase[3]				
Ending BW, kg	415	464	5	<0.01
ADG, kg	0.62	0.92	0.03	<0.01
Feedlot phase[4]				
Final BW[5], kg	650	650	9	0.92
Days on feed, d	130	106	1	<0.01
ADG, kg	1.81	1.76	0.12	0.07
DMI, kg/d	13.71	13.56	0.21	0.16
G:F	0.132	0.130	0.007	0.22
Carcass Characteristics				
HCW, kg	410	409	6	0.92
LM area[6], cm^2	87.92	90.11	1.53	0.01
12^{th} rib fat thickness, cm	1.28	1.26	0.06	0.57
Marbling score[7]	613	557	12	<0.01
Calculated yield grade[8]	3.33	3.06	0.16	<0.01

[1]CON = cattle grazing native range during the summer with no supplementation.
[2]SUPP = cattle grazing native range during the summer with modified wet distillers grains with solubles supplementation at 0.6% BW.
[3]Summer phase = 23 d grazing brome grass + 136 d grazing native range; Initial BW = Ending BW from previous phase.
[4]Initial BW = Ending BW from previous phase.
[5]Final BW = HCW ÷ 0.63.
[6]Longissimus muscle area.
[7]$Small^{00}$ = 500.
[8]Calculated yield grade = (2.5 + (2.5 x 12th rib fat thickness) - (0.32 x longissimus muscle area) + (0.2 x 2.5 KPH) + (0.0038 x HCW)).

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INFLUENCE OF WEANING DATE AND PRE-PARTUM PLANE OF NUTRITION ON COW-CALF PRODUCTIVITY

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ABSTRACT: A 3-yr trial was conducted to elucidate effects of weaning date and pre-partum plane of nutrition on cow-calf productivity in a spring calving system. Treatments were imposed on crossbred beef cows (n= 156; BW = 477 ± 68 kg) in a factorial arrangement: 1) cows were weaned in early October or early December; 2) during the last trimester of pregnancy, cows received either 0.00, 0.45, or 0.91 kg DM/animal daily of a 32% CP supplement on dormant upland range. An additional winter treatment was cornstalk grazing with no supplement. October weaned cows grazing winter range had greater ($P < 0.01$) average BCS and BW compared to December weaned counterparts. Level of supplementation on upland range did not impact ($P > 0.11$) BCS or BW. Subsequent pregnancy rates (96.5% - 98.5%) were not influenced ($P > 0.54$) by weaning date or any winter treatments. Calves born to October weaned dams grazing winter range had greater ($P = 0.02$) birth BW than December weaned contemporaries; BW in October was also greater ($P = 0.02$). The first year of steer progeny showed no differences ($P > 0.14$) in feedlot entry BW, final BW, feedlot DMI, feedlot ADG, or carcass characteristics. Average pre-breeding BW of heifers born to October weaned dams on upland range was greater ($P < 0.01$) than December weaned contemporaries. However, there were no differences ($P > 0.31$) in percent cycling before breeding or pregnancy rates. Body condition and BW of cows grazing corn residue were greater ($P < 0.01$) than cows on winter range. Calves born to dams grazing corn residue had greater ($P < 0.01$) birth and weaning BW, compared to progeny from cows on upland range without supplement; however steer feedlot performance and heifer reproduction were similar ($P > 0.19$). Cows weaned in December had decreased BW and BCS with similar pregnancy rates as cows weaned in October. Weaning date and supplementation had minimal affect on steer and heifer progeny.

Key words: Beef Cattle, Maternal Nutrition, Supplementation

Introduction

Harvested forages are one of the greatest costs accrued during winter grazing for a spring calving system. Dormant forage, however, does not meet the high nutrient demands of the pregnant cow in the last trimester of pregnancy (NRC, 2000). Research has determined that only 0.14 kg DM·animal^{-1}·d^{-1} of supplemental ruminally degradable protein is necessary to maintain body condition score **(BCS)** of gestating cows grazing winter range (Hollingsworth-Jenkins et al., 1996). Supplementation of 0.45 kg DM·animal^{-1}·d^{-1} (42% CP) has been shown to increase BCS and percentage of live calves at weaning compared to cows not receiving supplemental protein, but has minimal impact on pregnancy rate (Stalker et al., 2006). Adjusting weaning date of a spring calving system may also help maintain cow BCS on winter range (Stalker et al., 2007). However, in that study, researchers were unable to detect a difference in pregnancy rates, possibly because cows were not weaned late enough in the year.

Undernutrition causes suboptimal conditions in the maternal uterine environment which translates into depressed progeny performance. Unfortunately, the exact mechanisms causing these deleterious responses are complex and not well understood (Funston et al., 2010a). Focusing on specific management practices may be the most practical approach for beef cattle research to evaluate these interactions from a systems context.

The objectives of the current study were to evaluate long-term effects of prepartum protein supplement and weaning date, and the interactions, on cow reproduction, heifer progeny growth and reproduction, and steer progeny growth, feedlot performance, and carcass characteristics.

Materials and Methods

Cow-calf Management. All procedures and facilities were utilized under the approval of the University of Nebraska-Lincoln Institutional of Animal Care and Use Committee.

Two years of a 3-yr trial used crossbred, March calving cows and calves at Gudmundsen Sandhills Laboratory (near Whitman, NE). Cows were stratified by age and treatments were assigned in a factorial arrangement: 1) cows were weaned in early October or early December; 2) approximately December 1 to February 28, cows received the equivalent of 0.0, 0.45, 0.91 kg DM ·animal^{-1}·d^{-1} of a protein supplement (Table 1) on dormant upland range, or corn residue grazing with no supplement. Supplement was delivered 3 times·wk^{-1} on a pasture (35.6 ha) basis. After the December weaning each year, dams were relocated to dormant upland range pastures, or transported to corn residue fields. Cows were managed in a common group for calving, fed *ad libitum* hay and protein supplement. At the time of breeding, cows were relocated to upland range pastures and managed as a common group until subsequent weaning date. Cows were estrus synchronized and artificially

inseminated with semen from the same two bulls each year. Estrus was synchronized with a two injection of prostaglandin $F_{2\alpha}$ (Prostamate, Agri Laboratories, St. Joseph, MO) two weeks apart, followed by heat detection and artificial insemination for 6 d. Cows were then placed with bulls (1:20 bull:cow) for 45 d. Pregnancy was determined via rectal palpation or ultrasonography by a veterinarian at the October weaning date each year.

Cows were removed from the study only if reproductive failure, calf death, or injury occurred. Replacement females were stratified by age and allotted randomly to treatment of removed cows. Influences of dam treatments on progeny performance were of interest; therefore, no further treatments were imposed on heifer or steer calves. October weaned calves were relocated to cool season meadows and supplemented to gain the equivalent of unweaned contemporaries until early December. Data reported were collected in 2009 (n = 144), and 2010 (n = 161).

Table 1. Composition and nutrient analysis of supplement

Item	DM, %
Ingredient	
Dried distillers grains with solubles	62.0
Wheat middlings	11.0
Cottonseed meal	9.0
Dried corn gluten feed	5.0
Molasses	5.0
Calcium carbonate	3.0
Trace minerals and vitamins[1]	3.0
Urea	2.0
Nutrient	
CP	31.6
Undegradable intake protein, % CP	47.6
TDN	89.4

[1]formulated inclusion of 80 mg·animal^{-1}·d^{-1} monensin.

Heifer Management. After December weaning, October and December weaned heifers were relocated to subirrigated meadows and fed 0.45 kg DM·animal^{-1}·d^{-1} of supplement (Table 1) as a common group. At the time of breeding, heifers were moved to upland range pastures to graze for the remainder of the year. Blood samples were collected twice, 10 d apart prior to placement with bulls. Heifers were considered cycling if blood serum progesterone concentrations were > 1 ng/mL. Estrus was synchronized with a single injection of prostaglandin $F_{2\alpha}$ (Prostamate, Agri Laboratories, St. Joseph, MO) administered 108 h after bulls were initially placed with heifers. An adequate bull-to-heifer ratio (1:20) was maintained throughout the breeding season (45 d). Pregnancy was determined via rectal palpation or ultrasonography by a veterinarian on approximately August 30 each year. Data reported were collected in 2010 (n = 68).

Steer Management. After December weaning, October and December weaned steers were fed *ad libitum* hay in a dry lot for approximately 14 d as a common group. Steers were then transported to the feedlot at West Central Research and Extension Center, in North Platte, NE; where they were limit fed 5 d at 2.0% BW, weighed 2 d consecutively, and adapted (21 d) to a common finishing diet of 48% dry rolled corn, 40% corn gluten feed, 7% prairie hay, and 5% supplement. Steers were assigned to one of eight pens based on weaning date and winter grazing treatment of dam. Synovex S (200 mg progesterone and 20 mg estradiol benzoate; Wyeth Inc., Fort Dodge, IA) was administered at feedlot entry, followed by Revalor S (120 mg trenbolone acetate and 8 mg estradiol; Intervet Inc., Millsboro, DE) approximately 100 d before harvest. Dry matter intake and G:F of treatment group within pen was adjusted by % BW DMI of feedlot pen. Harvest date was targeted for 1.27 cm 12th rib fat thickness. A commercial abattoir was used for slaughter, and carcass data were collected after a 24 h chill. Final BW was calculated from HCW and an assumed dressing percentage (63%). Data reported were collected in 2010 (n = 64).

Statistical Analyses. The experiment was completely randomized with treatments arranged in an unstructured 2x4 factorial design. Winter treatments were applied on a pasture basis, and both October and December weaned dams were maintained in a single pasture; pasture or cornstalk residue was not limiting at anytime. Therefore, each group of weaned cows within pasture served as the experimental unit. Data were analyzed with the GLIMMIX procedure of SAS (SAS Inst., Inc., Cary, NC). Model fixed effects included weaning date, winter grazing treatment, and weaning date x winter grazing treatment interaction where appropriate ($P < 0.05$). Year was considered a random effect for cow and calf variables.

Results and Discussion

The interaction between weaning date and winter grazing treatment was not significant ($P > 0.06$) for variables measured in the dams. Therefore, only main effects of weaning date and winter grazing treatment will be discussed in detail (Table 2). Body condition of cows was not different ($P > 0.15$) between weaning treatments at the time of October weaning. However, dams weaned in October maintained BCS until the time of December weaning; whereas nursing cows lost BCS during that time. Similar results were found in dams weaned an average of one month earlier (Stalker et al., 2007). Body weight had a similar pattern, where there were no differences ($P = 0.30$) in October, but October weaned dams were heavier ($P < 0.01$) in December. October weaned dams had lower BW and BCS ($P < 0.02$) before calving, but were not different ($P > 0.25$) from December weaned dams at the time of breeding. Thus, subsequent pregnancy rates for cows were similar ($P > 0.68$) among weaning treatments. Winter grazing treatment did not affect ($P > 0.13$) cow BCS or BW in October or December. Prior to calving and breeding, cows grazing corn residue had the greatest ($P < 0.01$) BCS and BW. However, subsequent pregnancy rates were not different ($P = 0.54$), regardless of winter grazing treatments applied during the third trimester of gestation.

An interaction ($P = 0.03$) for effects of weaning date and winter grazing treatment were found for calf birth BW and calf BW in December. Progeny born to October weaned dams receiving 0.91 kg supplement on upland range had the heaviest ($P < 0.01$) birth BW, except when compared to contemporaries born to dams on winter range receiving 0.45 kg supplement. Whereas progeny born to December weaned dams on winter range without supplementation had the lightest birth BW ($P < 0.01$), except when compared to progeny born to December weaned dams receiving 0.45 kg supplement on winter range. In October, progeny born to October weaned dams had greater ($P = 0.02$) BW than progeny born to December weaned dams. Cows grazing winter range without supplement had the lightest ($P < 0.01$) calves in October, when all other winter grazing treatments were similar. Despite previous differences, weaning date and winter grazing treatment during the third trimester of gestation of dams did not impact ($P > 0.76$) percent live calves weaned.

An interaction ($P < 0.01$) for effects of weaning date and winter grazing treatment was found for steer progeny G:F in the feedlot. However, only main effects of weaning date and winter grazing treatments are presented and will be discussed (Table 3). The first year of steer progeny were similar ($P > 0.14$) in feedlot entry BW, final BW, feedlot DMI, feedlot ADG, and carcass characteristics. Larson et al. (2009) reported steers born to protein supplemented dams on winter range to have greater final BW, marbling score, and percent grading USDA Choice or greater than unsupplemented cows. Numerically these data agree with Larson et al. (2009), however statistical significance was not found.

December and pre-breeding BW of heifers born to October weaned dams were greater ($P < 0.01$) than December weaned contemporaries. However, there were no differences ($P > 0.31$) in percent cycling before breeding or pregnancy rates. Level of supplement provided to dams had no effect ($P > 0.71$) on post-weaning heifer progeny growth or reproduction. Funston et al. (2010b) found a trend for heifers born to dams receiving 0.40 kg DM·animal^{-1}·d^{-1} supplemental protein to have greater pregnancy rates than unsupplemented dams, when three years of data were evaluated. A similar numerical trend was observed in these data, despite a lack of statistical significance. Perhaps these statistical contradictions are due to the lack of power in a single year of data.

Cows weaned in December had decreased BW and BCS with similar pregnancy rates compared to cows weaned in October. Winter grazing management of cows in the third trimester of pregnancy had minimal impact on pregnancy rates and percent live calves weaned. One year of progeny data indicate that weaning date, level of supplementation, and any corresponding interactions may have minimal effect on steer and heifer calves.

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Table 2. Effects of winter wean date and winter grazing treatment during the last third of gestation on cow body condition score (BCS), BW, pregnancy rate, calf BW, and percent calves weaned

	Wean date		Winter grazing treatment[1]					*P*-value[2]	
Item	October	December	WR0	WR1	WR2	CR	SE[3]	Wean	Winter
Cow BCS									
October	5.2	5.2	5.2	5.2	5.1	5.3	0.1	0.15	0.13
December	5.2	4.8	5.0	5.0	5.0	5.0	0.1	< 0.01	0.89
Pre-calve	5.1	5.0	4.6	4.9	5.2	5.4	0.1	0.01	< 0.01
Pre-breed	5.1	5.1	4.9	5.0	5.1	5.2	0.2	0.82	< 0.01
Cow BW									
October, kg	494	488	488	488	485	503	8	0.30	0.15
December, kg	478	447	462	461	457	472	11	< 0.01	0.33
Pre-calve, kg	511	488	471	482	505	540	8	< 0.01	< 0.01
Pre-breed, kg	459	450	439	448	454	477	17	0.26	< 0.01
Pregnancy rate, %	97.7	98.1	96.5	98.5	98.5	97.5	3.7	0.68	0.54
Calf BW									
Birth[4], kg	35	34	33	34	36	35	1	0.02	< 0.01
October, kg	213	206	195	210	216	218	3	0.02	< 0.01
December[4], kg	241	217	217	229	235	237	4	< 0.01	< 0.01
Weaning rate, %	97.7	97.9	98.0	96.7	97.8	98.4	3.3	0.82	0.76

[1]Winter grazing treatments: WR0 = dams grazed winter range without supplement; WR1 = dams grazed winter range and received 0.45 kg DM·animal^{-1}·d^{-1} 32% CP supplement; WR2 = dams grazed winter range and received 0.91 kg DM·animal^{-1}·d^{-1} 32% CP supplement; CR = dams grazed corn residue without supplement.
[2]Wean = weaning date main effect; Winter = winter grazing treatment main effect.
[3]Pooled standard error of treatment means.
[4]Wean date x Winter grazing treatment interaction ($P < 0.05$).

Table 3. Effects of wean date and winter grazing treatment during the last third of gestation of dams on progeny growth and performance

	Wean date		Winter grazing treatment[1]					*P*-Value[2]	
Item	October	December	WR0	WR1	WR2	CR	SE[3]	Wean	Winter
Steer progeny									
Initial BW, kg	237	232	227	235	234	243	10	0.59	0.71
DMI, kg/d	10.4	11.0	10.5	10.5	10.8	10.9	0.4	0.22	0.82
ADG, kg	1.61	1.66	1.55	1.62	1.69	1.68	0.06	0.41	0.26
G:F[4]	0.154	0.152	0.147	0.153	0.157	0.154	0.003	0.36	0.14
HCW, kg	357	360	342	357	365	369	12	0.82	0.40
LM area, cm^2	87.07	85.18	84.81	85.41	86.85	87.63	2.35	0.41	0.81
12th rib fat, cm	1.46	1.46	1.28	1.42	1.53	1.62	0.11	0.98	0.17
Marbling score[5]	502	516	500	533	496	506	18	0.44	0.47
Yield grade	2.56	2.53	2.33	2.55	2.61	2.68	0.19	0.86	0.58
Heifer progeny									
December BW, kg	228	209	201	218	228	227	6	< 0.01	0.03
Pre-breed BW, kg	297	270	267	286	290	293	6	< 0.01	0.02
Post-wean ADG[6], kg	0.43	0.38	0.39	0.42	0.39	0.41	0.03	0.08	0.80
Pregnancy BW, kg	358	330	331	350	337	358	9	< 0.01	0.13
Summer ADG[7], kg	0.44	0.42	0.47	0.45	0.33	0.47	0.05	0.74	0.20
Pregnancy BCS[10]	5.8	5.7	5.6	5.9	5.5	5.9	0.1	0.41	0.22
Cycling rate[8], %	45.6	33.2	40.6	27.6	47.1	42.8	13.0	0.31	0.71
Pregnancy rate, %	69.0	71.3	60.8	73.8	71.4	73.6	12.1	0.85	0.79

[1]Winter grazing treatments: WR0 = dams grazed winter range without supplement; WR1 = dams grazed winter range and received 0.45 kg DM·animal^{-1}·d^{-1} 32% CP supplement; WR2 = dams grazed winter range and received 0.91 kg DM· animal^{-1}·d^{-1} 32% CP supplement; CR = dams grazed corn residue without supplement.
[2]Wean = weaning date main effect; Winter = winter grazing treatment main effect.
[3]Pooled standard error of treatment means.
[4]Wean date x Winter grazing treatment interaction ($P < 0.05$).
[5]Small^{00} = 400.
[6]Calculated from December weaning date to subsequent average breeding date (161 d).
[7]Calculated from average breeding date to subsequent October weaning date (139 d).
[8]Considered cycling if blood serum progesterone concentrations were > 1 ng/mL.

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EFFECTS OF PRE-PARTUM AND POST-PARTUM BOLUS INJECTIONS OF TRACE MINERALS ON PERFORMANCE OF BEEF COWS AND CALVES GRAZING NATIVE RANGE

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ABSTRACT: Our objective was to evaluate the effects of pre- and post-partum bolus injections of a trace mineral solution on beef cow reproductive performance, BW change, and BCS change and on performance of suckling calves. Mature beef cows (n= 460; initial BW = 497 ± 89 kg, initial BCS = 5.4 ± 0.74) were stratified by BCS, age, parity, and predicted calving date and assigned randomly to 1 of 2 treatments: 1) s.c. trace mineral (TM) injection containing 15 mg/mL Cu, 5 mg/mL Se, 10 mg/mL Mn, and 60 mg/mL Zn or 2) s.c. injection of physiological saline (SA). Injections were administered to cows (1 mL/ 90 kg BW) 105 d before the first projected calving date and again 30 d before fixed-time AI. Calves received the same treatment as their dams and were injected (1 mL / 45 kg BW) at birth and again at 71 ± 21 d of age. Cows grazed native pastures for the duration of the study; trace mineral supplements and white salt were available to all cattle *ad libitum* before and during the study. Ovulation was synchronized using a 5-d CO-Synch + CIDR protocol and cows were inseminated 60 to 64 h after CIDR removal. Cows were exposed to fertile bulls for natural-service breeding 10 d after AI for 35 to 50 d. Conception to AI and final pregnancy rate were assessed 36 d after AI with ultrasound and 120 d after AI via rectal palpation. Change in BW and BCS from initiation of the study to calving and from AI to weaning did not differ ($P \geq 0.15$) between TM and SA cows. Conversely, TM cows had greater ($P = 0.04$) BCS increase than SA cows between calving and AI. Calf BW at birth, ADG, and age-adjusted weaning BW did not differ ($P \geq 0.36$) between treatments. Proportion of cows with estrus cycles 21 d before ovulation synchronization was similar ($P = 0.51$) between treatments. Conception to AI was greater ($P = 0.05$) for cows receiving TM (60.2%) than for cows receiving SA (51.2%); however, overall pregnancy did not differ ($P = 0.24$) between treatments and averaged 92%. Under the conditions of our study, pre- and post-partum TM injections improved conception to fixed-time AI by beef cows.

Key Words: beef cows, fixed-time AI, trace minerals

Introduction

Adequate dietary intakes of trace minerals are thought necessary in order to maximize cow reproduction, calf health, and calf performance. Diets grazed by beef cattle are generally deficient to marginal in Cu, Mn, Se, and Zn concentrations; therefore, these trace minerals are usually added to the diet in supplement form.

The most widely used means of trace-mineral supplementation for grazing cattle is the self-fed, salt-based, granular supplement (Greene, 2000). Even though cattle do not balance their mineral needs perfectly when consuming a self-fed mineral supplement, there is usually no other practical way of supplying mineral needs under grazing conditions (McDowell, 1985). The greatest limitation to using self-fed mineral supplements is variation in animal intake. More direct methods of mineral supplementation include adding minerals to drinking water or feed, oral drenching, ruminal boluses, and injection. Variation in mineral intake is reduced relative to self-fed supplementation and the additional labor requirement and expense are relatively small (Olson, 2007).

Delivery of supplemental trace minerals using an injectable solution may be a more reliable means of achieving adequate trace-mineral status than using self-fed, salt-based, granular mineral supplements alone. The objective of our study was to evaluate the effects of pre- and post-partum bolus injections of a trace mineral solution on beef cow reproductive performance, BW change, and BCS change and on performance of suckling calves.

Materials and Methods

All procedures involving the handling and care of animals used in our experiment were approved by the Kansas State University Institutional Animal Care and Use Committee (Protocol # 2650).

Angus × cows and heifers (n=460; initial BW 497 ± 89 kg) managed in 2 locations were used in our study (193 cows and 81 heifers at Manhattan, KS and 132 cows and 54 heifers at Hays, KS). At the end of December 2009, cows were stratified by BCS (1 = emaciated, 9 = obese), age, parity, and predicted calving date and assigned randomly to 1 of 2 treatments: 1) s.c. injection with a trace mineral solution (**TM;** Table 1) or 2) s.c. injection of physiological saline (**SA**). Injections were administered to cows (1 mL/90 kg BW) 105 d before the first projected calving date and again 30 d before fixed-time AI. Calves received the same treatment as their dams and were injected (1 mL/45 kg BW) at birth and again at 71 ± 21 d of age.

Within location, cows and heifers were managed as a single group from 12/17 through the end of the calving season. In Manhattan, cows were evenly distributed by treatment and parity into 5 native pastures on May 1; in Hays, cows were divided by parity (2-yr old or mature) and managed as parity groups during the summer grazing season. Cows grazed assigned pastures until October 5; trace mineral supplements and white salt were available to all cattle *ad libitum* before and during the study.

Table 1. Composition of injectable trace mineral solution administered pre- and post-partum to beef cows and at birth and 71 ± 21 d of age to beef calves.

Item	Multimin®90[a]
Zinc	60 mg/ml
Manganese	10 mg/ml
Selenium	5 mg/ml
Copper	15 mg/ml

[a]Multimin USA, Ft Collins, CO

Cow BW and BCS measurements were obtained 105 d before the first projected calving date, at calving, 30 d before fixed-time AI, and at weaning. Calf BW measurements were recorded at birth, on 06/16, and at weaning.

Blood samples were collected from each cow 17 and 8 d before fixed-time AI via jugular venipuncture and immediately placed on ice. Samples were allowed to clot for 24 h at 4 °C and centrifuged (1,500 × *g*) for 10 min. Serum was decanted into12 × 75 mm plastic tubes and immediately frozen (-20º C). Concentration of progesterone (P4) in serum was subsequently quantified by RIA (Skaggs et al., 1986). Intra- and inter-assay CV were 6.4 and 6.7%, respectively. When samples contained concentrations of P4 ≥ 1 ng/mL cows were considered to be cycling.

Ovulation was synchronized using a 5-d Co-synch + CIDR protocol and cows were inseminated 60 to 64 h after CIDR removal. Cows were exposed to fertile bulls for natural service breeding 10 d after fixed-time AI for the remainder of a 35-d breeding season in Hays and a 50-d breeding season in Manhattan. Conception to fixed-time AI was determined via ultrasound 36 d after AI and final pregnancy rate was determined via rectal palpation 120 d after insemination.

Cow and calf performance were analyzed as a randomized complete block. The original model included effects for treatment, origin, and pasture. Treatment within origin was considered the experimental unit. Pasture effects and associated interactions were not significant and were removed from the model. Treatment × location effects were not detected. When protected by a significant F-test ($P < 0.05$), Least Squares treatment means were separated using the method of Least Significant Difference.

Pregnancy rates were analyzed using PROC CATMOD (SAS Inst. Inc., Cary, NC). The original model used to assess differences in fixed-time AI pregnancy rates and overall pregnancy rates included effects for treatment, parity, origin, and pasture. Pasture effects and associated interactions were not significant and were removed from the model. Treatment × location effects were not detected. Least Squares means for pregnancy rates were reported. Treatment differences in performance and pregnancy data were discussed when $P < 0.05$; trends and tendencies were discussed when $P > 0.05$ and < 0.10.

Results and Discussion

Change in cow BW and BCS from initiation of the study to calving and from AI breeding to weaning did not differ ($P \geq 0.15$) between TM and SA cows (Table 2). Conversely, TM cows had greater ($P = 0.04$) BCS increase than SA cows between calving and AI. Proportion of cows with estrus cycles before ovulation synchronization was similar ($P = 0.51$) between treatments. Conception to fixed-time AI was greater ($P = 0.05$) for cows receiving TM (60.2%) than for cows receiving SA (51.2%); however, overall pregnancy did not differ ($P = 0.24$) between treatments and averaged 92%.

Calf BW at birth was not different ($P > 0.91$) between treatments (data not shown). Calf ADG from birth to 06/16, from 06/16 to weaning, and from birth to weaning were not different ($P \geq 0.36$) between TM and SA (Table 3). Similarly, adjusted 205-day BW was not different ($P = 0.48$) between treatments.

Daugherty et al. (2002) treated crossbred beef cows with a bolus injection of trace minerals to provide 0.18, 0.18, 0.09, and 0.05 mg/lb BW of Cu, Zn, Mn, and Se, respectively and 2.8 IU/lb BW of vitamin E. Cows receiving trace mineral + vitamin E had greater serum concentrations of copper than control cows. Despite increased copper status, the trace mineral + vitamin E treatment had no effect on conception rates of cows, survival rates of calves, or passive immune transfer.

Ahola et al. (2004) reported that supplementing grazing beef cows with copper, zinc, and managanese increased liver concentrations of all supplemented minerals in the first year but cows exhibited only increased liver copper concentrations in the second year. Similar to our results, supplemented cows had greater AI pregnancy rates than unsupplemented cows.

Sales et al. (2011) reported that crossbreed heifers subcutaneously injected with trace minerals had a 1.72 fold greater chance of being pregnant after timed embryo transfer compared to their control counterparts. In addition, trace mineral-treated heifers had greater implantation rates 23 and 48 d after embryo transfer. In contrast, Vanegas et al. (2004) reported that treatment of dairy cows with an injectable trace mineral solution before calving and before breeding resulted in decreased first-service conception compared to untreated dairy cows.

Stanton et al. (2000) reported greater pregnancy rates for cows receiving supra-nutritional amounts of organic trace minerals to compared cows receiving inorganic trace-mineral supplements alone. These researchers also reported increased ADG by calves receiving organic trace minerals compared to those consuming inorganic supplements alone; however, cow BW change, cow BCS change, calf BW at birth, and calf immune function were not affected by supra-nutritional supplementation with organic trace minerals.

Implications

Pre- and post-partum bolus injections of trace minerals had no effect on cow BW change, proportion cycling before ovulation synchronization, or overall pregnancy. Conversely, trace mineral-treated cows had more favorable BCS change between calving and breeding and greater conception to fixed-time AI. Treatment of calves with an injectable trace mineral solution had no effect on birth weight, ADG, or adjusted 205-day BW. Under the conditions of our study, pre- and post-partum TM injections improved conception to fixed-time AI by beef cows. Delivery of supplemental trace minerals to beef cows using an injectable solution may be more reliable means of achieving adequate trace-mineral status than using self-fed, salt-based, granular mineral supplements alone.

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Table 2. Performance of beef cows treated pre- and post-partum with bolus injections of either a trace mineral solution or physiological saline (1 mL/90 kg BW).

Item	Trace Mineral[a]	Saline	SE	*P*-value
Cow BW change, kg				
Pregnancy Check (12/17) to Calving	-8.7	-7.9	0.04	0.85
Calving to AI Breeding	39.1	38.4	1.51	0.81
AI Breeding to Weaning	45.9	43.5	2.72	0.59
Cow BCS Change (1 to 9 scale)				
Pregnancy Check (12/17) to Calving	-0.37	-0.34	0.013	0.57
Calving to Breeding	0.38	0.26	0.021	0.04
Breeding to Weaning	0.19	0.10	0.008	0.15
Cows Cycling 30 d before Timed AI, %	59.5	56.3	0.04	0.51
Timed-AI Pregnancy (%)	60.2	51.2	0.03	0.05
Overall Pregnancy (%)	93.0	89.9	0.02	0.24

[a] Multimin®90, Multimin USA, Ft Collins, CO

Table 3. Performance of beef calves treated at birth and at 71 ± 21 d of age with bolus injections of either a trace mineral solution or physiological saline (1 mL/45 kg BW).

Item	Trace Mineral[a]	Saline	SE	*P*-value
Early ADG (birth to 06/16), kg	0.94	0.94	0.004	0.89
Late ADG (06/16 to weaning), kg	0.89	0.91	0.010	0.36
Overall ADG (birth to weaning), kg	0.93	0.94	0.005	0.48
Adjusted 205-d BW, kg	229	232	0.9	0.48

[a] Multimin®90, Multimin USA, Ft Collins, CO

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RESPONSE OF BEEF COWS AND CALVES AFTER SUPPLEMENTATION WITH A NOVEL DISTILLER'S GRAIN DURING GESTATION

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ABSTRACT: Refinements in ethanol manufacturing create novel co-products. Dry milling to remove the germ and bran of corn grain before fermentation creates distiller's grains with reduced oil and higher CP [deoiled, dried distiller's grains with solubles (**dDGS**), 42% CP, 80.3% TDN]. Our objective was to determine the suitability of dDGS as an iso-nitrogenous alternative to soybean meal (SBM, 51.7% CP, 82.1% TDN) to provide supplemental CP to gestating beef cows consuming low-quality forage. In experiment 1, 2- and 3-yr-old beef cows (n = 84) in the last trimester of gestation were used in a randomized complete block design with a 2 supplement × 2 cow-age factorial treatment structure. The study was conducted in 12 drylot pens, creating three pen replicates of each supplement × cow age combination. All cows had ad libitum access to a mixture of grass straw and grass hay (5.25% CP, 50.5% TDN). Responses measured were cow BCS and cow and calf BW after the supplementation period, and subsequent fall pregnancy rate. In experiment 2, six ruminally cannulated 2-yr-old beef cows were used in a completely randomized crossover design to evaluate the influence of dDGS vs. SBM on in-situ rate of CP and NDF degradation from straw, hay, dDGS, and SBM. Protein supplement did not affect ($P > 0.1$) cow BW at breeding or weaning, cow BCS at breeding, cow BW gain and BCS change from breeding to weaning, cow pregnancy rate, calf BW at weaning, calf BW gain from breeding to weaning, and hay and straw NDF and CP rates of degradation. Calves of SBM-supplemented cows had greater ($P = 0.03$) BW at breeding than those of dDGS-supplemented cows (100.2±1.928 and 105.6±1.928 kg, respectively). Cow BCS at weaning was different ($P = 0.09$) between dDGS and SBM (4.808 ± 0.084 and 4.686 ± 0.084, respectively). There tended to be a difference ($P = 0.13$) in rate of CP degradation between dDGS and SBM (11.4 ± 4.79 and 23.8 ± 5.28 %/hr, respectively). In conclusion, dDGS can be used as a substitute for SBM as a protein supplement for low-quality forages.

Keywords: Beef cows, Protein supplement, Ethanol coproducts

Introduction

Refinements in ethanol manufacturing create novel co-products. Dry milling to remove the germ and bran of corn grain before ethanol fermentation creates distiller's grains with reduced oil and higher CP [deoiled, dried distiller's grains with solubles (**dDGS**)], such as Dakota Gold® HP™ (42% CP, 4.1% CF, Poet Nutrition, Sioux Falls, SD). This product is a highly digestible source of energy, natural protein, and phosphorus and is low in fat (Poet Nutrition, 2010). Bowman and Sanson (2000) stated that some by-products of the milling industry are low in nonstructural carbohydrates (**NSC**) but are highly digestible. They indicated that byproduct feeds that are lower in NSC have a less negative effect on fiber digestion than supplementation with the original grains (Bowman and Sanson, 2000). Surber et al. (1996) compared cows fed protein supplements with varying levels of NSC and cows that were not supplemented; cows receiving the supplement with the highest levels of NSC and non-supplemented cows experienced the most weight loss. In the same study, cows receiving the lowest NSC supplement, soyhulls and SBM, increased total diet intake.

The objective of this study was to determine the suitability of dDGS as an iso-nitrogenous alternative to soybean meal (SBM) to provide supplemental CP to gestating beef cows consuming low-quality forage. The hypothesis was that supplemental protein from dDGS would influence ruminal forage digestion and cow-calf performance similar to that of SBM, a traditional and well-understood source of supplemental protein. Cow and calf performance subsequent to the supplementation period is reported herein. Weights and BCS during the supplementation period were previously reported by Hojer et al. (2011).

Materials and Methods

All animal procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee.

Experiment 1

Design and Treatments. This experiment was a randomized complete block with a 2 supplement × 2 cow-age factorial treatment structure using 2- and 3-yr-old spring-calving beef cows (n=42 in each age group) in the last trimester of gestation. The study was conducted in 12 drylot pens, creating three pen replicates of each supplement × cow age combination. Cows received 0.43 $kg \bullet d^{-1}$ of supplemental protein as either SBM or dDGS (Table 1). To provide this, SBM-supplemented cows received 0.93 kg (as-fed) of $SBM \bullet cow^{-1} \bullet d^{-1}$and dDGS-supplemented cows received 1.13 kg (as-fed) of $dDGS \bullet cow^{-1} \bullet d^{-1}$. This was prorated and delivered 3 times per week (Monday, Wednesday, and Friday). Bunks were

cleaned at 0700 each day to measure forage refusal for the previous day (data not reported herein) and then supplement was delivered in the clean bunk. All supplements were consumed within 15 minutes. After that, a 50:50 (as-fed) mixture of grass straw and hay (Table 1) was delivered at 105% of the previous day's consumption. Grass straw and hay were chopped to a mean length of 8 cm in a tub grinder and equal amounts of both forages were mixed in a feeder wagon to deliver to the cows. The supplement-feeding period was initiated on January 13, 2010. It was concluded on February 25, 2010 for the 2-yr-old cows in anticipation of their initiation of calving on March 8 and concluded on March 11 for the 3-yr-old cows in anticipation of their initiation of calving on April 1.

Collections. Cow and calf BW were recorded on June 21, 2010 (initiation of breeding) and September 27, 2010 (weaning). Cow BCS was scored at the same time by two trained technicians and reported as the mean of those scores. Pregnancy status was detected on September 27, 2010 via rectal palpation.

Statistics. This experiment was analyzed as a randomized complete block design using the Mixed procedure of SAS (PROC MIXED, SAS Institute, Cary, NC). Fixed effects included supplement treatments, cow age, and their interaction. Denominator degrees of freedom were calculated using the Kenward-Roger option. Block was considered a random effect. Response variables included cow and calf BW at breeding and weaning, cow and calf BW gain from breeding to weaning, cow BCS at breeding and weaning, cow BCS change from breeding to weaning, and cow pregnancy rate. Least squares means were calculated and were separated by F-tests for main effects and protected LSD for significant treatment × age interactions.

Experiment 2

Design and Treatments. This experiment was a completely randomized crossover design using six ruminally cannulated 2-yr-old beef cows to evaluate the influence of dDGS vs. SBM on in-situ rate of CP and NDF degradation of grass straw, grass hay, dDGS, and SBM (Table 1). The cows had ad libitum access to the same 50:50 grass straw and grass hay mixture (Table 1) and it was delivered the same as experiment one. During the first period of the crossover design, 3 cows received SBM as the supplement and 3 cows received dDGS as the supplement. The same supplements that were used in experiment 1 (Table 1) were used at the same levels in experiment 2. Cows were fed their supplement for 10 days to adapt to the rations.

Samples of grass straw, grass hay, SBM, and dDGS were used for in situ procedures. The grass straw and grass hay samples were ground in a Wiley mill to pass a 2-mm screen. Duplicate 5-g samples of each were weighed into 10 × 20 cm nylon bags (Ankom, Fairport, NY) with a 50 ± 15μm pore size, and heat-sealed using an impulse sealer for each time point and each animal. All samples for each time point were placed in 36 × 42 cm polyester mesh bags to ensure similar location within the rumen and to assist in retrieval. Grass straw and hay samples were incubated in all 6 cows, while SBM and dDGS were only incubated in the 3 cows receiving the respective supplement. Bags were placed in reverse order to allow 0 (never placed in the rumen), 2, 6, 12, 18, 24, 48, 72, and 96 h of fermentation. Bags for 96 h were placed into the rumen of each animal at the end of the 10-d dietary adaption period. All bags were removed simultaneously at 0 h and placed immediately into an ice-water bath to stop microbial activity. Bags were then immediately rinsed in a top-loading washing machine for 10 cold-water rinse cycles. Each rinse cycle consisted of a one-minute agitation and a two-minute spin per rinse. The 0-h time bags were treated identically to the rest of the samples except for not being placed in the rumen. After rinsing, the bags were dried at 105º C for 48 h.

After period 1 was completed, the cows were immediately crossed over to the opposite supplement treatment to start period 2 after another 10-day adaptation period. During the adaptation period, one cow died because of non-trial-related problems.

Laboratory Analysis. All samples were dried for 48 h at 105ºC to determine DM (AOAC, 1996). In situ residues of straw, hay, dDGS, and SBM were analyzed for NDF content using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). In situ residues were also analyzed for N content with the combustion method (AOAC, 1996) using a N analyzer (SBM and dDGS: Leco, St. Joseph, MI; straw and hay: Elementar, Mt. Laurel, NJ) and N was multiplied by 6.25 to determine CP.

Statistics. The nonlinear regression procedure of SAS (PROC NLIN, SAS Institute, Cary, NC) was used with the in situ residue data to calculate ruminal rate of disappearance of NDF and CP. Rates of disappearance of NDF and CP for each feedstuff were analyzed using the MIXED procedure of SAS in a completely randomized crossover design. The model included supplement treatment, period, and cow. Cow was designated a random effect and period was designated a repeated measure.

Results and Discussion

Experiment 1

Protein supplement did not affect cow BW at breeding or weaning (Table 2). Cow BW gain from breeding to weaning was also not affected by supplement. Three-yr-olds were heavier than 2-yr-olds at both breeding and weaning (Table 3). Cow BW gain from breeding to weaning did not differ by age (Table 3). There was no ($P >$ 0.1) age × treatment interaction effects on cow BW at breeding and weaning, or cow gain from breeding to weaning.

Cow BCS at breeding was the same across treatments. Additionally, there was no supplement × age interaction (P

> 0.1) for cow BCS at breeding. However, supplement and cow age interacted for cow BCS at weaning and the change in BCS from breeding to weaning (Table 4). All cows except 3-yr-olds that had received dDGS lost BCS from breeding to weaning while the dDGS-supplemented 3-yr-olds maintained BCS. This resulted in them having higher BCS than the other 3 groups at weaning.

Supplement interacted with cow age for calf BW at breeding (Table 4). Calves of 2-yr-old cows were similar among supplement treatments and heavier than calves of 3-yr-old cows. Additionally, calves of 3-yr-old cows supplemented with SBM were heavier than calves of 3-yr-old cows supplemented with dDGS. There was no interaction ($P > 0.1$) for calf BW at weaning or calf gain from breeding to weaning. Additionally, calf BW at weaning and gain from breeding to weaning did not differ among supplement treatments (Table2) or cow age groups (Table 3).

Pregnancy rate was similar ($P = 0.55$) between SBM and dDGS supplemented cows (94.8 and 92.1 ± 3.2%, respectively). This was interesting because cows fed dDGS had a higher ($P = 0.03$) percentage of cows cycling at the beginning of the breeding season compared to cows supplemented with SBM (32.6 and 14.1 ± 7.4%, respectively; Hojer et al., 2011). Pregnancy rate tended ($P = 0.10$) to be different between 2- and 3-yr-old cows (89.27 and 97.62 ± 3.18%, respectively).

Experiment 2

Protein supplement treatment did not affect grass hay or grass straw NDF and CP rates of degradation. We also found no difference in rate of DM degradation of grass hay ($P = 0.79$) or straw ($P = 0.48$) between SBM or dDGS as a protein supplements (Hojer et al., 2011). There tended to be a difference in rate of CP degradation between SBM and dDGS (Table 5). This was expected because SBM is high in DIP (DIP = 80%, NRC, 2000) and dDGS is high in UIP (DIP = 27%, NRC, 2000). There was also a significant difference in the rate of NDF degradation in SBM and dDGS (Table 5).

Implications

Past research has clearly indicated that cattle grazing dormant range or consuming other low-quality forages have increased performance when they receive adequate protein supplementation compared to cattle that do not receive supplement or receive an NSC-based energy supplement. These results suggest that dDGS has adequate amounts of available CP to not only help with cow performance compared to SBM, but may also improve reproductive soundness. With input costs constantly changing, beef producers need to continually determine what the most economical protein source available to them is. If dDGS is the most economical choice, it can be used as a protein supplement for cows on dormant range or receiving low quality forage.

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Table 1. Nutrient analyses of grass straw, grass hay, SBM[1], and dDGS[2] used in this study.

	Feedstuff			
Item	Straw	Hay	SBM	dDGS
DM, %	93.5	87.4	89.6	92.5
		----- % of DM -----		
CP, %	3.2	6.4	52.5	40.7
NDF, %	76.4	65.2	9.7	26.9
TDN, %	47.1	55.2	83.0	78.5
EE, %	2.2	1.6	1.5	4.5
Ca	0.30	0.62	0.25	0.02
P	0.09	0.14	0.71	0.40
S	0.06	na[3]	0.38	0.65

[1]SBM = soybean meal
[2]dDGS = deoiled dried distiller's grains with solubles
[3] na= not available

Table 2. Effect of SBM[1] or dDGS[2] as protein supplements during late gestation on cow BW and BCS and calf BW from breeding to weaning.

	Supplement			
Item	SBM	dDGS	SEM	*P*-Value
Cow BW @ breeding, kg	468.8	474.3	3.53	0.260
Cow BW @ weaning, kg	469.5	478.0	5.82	0.325
Cow BW gain, kg	1.0	4.2	3.26	0.506
Cow BCS @ breeding	5.01	5.05	0.031	0.425
Cow BCS @ weaning	4.68	4.81	0.084	0.099
Change in cow BCS	-0.34	-0.24	0.067	0.127
Calf BW @ breeding, kg	105.6	100.3	1.93	0.034
Calf BW @ weaning, kg	199.1	200.1	5.81	0.888
Calf BW gain, kg	94.7	97.4	3.82	0.615

[1]SBM = soybean meal
[2]dDGS = deoiled dried distiller's grains with solubles

Table 3. Effects of cow age on cow BW and BCS and calf BW from breeding to weaning.

	Cow Age			
Item	2-yr-old	3-yr-old	SEM	*P*-value
Cow BW @ breeding, kg	438.3	504.7	3.53	< 0.001
Cow BW @ weaning, kg	437.8	509.8	5.82	< 0.001
Cow BW gain, kg	-0.3	5.4	3.26	0.254
Cow BCS @ breeding	5.01	5.04	0.031	0.516
Cow BCS @ weaning	4.58	4.91	0.084	0.003
Change in cow BCS	-0.44	-0.14	0.067	0.001
Calf BW @ breeding, kg	111.2	94.6	1.93	<0.001
Calf BW @ weaning, kg	203.0	196.2	5.81	0.368
Calf BW gain, kg	91.6	100.4	3.82	0.139

Table 4. Effect of supplement treatment × cow age interaction on cow BW at weaning, change in BCS, and calf BW at breeding.

Trt	SBM		dDGS			
Cow age	2-yr-olds	3-yr-olds	2-yr-olds	3-yr-olds	SEM	*P*-value
Cow BCS @ weaning	4.61^{a}	4.74^{a}	4.54^{a}	5.07^{b}	0.096	0.025
Change in cow BCS	-0.39^{a}	-0.28^{ab}	-0.48^{a}	0.00^{c}	0.077	0.014
Calf BW @ breeding	111.7^{c}	99.4^{b}	110.7^{c}	89.8^{a}	2.37	0.071

[1] SBM = soybean meal
[2] dDGS = deoiled dried distiller's grains with solubles
[a,b,c] Within a row, means without common superscripts differ at $P < 0.10$

Table 5. Effects of SBM[1] or dDGS[2] as protein supplements on the rate of NDF and CP degradation of grass straw, grass hay, SBM and dDGS.[1]

	NDF Rate			CP Rate		
Item	SBM	dDGS	*P*-Value	SBM	dDGS	*P*-Value
Grass straw, %	2.92 ± 0.529	3.34 ± 0.471	0.638	6.9 ± 1.52	4.2 ± 1.64	0.361
Grass hay, %	3.74 ± 0.422	3.44 ± 0.379	0.685	6.2 ± 1.25	6.2 ± 1.13	0.971
Concentrate[3], %	24.6 ± 1.57	6.4 ± 1.45	<0.001	23.8 ± 5.28	11.5 ± 4.79	0.128

[1] SBM = soybean meal
[2] dDGS = deoiled dried distiller's grains with solubles
[3] Concentrate is the respective SBM or dDGS for each supplement treatment.

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CAMELINA MEAL SUPPLEMENTATION TO BEEF CATTLE: II. EFFECTS ON DMI, FORAGE IN SITU DIGESTIBILITY, AND PLASMA CHOLECYSTOKININ CONCENTRATIONS

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ABSTRACT: Nine Angus × Hereford steers, ranked by initial BW (average 250 ± 9 kg), were assigned (d 0) to receive: 1) supplement based (as-fed basis) on 84% corn, 14% soybean meal, and 2% mineral mix (CO); and 2) supplement based (as-fed basis) on 70% corn, 28% camelina meal, and 2% mineral mix (CAM). Treatments were offered daily (0700 h) at a rate of 2.20 and 2.04 kg of DM/steer for CO and CAM, respectively. Treatment intakes were formulated to be iso-caloric and iso-nitrogenous. Mixed alfalfa-grass hay was offered ad libitum from d 0 to 15, and hay DMI was recorded daily. Intake recorded from d 8 to 15 was used to determine treatment effects on hay and total DMI. From d 16 to d 19, steers were restricted to receive 90% of their voluntary hay DMI (BW basis). Immediately before treatment feeding on d 16, polyester bags (pore size 50-60 μm) containing 4 g of hay (DM basis) were suspended within the rumen of each steer, and incubated in triplicate for 0, 1, 3, 5, 8, 12, 24, 36, 48, 72 and 96 h. After removal, triplicates were washed, dried for 96 h at 50°C, weighed, and combined for NDF analysis. From d 20 to 21, steers received hay ad libitum and blood samples were collected on d 21 at 0, 1, 2, 3, 4, 5, 6, 9, and 12 h relative to treatment feeding for determination of plasma cholecystokinin (CCK) concentrations. Hay DMI tended (P = 0.15) to be reduced whereas total DMI was reduced (P = 0.01) in CAM vs. CO steers (2.71 vs. 2.91% of BW for hay and 3.46 vs. 3.76% of BW for total DMI, respectively). No treatment effects were detected (P > 0.35) for rate of ruminal degradation of DM (7.91 vs. 8.58%/h for CAM and CO) and NDF (7.49 vs. 7.39%/h for CAM and CO). Similarly, no treatment effects were detected (P > 0.55) for effective ruminal degradability of DM (64.3 vs. 64.9% for CAM and CO) and NDF (70.1 vs. 71.0% for CAM and CO). No treatment effects were detected (P = 0.35) for plasma CKK concentrations (22.7 vs. 26.8 pg/mL for CAM and CO). In conclusion, camelina meal supplementation did not impact forage digestibility and plasma CCK, but decreased total DMI in forage-fed beef steers.

Key Words: Beef steers, camelina meal, digestibility

Introduction

Supplemental polyunsaturated fatty acids (PUFA) sources, such as camelina meal, are nutritional alternatives to alleviate the bovine acute-phase response stimulated by transport and feedlot entry (Araujo et al., 2010). However, feeder calves supplemented with PUFA may experience decreased DMI, ADG (Araujo et al., 2010; Cooke et al., 2010a) and feed efficiency (Araujo et al., 2010) compared to cohorts offered control diets. Several factors may be associated with these outcomes, including altered dietary palatability (Grummer et al., 1990), impaired dietary digestibility and consequent feed intake (Schauff and Clark, 1989), reduced gut motility and increased cholecystokinin (CCK) synthesis and release (Drackley et al., 1992; Allen et al., 2000). Therefore, the objective of the present study was to compare DMI, in situ forage digestibility, and plasma CCK concentrations in beef steers offered diets with or without camelina meal.

Materials and Methods

This experiment was conducted at the Eastern Oregon Agricultural Research Center – Burns, in accordance with an approved Oregon State University Animal Care and Use Protocol. Nine Angus x Hereford steers were ranked by initial BW (average = 250 ± 9 kg), and assigned on d 0 to 1 of 2 treatments: 1) supplement based (as-fed basis) on 84 % corn, 14 % soybean meal, and 2 % mineral mix (CO); and 2) supplement based (as-fed basis) on 70 % corn, 28 % camelina meal, and 2 % mineral mix (CAM). Treatment intakes were formulated to be iso-caloric and iso-nitrogenous, and offered individually and daily (0700 h) at a rate of 2.20 and 2.04 kg of DM/steer for CO and CAM, respectively (Table 1).

Mixed alfalfa-grass hay was offered in amounts to ensure ad libitum access from d 0 to 15 of the study, and hay DMI was recorded daily by measuring refusals. Samples of the offered hay and treatment ingredients were collected weekly to determine nutrient composition (Dairy One Forage Laboratory, Ithaca, NY) and DM, whereas samples of refusals were collected daily to determine DM content only. Hay samples were dried for 96 h at 50°C in forced-air ovens. Intake data collected from d 8 to 15 were used to determine treatment effects on hay and total DMI. From d 16 to 19, steers were restricted to receive 90% of their voluntary hay DMI (BW basis).

Immediately before treatment feeding on d 16, polyester bags (pore size 50-60 μm) containing 4 g (DM basis) of mixed alfalfa-grass hay were suspended within the rumen of each steer, and incubated in triplicates for 0, 1, 3, 5, 8, 12, 24, 36, 48, 72 and 96 h. Prior to incubation, all bags were soaked in warm water (37 °C) for 15 min. The 0-h bags were not incubated in the rumen, but were subjected to the same rising procedure used for ruminally incubated bags. After removal, bags were washed repeatedly until the rinse water was colorless, dried for 96 h at 50°C in forced-air ovens, and weighed. Triplicates were combined and analyzed for NDF (Robertson and Van Soest, 1981) using

procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom CO., Fairport, NY).

From d 20 to 21, steers received hay ad libitum and blood samples were collected on d 21 at 0, 1, 2, 3, 4, 5, 6, 9, and 12 h relative to treatment feeding for determination of plasma CCK concentrations (KT-10170; Kamiya Biomedical Company, Seattle, WA)

Table 1. Composition and nutrient profile of supplements offered during the study.

Item	CO	CAM
Ingredient, DM basis		
Corn, kg	1.82	1.39
Soybean Meal, kg	0.32	--
Camelina, kg	--	0.59
Mineral Salt, kg	0.06	0.06
Nutrient profile, DM basis		
DM, %	87.0	88
TDN, %	94	95
CP, %	14.7	15.6
NDF, %	9.6	14.7
Ether extract, %	4.5	9.8
Ca, %	0.1	0.3
P, %	0.4	0.5

Voluntary forage, total DMI, and plasma CCK concentrations were analyzed using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statements contained the effects of treatment, day, and the interaction. Data were analyzed using steer(treatment) as the random variable. Kinetic parameters of hay DM and NDF disappearance were estimated using nonlinear regression procedures of SAS, as described by Vendramini et al. (2008). Treatment effects on ruminal degradation rate and effective ruminal degradability (Coblentz and Hoffman, 2009) were analyzed using the PROC MIXED procedure of SAS and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement contained the effect of treatment. Data were analyzed using steer(treatment) as random variable. Results are reported as least square means and were separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.15. Results are reported according to treatment effects if no interactions were significant.

Results and Discussion

Steers receiving CAM had decreased (P = 0.01) total DMI compared to CO cohorts, whereas a trend (P = 0.15) was observed for forage DMI (Figure 1). Our results support previous efforts reporting that PUFA supplementation reduced DMI in cattle (Araujo et al., 2010; Cooke et al., 2010a; Cooke et al., 2010b). The reasons for this outcome may include impaired dietary digestibility (Schauff and Clark, 1989), as well as reduced gut motility and increased CCK synthesis and release (Drackley et al., 1992; Allen et al., 2000).

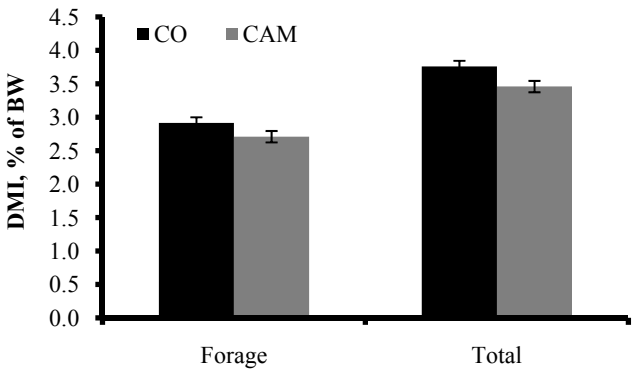

Figure 1. Forage and total DMI, as percentage of BW, of steers offered supplements containing (CAM) or not (CO) camelina meal. A trend (P = 0.15) was detected for forage DMI, whereas a treatment effect was detected (P = 0.01) for total DMI.

However, no treatment effects were detected (P > 0.35) on ruminal degradation rate (K_d) of hay DM and NDF (Table 2). Similarly, no treatment effects were detected (P > 0.55) for effective ruminal degradability of hay DM and NDF (Table 2). Accordingly, previous research from our group reported that ruminal digestibility parameters are not affected by PUFA supplementation, even when forage and total DMI are impaired (Cooke et al., 2010b); Plasma CCK concentrations were not different (P = 0.35) between CAM and CO steers (Figure 2). These results were not expected, since CCK is related to satiety (Baile et al., 1986) and fat supplementation has been shown to decrease DMI while increasing plasma CCK concentrations (Choi et al., 2000).

Table 2. In situ disappearance kinetics of DM and NDF of mixed alfalfa-grass hay incubated in steers offered supplements containing (CAM) or not (CO) camelina meal.

Treatment	K_d, /h	Effective degradability, [1] %
DM analysis		
CO	0.085	64.95
CAM	0.079	64.30
SEM	0.005	0.008
P-value	0.35	0.57
NDF analysis		
CO	0.074	70.98
CAM	0.075	70.15
SEM	0.006	0.01
P-value	0.91	0.55

[1] Calculated as $A + B \times [(K_d + K_p)/K_d]$, where K_p was the ruminal passage rate, which was arbitrarily set at 0.025/h (Coblentz and Hoffman, 2009).

Implications

These results indicate that camelina meal supplementation did not impact forage digestibility and

plasma CCK concentrations, but decreased total DMI in beef steers. Therefore, additional research is needed to understand the mechanisms by which PUFA supplementation reduces feed intake in cattle.

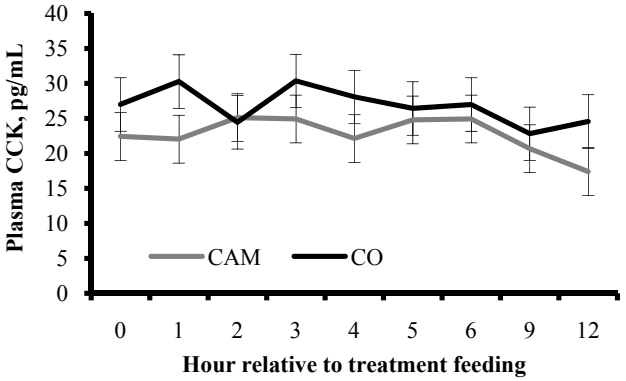

Figure 2. Plasma cholecystokinin (CCK) concentrations of steers offered supplements containing (CAM) or not (CO) camelina meal (P = 0.35).

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SUPPLEMENTAL RUMEN-PROTECTED FISH OIL INCREASES CONCENTRATIONS OF LONG-CHAIN *N*-3 FATTY ACIDS IN TISSUES OF GRASS-FED BEEF

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ABSTRACT: Our hypothesis was dietary rumen-protected *n* -3 PUFA will increase concentrations of these fatty acids in tissues of grass-fed beef cattle. Forty half-blood LowLine Angus steers (290.5 ± 6.6 kg initial BW) were allotted to either a control (**CON**; no supplemental fat), saturated fatty acid Ca salt (**SAT**), or fish oil fatty acid Ca salt (**N3**) treatment in a completely randomized designed experiment. Beet pulp supplements that contained 7.6% molasses, 4.0% $CaCO_3$ for CON, 4.4% mineral mix, and 1.8% Poloxalene were individually fed and formulated to provide 0.25% of BW as supplement and 2.0% of DM as fat for SAT and N3. Irrigated pasture consisted of 25% bromegrass, 25% wheatgrass, and 50% alfalfa (CP = 20.9%; 36.7 kg DM • $head^{-1}$ • d^{-1}), and was rotated weekly from June 1 through October 15, 2008 when steers were fed forage harvested from the same pastures until December 8. Blood was sampled at 45 and 93 d. Steers were shipped 137 km for slaughter at a commercial plant; liver was sampled upon evisceration. Twelve days post mortem, 100 g each of longissimus (12^{th} rib), supraspinatus, and semitendinosus muscles were obtained. Fatty acids extracted from serum, liver, and muscles were analyzed by GLC with C13:0 as internal standard. In liver compared with CON, SAT caused increased ($P < 0.01$) C16:0, C18:0, C18:1 *n*-9, C18:2 *n*-6, C20:3 *n*-3, and C20:4 *n*-6, and decreased ($P = 0.01$) C18:3 *n*-3. Compared with CON, N3 supplementation resulted in greater ($P < 0.01$) C18:1 *t*-11, C20:5 *n*-3 (eicospentaenoic acid, **EPA**) and C22:6 *n*-3 (docoshexaenoic acid, **DHA**), and less ($P < 0.01$) C18:1 *n*-9, C18:2 *n*-6, C20:3 *n*-3, C20:4 *n*-6 in liver. In muscle, concentrations of C18:2 *n*-6 and C20:4 *n*-6 increased ($P < 0.01$) for SAT compared with CON. For each muscle, N3 resulted in greater ($P < 0.01$) EPA and DHA compared with CON. Serum concentrations of fatty acids reflected differences in supplemental intake of C16:0 and C18:1 *n*-9 of SAT, as well as EPA and DHA of N3. Overall, supplementation of N3 resulted in 86.4% and 85.6% increases in concentration of EPA + DHA in liver and muscle, respectively.

Key Words: Grass-Fed Beef; Supplemental Omega-3 Fatty Acids; Serum;

Introduction

Grass-fed beef offers consumers a lean product with a fatty acid profile that generally reflects that of the forage consumed. Grass-fed beef producers market this product, in part, on the contents of *n*-3 fatty acids and CLA (predominately the *cis*-9, *trans*-11 isomer). Although concentrations of *n*-3 fatty acids and CLA are higher in meat of grass-fed beef than in feedlot finished beef (Rule et al., 2002; Nuernberg et al., 2005) their concentrations reported in the scientific literature do not support the contention that grass-fed beef is a particularly rich source of either class of fatty acid. Moreover, while α-linolenic acid (C18:3 *n*-3) is increased in grass-fed compared to feedlot beef, its conversion to eicosapentaenoic acid (**EPA**, C20:5 *n*-3) and docosahexaenoic acid (**DHA**, C22:6 *n*-3) is inefficient because of the low rates of elongation and desaturation of C18:3 *n*-3. Attempts to increase the tissue concentration of EPA and DHA by fish oil supplementation have shown mixed results, largely because of ruminal biohydrogenation (Scollan et al., 2001). By using a by-pass source of EPA and DHA the concentration of these fatty acid could be increased in beef; however, the extent to which supplemental *n*-3 fatty acids can be increased in tissues of grass-fed beef has not been reported. Calcium salts of unsaturated fatty acids may provide limited ruminal by-pass characteristics, which could be an effective alternative to dietary supplementation with untreated oils (Castaneda-Gutierrez et al., 2006). Our hypothesis was dietary rumen-protected *n* -3 PUFA will increase concentrations of these fatty acids in tissues of grass-fed beef cattle. The objective of this study was to supplement Ca salts of fish oil fatty acids to half-blood LowLine Angus steers grazing irrigated pasture to determine extent of

deposition of EPA and DHA in liver, muscle, and serum lipids.

Materials and Methods

The University of Wyoming Institutional Animal Care and Use Committee approved all procedures for the following study. Forty half-blood LowLine Angus steers (initial BW 290.5 ± 6.62 kg) were allotted to either a control (**CON**; no supplemental fat), a palm oil-based saturated fatty acid Ca salt (**SAT**), or a fish oil-based fatty acid Ca salt (**N3**). Calcium salts of fatty acids were provided by Virtus Nutrition (Corcoran, CA). Supplements were beet pulp-based and contained 7.6% molasses, 18.2% fatty acid Ca salts, 4.0% $CaCO_3$ for CON (to balance for Ca in SAT and N3), 4.4% mineral mix, and 1.8% Poloxalene. Supplements were fed at 0.25% of BW and formulated to limit supplemental fat to 2.0% for SAT and N3 to avoid negative associative effects of dietary fat in cattle consuming high-forage diets (Hess et al., 2008). Supplements were offered to individual steers on alternate days such that supplement intake could be recorded. Steers were raised on irrigated pasture of 25% bromegrass, 25% wheatgrass, and 50% alfalfa (CP = 20.9%; available forage was 36.7 kg DM • $head^{-1}$ • d^{-1}). Steers were weighed monthly and blood sampled at 45 and 93 d. During early October of the grazing study, four steers died leaving 12 steers in each treatment group. Pasture was rotated weekly over a period beginning June 1, 2008 and ending October 15, 2008; steers were fed forage harvested from the same pastures until December 8 when steers were shipped 137 km for harvest at a commercial slaughter plant. Liver was sampled upon evisceration 5 cm lateral of the gall bladder at the left lobe. Carcasses were graded 48 h postmortem followed by fabrication and vacuum packaging of primals. Final fabrication occurred 12 d post mortem, when 100 g each of *M. longissimus dorsi* (**LD**; 12^{th} rib), *supraspinatus*, and *semitendinosus* muscles were obtained. For fatty acid analysis of serum, total lipid extracts from 2.5 mL were reacted with 4.0 mL of 0.545 *N* HCl in methanol for fatty acid methyl ester (FAME) preparation. Muscle and liver FAME were prepared by direct transesterification with 0.2 *N* KOH in methanol (Murrieta et al., 2003). Forage and supplement FAME were prepared using methanolic-HCl as described by Weston et al. (2008). All FAMEs were analyzed by GLC; all tissue samples contained 1.0 mg of C13:0; whereas, feed samples contained 1.0 mg of C21:0 as internal standard.

Fatty acid data were analyzed using the GLM procedure of SAS for a completely randomized designed experiment. Initial BW was used as covariate in the analysis. Least squared means were compared using Tukey critical difference.

Results and Discussion

By design, steers fed CON did not have substantial intake of fatty acids. Steers fed N3 consumed modest amounts of C16:0 and C18:0, and were the only treatment offered EPA or DHA. Steers fed SAT consumed substantial amounts of C16:0, C18:0, and modest amounts of C18:2 *n*-6.

Table 1. Intake of fatty acids by steers fed Control (CON), Ca-salts of fish oil (N3), or Ca-salts of palm oil (SAT) supplements

Intake, g/d	Treatment		
Fatty acid	CON	N3	SAT
C16:0	2.4 ± 0.1	12.2 ± 1.0	40.3 ± 1.7
C18:0	0.2 ± 0.0	1.6 ± 0.1	3.1 ± 0.1
C18:1*n*-9	1.4 ± 0.0	6.2 ± 0.5	28.0 ± 1.2
C18:2*n*-6	2.0 ± 0.1	2.0 ± 0.2	8.4 ± 0.4
C18:3*n*-3	0.2 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
C20:5*n*-3	0.0	4.26 ± 0.3	0.0
C22:6*n*-3	0.0	1.2±0.1	0.0

Intake of grazed forage was not determined. Fatty acid concentrations (mg/g DM) of the forage were: C16:0, 2.9 (12.3% of total fatty acids); C18:0, 0.4 (1.6%); C18:1 *n*-9, 0.3 (1.3%); C18:2 *n*-6, 3.45 (14.6%); and C18:3 *n*-3, 13.8 (58.2%). Fatty acid concentrations and percentages were of similar magnitude as those previously reported (Weston, et al., 2008), and concentration of total fatty acids was 23.7 mg/g DM, or approximately 2.4% of DM as fatty acids. Initial BW, final BW, and ADG were similar ($P \geq 0.31$) for grazing steers consuming the CON, N3, and SAT supplements (Table 2).

Table 2**.** Body weights and ADG of steers fed Control (CON), Ca-salts of fish oil (N3), or Ca-salts of palm oil (SAT) supplements

	Treatment				
Item	CON	N3	SAT	SEM[a]	*P*
Initial BW[b]	292.1	290.7	288.6	6.6	0.93
Final BW[b]	433.8	420.0	416.3	9.4	0.39
ADG[b,c]	0.74	0.69	0.70	0.03	0.31

[a]n=14, 13, and 13 for initial BW for CON, NE, and SAT, respectively and n=12 for each treatment for final BW.
[b]Kg.
[c]ADG is for entire trial, 187 d.

Fatty acid concentrations (mg/100 g) in liver of steers fed the experimental diets are shown in Table 3. Steers fed the SAT supplement had the greatest ($P <$ 0.01) liver concentrations of C16:0, C18:0, C18:1 *n*-9, and C18:2 *n*-6, which was consistent with the intake of these fatty acids from the supplement. The greater

concentration of C18:2 *n*-6 in liver of SAT-fed steers also was consistent with greater ($P < 0.01$) concentration of C20:4 *n*-6, the elongation and desaturation product of the former. Concentrations of C18:1 *n*-9 and C18:2 *n*-6 were greater ($P < 0.01$) in liver of CON steers than N3 steers. Interestingly, average daily intake of these fatty acids was the same or greater for N3 steers suggesting that in liver of N3 steers more of them were metabolized through oxidation or transported out of the liver. Liver concentrations of C18:3 *n*-3 were similar ($P > 0.05$) for CON and N3, but was less ($P < 0.01$) for SAT steers. Without forage intake determination, we cannot conclude that intake of C18:3 *n*-3 was less for SAT steers because metabolism of this fatty acid could also have been greater. Concentration of C20:4 *n*-6 was greater ($P < 0.01$) for CON than N3, which was consistent with greater ($P < 0.01$) C18:2 *n*-6 for CON. Concentrations of EPA and DHA were greatest ($P < 0.01$) for N3, and similar ($P > 0.05$) for CON and SAT, which was expected because N3 steers had consumed these fatty acids from a source providing protection from ruminal biohydrogenation. Although neither CON nor SAT was fed EPA or DHA, liver concentrations seemed substantial indicating that elongation and desaturation of C18:3 *n*-3 occurred.

Although three muscles were obtained and analyzed, concentrations of fatty acids were affected similarly by dietary treatment; therefore, only fatty acids of the LD are reported (Table 3). Concentrations of C18:2 *n*-6 and C20:4 *n*-6 were greater ($P < 0.01$) for steers consuming SAT compared with either CON or N3. This response was similar to that observed with liver, suggesting elongation and desaturation of C18:2 *n*-6 also occurred in LD in response to greater intake of this fatty acid. Concentrations of EPA and DHA were greater ($P < 0.01$) for N3 compared with CON and SAT indicating that muscle fatty acids were affected by supplementation with Ca-salts of fish oil fatty acids. Concentrations of EPA and DHA in muscle of CON and SAT steers also indicate that conversion of C18:3 n-3 to these two fatty acids occurred. For LD, supplementation with N3 resulted in 70.7% and 225% increases in EPA and DHA, respectively, compared with CON, while no change in concentration of C18:3 *n*-3 ($P = 0.51$) occurred.

Changes in serum fatty acid concentrations (Table 3) were consistent with dietary intakes of the fatty acids. Elongation and desaturation of C18:2 n-6 to C20:4 n-6 continued to be apparent as the latter fatty acid was also greater in serum of the SAT steers. Compared with LD, the increase in EPA in N3 over CON was greater in serum (70.7% vs. 161%) but less for DHA (225% vs 117%). The lipid fraction in which fatty acids occurred in serum was not determined; thus, it is difficult to suggest why these shifts were observed. Nonetheless, feeding Ca-salt of fish oil increased EPA and DHA concentrations in serum, which resulted in greater amounts deposited in total lipids of liver and LD.

When Ca-salts of fish oil were fed to lactating dairy cows, no increase in milk fat concentration of EPA or DHA occurred; however, negative effects of oil feeding on DM intake were inhibited by feeding the Ca-salt over unprotected fish oil (Castaneda-Gutierrez, et al., 2007). Thus, results with dairy cows may be conflicting with those of the current study. Diet, breed, and stage of maturity likely are involved in this difference. In the present study, intake of the N3 supplement was quite variable (196.8 to 456.3 g/d, SD = 82.4) and caused the concentration of EPA and DHA to vary in LD (17.8 to 35.9 mg/100 g, SD = 6.3). This observation suggests that LD concentration could be greater if palatability of the supplement is improved and intake is greater and more uniform.

Implications

Feeding growing steers a supplement containing calcium salts of fish oil fatty acids resulted in increased muscle deposition of eicosapentaenoic and docosahexaenoic acids, two important omega-3 fatty acids. Such a supplementation strategy could be used to increase important fatty acids in beef of grass-fed cattle.

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Table 3. Effect of dietary Ca salts of fish oil or palm oil on fatty acid concentrations of liver, LD (mg/100g), and serum (mg/100mL) of half blood LowLine Angus steers

	Treatment[a]				
Item	CON	N3	SAT	SEM[c]	*P*-value
Liver fatty acid:[b]					
C16:0	263.1[f]	262.3[f]	314.2[e]	9.27	< 0.01
C18:0	943.2[f]	932.9[f]	1,058.3[e]	24.9	< 0.01
C18:1 *n*-9	201.6[f]	166.6[g]	260.0[e]	10.1	< 0.01
C18:2 *n*-6	161.1[f]	143.3[g]	180.5[e]	5.7	< 0.01
CLA[d]	4.2	4.0	4.3	0.2	0.55
C18:3 *n*-3	38.6[e]	36.3[e]	30.6[f]	1.8	0.01
C20:4 *n*-6	214.6[f]	154.4[g]	254.5[e]	7.5	< 0.01
C20:5 *n*-3	80.8[f]	123.3[e]	69.5[f]	5.0	< 0.01
C22:6 *n*-3	61.1[f]	141.2[e]	51.7[f]	5.8	< 0.01
LD fatty acid:[b]					
C16:0	552.8	647.5	728.4	57.2	0.12
C18:0	349.8	439.9	489.0	44.1	0.10
C18:1 *n*-9	632.1	705.6	836.8	61.6	0.08
C18:2 *n*-6	92.8[f]	105.2[f]	123.8[e]	3.8	< 0.01
CLA[d]	8.3	11.5	10.6	1.4	0.24
C18:3 *n*-3	35.2	39.1	37.4	2.3	0.51
C20:4 *n*-6	33.0[f]	33.5[f]	41.5[e]	1.1	< 0.01
C20:5 *n*-3	12.3[f]	21.0[e]	12.4[f]	0.9	< 0.01
C22:6 *n*-3	2.0[f]	6.5[e]	2.2[f]	0.3	< 0.01
Serum fatty acid:[b]					
C16:0	16.9[f]	18.4[f]	21.1[e]	0.6	< 0.01
C18:0	21.1[f]	22.0[f]	24.6[e]	0.9	0.02
C18:1 *n*-9	9.8[f]	8.2[f]	13.0[e]	0.5	< 0.01
C18:2 *n*-6	42.1[f]	46.9[f]	57.1[e]	1.9	< 0.01
CLA[d]	0.27[e]	0.21[f]	0.23[ef]	0.02	0.02
C18:3 *n*-3	18.4	19.2	18.8	0.86	0.77
C20:4 *n*-6	3.3[f]	3.5[f]	4.1[e]	0.2	< 0.01
C20:5 *n*-3	1.8[f]	4.7[e]	1.9[f]	0.2	< 0.01
C22:6 *n*-3	0.6[f]	1.3[e]	0.6[f]	0.0	< 0.01

[a]CON = control, no supplemental fat; N3 = supplement contained Ca salt of fish oil; SAT = supplement contained Ca salt of palm oil.

[b]Number of carbon atoms:number of carbon-carbon double bonds.

[c]Standard error of the least squared mean; n=12.

[d] C18:2$^{\Delta 9\text{-cis, } 11\text{-trans}}$.

[e-g]Within a row means without a common superscript differ (P < 0.05).

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COMPARISON OF TOTAL LIPID FATTY ACID PROFILES OF BOVINE SERUM AND PLASMA

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ABSTRACT: Our hypothesis was concentrations of fatty acids measured in lipid extracts of bovine liquid serum and plasma, as well as in freeze-dried serum and plasma will not be different. Blood was sampled from five beef cows maintained on a forage diet to obtain plasma and serum. Ten milliliters of each fraction from each cow were freeze-dried and mixed. For liquid fractions, lipids were extracted from duplicate 2.5-mL samples with 9.3 mL of 1:2 (vol/vol) chloroform and methanol. Extracts were then subjected to transesterification with 4.0 mL of 0.545 *N* HCl in methanol for 1 h at 80˚ C to prepare fatty acid methyl esters (**FAME**). Duplicate 200-mg samples of each freeze-dried fraction were either reacted directly with 4.0 mL of 0.545 *N* HCl in methanol for 1 h at 80˚ C for FAME preparation or extracted with 3.8 mL of 1:2:0.8 (vol/vol/vol) chloroform, methanol, and water overnight to obtain total lipids for subsequent FAME preparation. Fatty acids were analyzed by GLC with 1.0 mg of C13:0 as internal standard. Total lipid extracts of the liquid serum and plasma, as well as direct transesterification of freeze-dried serum and plasma yielded consistent FAME results. However, lipid extraction of the freeze-dried serum and plasma did not provide repeatable or reliable results; these data were not analyzed. Concentrations (mg/100 mL) of C16:0, C16:1 *n*-7, C18:0, C18:1 *n*-9, C18:2 *n*-6, C18:3 *n*-3, C20:4 *n*-6, C20:5 *n*-3, and C22:6 *n*-3 were not different (P = 0.47 to 0.98) when liquid fractions were compared. Direct transesterification of freeze-dried fractions also did not differ (P = 0.19 to 0.78) in concentrations (mg/g) of any of the aforementioned fatty acids. Concentrations expressed as mg/100 mg of total FAME were similar (P = 0.66 to 0.99) when liquid and freeze-dried fractions were compared. We conclude that FAME prepared from total lipid extracts of liquid serum and plasma, as well as by direct transesterification of freeze-dried serum and plasma will yield similar fatty acid profiles; however, total lipid extraction of freeze-dried serum and plasma will not provide adequate results when chloroform, methanol, and water are used as extraction solvent.

Key Words: Fatty Acid Profile; Serum; Plasma

Introduction

Circulating fatty acids would be associated with red blood cells, non-esterifed fatty acids carried by albumin, and lipoproteins such as chylmicrons, very-low-density-lipoproteins, and low-density-lipoproteins. Blood sampling for analyses of hormones and metabolites commonly incorporates harvest of either plasma or serum. Plasma is harvested upon addition of an anticoagulant followed by centrifugation; whereas, serum is harvested from blood by centrifugation after the blood has clotted. Except for removal of fibrinogen and clotting factors, the composition of serum and plasma is essentially the same (Ganong, 1975). The analysis of fatty acids of serum and plasma total lipids, therefore, should be the same. However, the potential for differences in fatty acid composition because of the differences between serum and plasma could be cause for concern with studies in which determination of low-abundance fatty acids or between treatments in which transport of dietary lipids are necessary. Moreover, comparison of data published where similar treatments were applied to animals, but in which neither report has used the same blood fraction could be a concern. Our study was conducted to directly compare fatty acid composition of total lipids of serum and plasma. Our hypothesis was that concentrations of fatty acids measured in lipid extracts of bovine serum and plasma will not be different. The objective of the study was to quantify fatty acid composition of liquid serum and plasma, as well as of freeze-dried preparations of each fraction from blood sampled from beef cows.

Materials and Methods

Blood samples were obtained from five mature beef cows that had been maintained on pasture and harvested forage. The cows were being used for a primary study in which multiple blood samples were taken during the course of a day; the primary study was approved by the University of Wyoming Institutional Animal Care and Use Committee. For the present study, blood samples were either collected in tubes that contained 68 units of Na-heparin/4 mL as anticoagulant for plasma harvest or no anticoagulant for serum harvest. Blood samples were obtained between 1700 and 1800 on the same day and immediately placed on ice overnight. For plasma and serum, blood was centrifuged at 2,500 • *g* for 20 min and fractions transferred to 16 x 125 mm borosilicate tubes equipped with screw caps. Plasma and serum samples were stored at -20˚ C. Ten milliliter aliquots of both serum and plasma were lyophilized for 5 d; dried samples were pulverized and homogenized by using mortar and pestle upon removal from the freeze-drier. For liquid fractions, lipids were extracted for 4 hr from duplicate 2.5-mL samples with 9.3 mL of 1:2 (vol/vol) chloroform and methanol. Chloroform was separated by mixing the extraction solvent with 2.5 mL of chloroform and 2.5 mL of a solution containing 0.05 *N* HCl and 1.0 M KCl. Chloroform phases were transferred to clean tubes and dried under a stream of N_2. Lipid extracts were then subjected to transesterification with 4.0 mL of 0.545 *N* HCl in methanol for 1 hr at 80˚ C to prepare fatty acid methyl esters (**FAME**). Duplicate 200-mg samples of each freeze-dried fraction were reacted directly with 4.0 mL of 0.545 *N* HCl in methanol for 1 hr at 80˚ C for direct transesterification for FAME preparation. After 1 hr tubes were allowed to cool and 1.0 mL of saturated NaCl and 1.0 mL of hexane were added and the tube vortex-mixed. The hexane phase was separated by centrifugation at 2,500 • *g* for 2 min and then transferred to GLC autosampler vials. Additional duplicate 200-mg samples of each freeze-dried fraction were extracted with 3.8 mL of 1:2:0.8 (vol/vol/vol) chloroform, methanol, and water overnight to obtain total lipids for subsequent FAME preparation. The total lipid extractions of the dried serum and plasma were treated as above to recover chloroform phases, dry the lipid extracts, and prepare FAMEs. Fatty acid composition was determined by using GLC with 1.0 mg of C13:0 as internal standard.

Data were analyzed as a completely randomized designed experiment using the GLM procedure of SAS. Concentration of fatty acids within the liquid serum and plasma fractions were compared separately from the concentrations of fatty acids within the dried fractions. Fatty acid proportions within the sample's total fatty acid pool allowed for comparison of serum and plasma within liquid and dry preparations. Least squared means were compared using Tukey critical difference.

Results and Discussion

Total lipid extracts of the liquid serum and plasma, as well as direct transesterification of freeze-dried serum and plasma yielded consistent FAME results. However, lipid extraction of the freeze-dried serum and plasma did not provide repeatable or reliable results; these data were not analyzed. The reason for the poor results with total lipid extraction of the freeze-dried material was not pursued. A more vigorous extraction or use of alternative extraction solvents would be necessary as a substitute for using the solvent extraction described in this study.

Concentrations (mg/100 mL) of fatty acids obtained from liquid plasma and serum were similar (P = 0.47 to 0.98) for each fatty acid compared (Table 1), indicating that both blood fractions had the same fatty acid profile, and each was equally extracted with the solvents employed. Because the liquid and dried preparations were extracted with the same solvent system, it was apparent that total lipids were more accessible to extraction solvents when in the liquid form.

Table 1. Comparison of fatty acid concentrations (mg/100 mL) of liquid plasma and serum of beef cows

	Blood fraction			
Fatty acid[a]	Plasma	Serum	SEM[b]	*P*
C16:0	14.6	15.0	0.4	0.47
C16:1	2.4	2.5	0.2	0.82
C18:0	18.5	18.9	0.9	0.78
C18:1 *n*-9	14.4	15.1	1.0	0.66
C18:2 *n*-6	25.8	26.4	5.9	0.95
C18:3 *n*-3	14.1	14.3	1.9	0.92
C20:4 *n*-6	3.3	3.3	0.3	0.90
C20:5 *n*-3	2.2	2.2	0.3	0.97
C22:5 *n*-3	2.3	2.3	0.2	0.98

[a]Number of carbon atoms:number of carbon-carbon double bonds.
[b]n=5.

Concentrations (mg/g) of fatty acids obtained from freeze-dried plasma and serum samples that were subjected to direct transesterification with methanolic-HCl were similar (P = 0.19 to 0.78) for the fatty acids compared (Table 2). Direct transesterification using hot

methanolic-HCl was effective in extracting and immediately esterifying non-esterified fatty acids to methanol, as well as transesterifications of fatty-acyl glycerols to the methanol during FAME preparation. Dried materials from both blood fractions were equally suitable for this type of FAME preparation method.

Table 2. Comparison of fatty acid concentrations (mg/g) of freeze-dried plasma and serum of beef cows

	Blood fraction			
Fatty acid[a]	Plasma	Serum	SEM[b]	*P*
C16:0	2.0	2.1	0.1	0.19
C16:1	0.3	0.3	0.0	0.59
C18:0	2.5	2.7	0.2	0.53
C18:1 *n*-9	1.9	2.1	0.2	0.42
C18:2 *n*-6	3.4	3.7	0.8	0.78
C18:3 *n*-3	1.9	2.1	0.2	0.53
C20:4 *n*-6	0.5	0.5	0.0	0.51
C20:5 *n*-3	0.3	0.3	0.0	0.69
C22:5 *n*-3	0.3	0.3	0.0	0.60

[a]Number of carbon atoms:number of carbon-carbon double bonds.
[b]n=5.

Concentrations of fatty acids within the total fatty acid pool (mg/ 100 mg of total fatty acids) represent a common means of expressing fatty acid data. By using this approach, fatty acid profiles from different extraction and (or) FAME preparation methods can be compared because the data indicate the proportion of each fatty acid relative to the total fatty acids observed. Proportions of the fatty acids compared were similar (P = 0.66 to 0.99) for serum and plasma liquid fractions subjected to total lipid extraction and dried fractions subjected to direct transesterification (Table 3). These data clearly show that within the fatty acid pool of each blood fraction and FAME preparation approach the resulting fatty acid composition was essentially the same.

Table 3. Comparison of fatty acid concentration (mg/100 mg of total fatty acids) of dry and wet serum and plasma of beef cows

	Blood fraction					
Fatty acid[a]	PD[b]	PW[c]	SD[d]	SW[e]	SEM[f]	*P*
C16:0	9.6	9.8	9.9	10.0	0.3	0.79
C16:1	1.5	1.6	1.5	1.7	0.1	0.66
C18:0	12.3	12.5	12.3	12.6	0.3	0.84
C18:1 *n*-9	9.4	9.7	9.8	10.0	0.4	0.80
C18:2 *n*-6	16.1	17.0	16.5	17.1	2.8	0.99
C18:3 *n*-3	9.4	9.6	9.7	9.7	1.0	0.99
C20:4 *n*-6	2.2	2.2	2.2	2.2	0.1	0.99
C20:5 *n*-3	1.5	1.5	1.5	1.5	0.2	0.99
C22:5 *n*-3	1.5	1.6	1.5	1.6	0.2	0.98

[a]Number of carbon atoms:number of carbon-carbon double bonds.
[b-e]Fractions: PD = plasma, dry; PW = plasma, wet; SD = serum, dry; SW = serum, wet.
[f]n=5.

Fatty acid composition of serum and plasma have been reported many times as an evaluation of dietary manipulation of lipid composition of milk and tissue of food-producing animals, as well as a determination of the effectiveness of diet as a means of influencing fatty acid dynamics in humans and animal models of biomedical relevance. The direct comparison of fatty acid composition of serum and plasma in the present study indicates that comparable results can be obtained from either fraction, and that data published using one fraction can be used to elaborate on results obtained from the other fraction. We conclude that FAME prepared from total lipid extracts of liquid serum and plasma, as well as by direct transesterification of freeze-dried serum and plasma will yield similar fatty acid profiles; however, total lipid extraction of freeze-dried serum and plasma will not provide adequate results when chloroform, methanol, and water are used as extraction solvent.

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IN VITRO EVALUATION MIMICS INFLUENCES OF WINTER COLD WATER INGESTION ON RUMINAL FUNCTION[1]

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ABSTRACT: Ingestion of cold feed and water may suddenly reduce ruminal temperature, which could result in decreased microbial activity and diet digestibility. The objective of this study was to investigate the association between critical rumen in vitro incubation temperature and activity of ruminal microorganisms to produce gases and degrade NDF. Lyophilized ruminal extrusa (0.25 g) collected from ruminally cannulated cows grazing winter range in November 2010 (81.13% NDF, OMB) was weighed into thirty 100-ml glass syringes. Warmed McDougall's buffer mixed 4:1 with rumen liquor donated by winter-grazing cows was added and syringes were placed in a 39°C water bath. After 12 h, syringes were randomly allocated to one of 3 water baths of different incubation temperatures, 39°C, 37°C or 35°C. These temperatures were selected based on previous findings that showed ruminal contents can drop intermittently below 35°C. Syringes were incubated for another 36 h. Rate and total gas production at 48 hours was reduced ($P < 0.05$) by lower incubation temperatures (rate: 0.63, 0.49, and 0.34 ± 0.01 mL/h; production: 29.3, 23.4, and 17.7 ± 0.65 mL/g of OM for 39°, 37° and 35°, respectively). Extent of NDF disappearance was reduced ($P < 0.001$) by incubation temperature (26.8, 22.9 and 21.26 $\pm$ 0.49% for 39°, 37° and 35°, respectively). Maximum gas production and NDF disappearance were found at 39°C. These data show the impact small differences in ruminal temperature due to cold water ingestion may have on rumen function.

Key words; range cows, water, temperature, in vitro

Introduction

When domestic species are grazing in a temperate climate it is generally thought the temperature of the rumen varies only 2°C (Dehority 2003, Hungate 1966). When grazing in the winter, ruminants have been shown to consume cold water and food which increase costs of thermoregulation and may affect fermentation (Crater and Barboza 2007). Cold water has been reported to drop the rumen temperature by 5-10°C in domestic cattle and sheep (Cunningham 1964 and Dehority 2003). Brod et. al (1982) reported reduced digestibility in sheep when consuming 0°C water compared to 30°C water (56.4 and 58.3 ± 0.3% DM for 0 and 30°C water, respectively). Conversely, Butcher (1966) reported no differences in feed consumed or average daily gain of sheep consuming ambient temperature water compared to sheep consuming only snow. Bewely et al. (2008) reported water intake by dairy cattle has an immediate dramatic effect on temperature measured in the reticulum. Therefore, microbial activity may be impaired by these drops in temperature and affect NDF digestion. Our objectives were to determine the effect of in vitro incubation temperature on rate and total gas production and extent of disappearance of winter range ruminal extrusa NDF.

Materials and Methods

This study was conducted at the USDA-ARS Fort Keogh Livestock and Range Research Laboratory near Miles City, MT. Ruminal extrusa was collected from 4 cows grazing native pasture in November, 2010. Ruminal contents were removed from the cows and stored in Rubbermaid tubs. The ruminal walls were sponged dry to remove any moisture, as described by Lesperance et al. (1960). The cows were allowed to graze in the experimental pastures for 30 minutes. After grazing the extrusa was removed from the rumen and thoroughly mixed. An aliquot was saved for analysis. The original ruminal contents were placed back in the cows. The aliquot was frozen at -20°C, lyophilized, and passed through a 1mm screen. Samples were analyzed for DM, OM (AOAC 1990) and NDF (Goering and Van Soest,

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1970). Extrusa sample collected was 93.1% DM, 90.2% OM, and 81.1% NDF. This sample of winter range extrusa and a laboratory forage control were weighed (0.25 g) into 100-ml glass syringes according to the procedure described by Blummel and Becker (1997). Rumen liquor was collected from winter-grazing ruminally cannulated cows, blended, saturated with CO_2 and strained through cheesecloth. McDougall's buffer was mixed 4:1 with rumen liquor and 20 ml added to each syringe. Syringes were placed upright in a 39°C water bath. At 12 hour incubation, gas production was recorded and syringes were randomly placed in water baths of 39°C, 37°C or 35°C. In a complementary study, Petersen et al. (2011) reported rumen temperatures can drop below 32°C during the winter after drinking water. Therefore, water bath temperatures were set to mimic changes in ruminal temperature during the winter. Gas production was recorded at 15, 18, 21, 24, 30, 36, 42, and 48 hours. At 48 hours, syringes were emptied into Berzelius beakers and rinsed with 50 ml heated neutral detergent solution to stop fermentation. Samples were refluxed in NDF solution for one hour and filtered, dried, weighed, ashed and re-weighed. In vitro disappearance of NDF was calculated. Due to restrictions in water bath capacity a second experiment was conducted using the same methods as experiment 1; but with water baths temperatures of 39°C, 33°C and 31°C.

Statistical Analysis. Rate of gas production was calculated using a linear model in Graphpad Prism. Data for both experiments were analyzed by MIXED procedures of SAS (SAS Inst. Inc., Cary, NC) using individual syringe as the experimental unit. The Kenward-Roger degrees of freedom method was used to adjust standard errors and calculate denominator degrees of freedom. Gas production data were analyzed as a split-plot design with repeated measures. Water temperature was the whole plot and hour and hour × treatment were the subplot. Significance was determined at $P \leq 0.05$.

Results and Discussion

Experiment 1. Gas production rate and total gas production at 39°C, 37°C, and 35°C incubation temperature were measured. A water temperature × hour interaction ($P < 0.05$;Figure 1) occurred for gas production. Therefore, gas production means were compared at each hour. At 12-hr all syringes produced the same ($P = 0.99$) quantity of gas with the range of gas production from 5.728 to 5.734 mL ± 0.410. This demonstrates that syringes had started a normal fermentation prior to movement to lower temperature water baths. However, the rate of gas production from 12 to 48-hr was reduced ($P < 0.001$; 0.63, 0.49, and 0.34 ± 0.01 mL/h for 39°, 37° and 35°, respectively) in the 37°C and 35°C water baths, compared to the 39°C water bath. Therefore, total gas production per g of substrate was also decreased ($P < 0.001$) in 37°C and 35°C water compared to 39°C. The differences in gas production and rate at different incubation temperatures were significant at 15-h and every incubation interval thereafter ($P < 0.01$). In vitro NDF disappearance was reduced ($P < 0.001$) by 15% or more with lower incubation temperatures compared to 39°C incubation temperature (26.8, 22.9 and 21.26 $\pm$ 0.61% for 39°, 37° and 35°, respectively). The NDF disappearance for in vitro cultures incubated at 37°C and 35°C were similar ($P = 0.07$). The rate of gas production and the total gas production may possibly be under reported due to a malfunction of the 39°C water bath between incubation h 21 and 24. By the end of the 48-hr period the laboratory control samples used had produced an expected amount of gas.

Experiment 2. Gas production rate and total gas production between incubation temperatures of 39°C, 33°C and 31°C were compared. A water temperature × hour interaction ($P < 0.01$;Figure 2) occurred for gas production therefore, gas production means were compared at each hour. The rate of gas production and total gas at 12 hr were similar for all syringe cultures as in Exp 1. The rate of gas production and total gas production was reduced ($P < 0.01$) at 48-hr for syringes in the 33°C and 31°C water baths, compared to the 39°C water bath (0.94, 0.45, and 0.20 ± .003 mL/h; 41.2, 23.4, and 4.4 ± .24 mL/g of OM for 39°, 33° and 31°C, respectively). NDF disappearance was reduced at least 33% with a decrease in incubation temperature (42.3, 28.2 and 21.33 $\pm$ 0.30% for 39°, 33° and 31°C, respectively). The extent of NDF disappearance was greater ($P < 0.01$) for culture incubated at 39° compared to 33° and 31°C. The extent of NDF disappearance also differed between 33° and 31°C ($P < 0.01$).

These data show the impact small differences in ruminal temperature due to cold water ingestion may have on rumen function.

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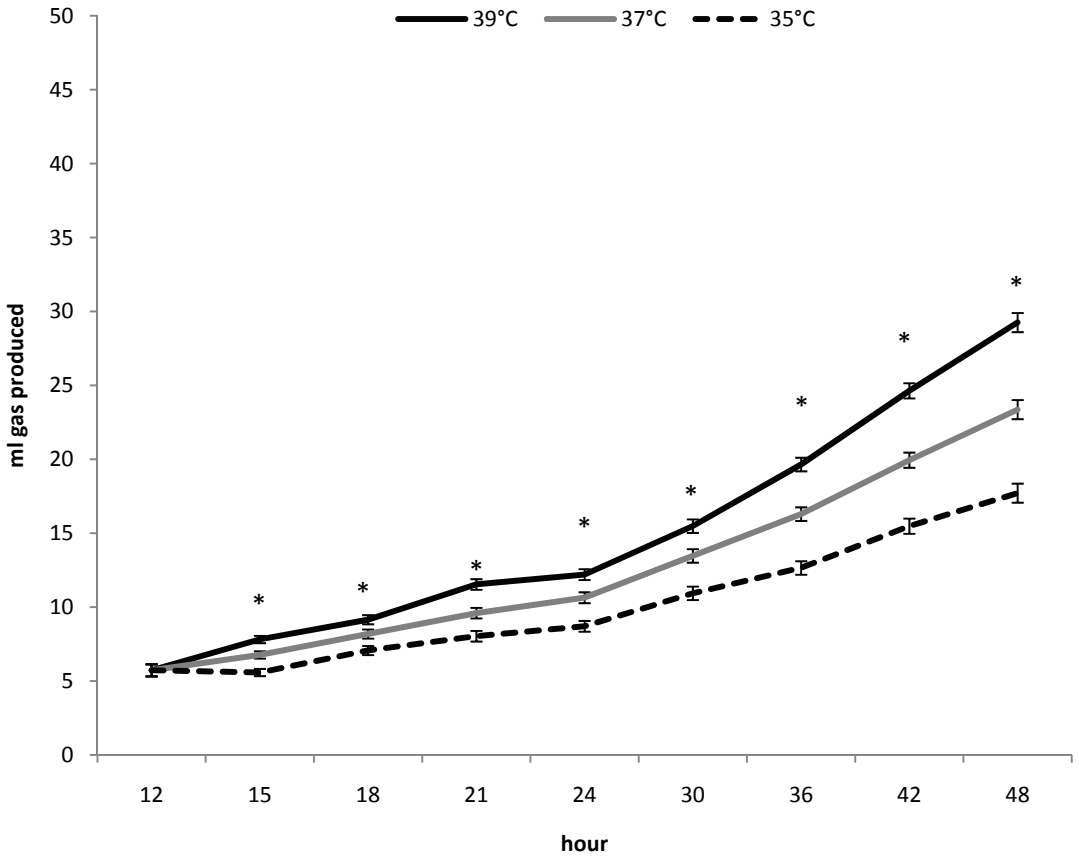

Figure 1. Gas production in a 48 hours incubation period with 3 different temperature water baths. A water temperature x hour interaction occurred (*P<.01)

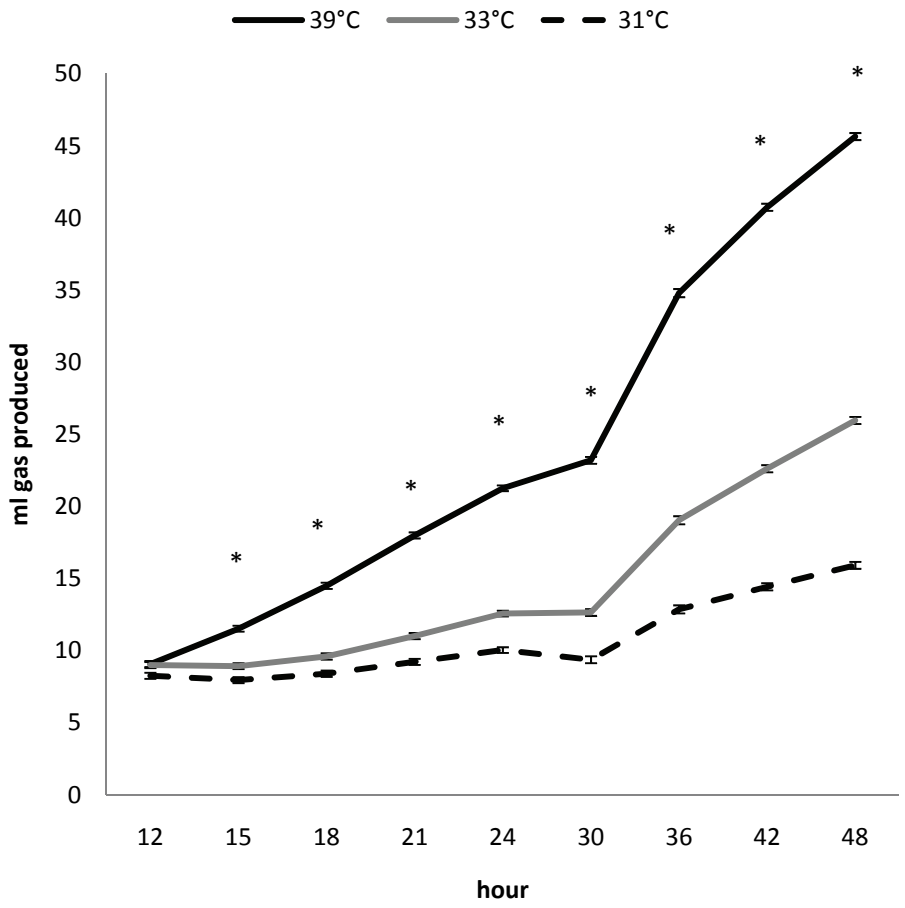

Figure 2. Gas production in a 48 hours incubation period with 3 different temperature water baths. A water temperature x hour interaction occurred (*P<0.01).

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PROTEIN AND ENERGY SUPPLEMENTATION OF BRAHMAN HEIFERS IN THE WESTERN PLAINS OF VENEZUELA

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ABSTRACT: To evaluate the effect of energy and energy-protein supplementation on BW, ADG, percent pregnancy (PP) and blood chemistry (BC), 57 Brahman heifers, with 309.1 ± 3.09 kg BW and 1,067 ± 8.49 d old were assigned to three treatments :1) pasture only (**P**); 2) P + 200 g·animal^{-1}·d^{-1} of bypass fat (**F**, 6.7 Mcal ME/kg DM); and 3) P + a protein-energy mix (**PE**) with 45% CP and 3.25 Mcal ME/kg DM (1 kg·animal^{-1}·d^{-1}) containing mainly hydrolyzed feather meal, corn meal and bypass fat. All animals had free access to a complete mineral supplement. The experiment lasted 102 d, located in the western plains of Venezuela. The animals were kept in a rotation pasture system of *Brachiaria arrecta,* with an average stocking rate of 0.75 animals/ha. Body weight and blood samples were taken every 28 d. The breeding period started 57 d after the beginning of the supplementation period and lasted for 45 d. Heifers were bred by AI. Data of BW, ADG, and BC were treated by ANOVA, in a complete randomized design, with measurements repeated in time. A contingency table with chi-squares was used for PP. Forage contained 8.0 ± 0.26% CP and 69.3 ± 0.55% NDF. Body weight showed a significant interaction for treatment x time (P<0.01), being similar during the initial 84 d of supplementation (335.6 ± 3.43 kg), but different at end of the experiment (347.5, 338.4, and 331.1 kg, respectively for F, PE, and P). Average daily gain (g/d) was greater (P<0.01) for F (358) than PE (285.4) and lower for P (206.3) in relation to the other treatments. Pregnancy (%) was improved (P<0.05) by protein-energy supplementation, with values of 35, 45 and 63 respectively for P, F, and PE. Concentration of blood urea was not influenced by treatment (P>0.05), with an overall mean of 31.2 ± 0.76 mg/100 mL, while cholesterol was higher (P<0.05) for PE. Energy and protein-energy supplementation improved BW and ADG of heifers, while PP and cholesterol levels were increased only by energy-protein supplement.

Key words: Brahman heifers, supplementation, energy, protein.

Introduction

Cattle production in Venezuela is a pastoral activity, being therefore subject to forage DM available to animals. This condition varies according to seasons (dry and rainy periods), and to low quality of tropical forages in terms of protein (Herrera et al., 2009; Mora et al., 2010) and energy content (Moss, 1993), in addition to high content of NDF (Herrera et al., 2009; Depablos et al., 2009; Mora et al., 2010). These conditions are limiting for growth, and, in case of young replacement heifers, puberty is delayed, consequently, the reproductive performance at mating time is negatively affected (Oyedipe et al., 1982).

These conditions imply the implementation of some technological arrangements for animal feeding, as the use of supplements to improve forage utilization or to correct dietary deficiencies to avoid productivity losses, especially when the nutrient demands of the animals are higher (Chicco and Godoy, 1987). For these reasons, the objective of this research was to evaluate the response of energy and protein-energy supplementation on body weight changes, pregnancy and some components of blood chemistry of Brahman heifers grazing in the western plains of Venezuela.

Materials and Methods

The experiment was carried out in the western plains of Venezuela (Barinas State) with 57 Brahman heifers at first mating, with 309.1 ± 3.04 kg BW and 1,067±8.49 d of age, that were divided in three BW uniform groups, and assigned to three treatments: 1) pasture only (**P**); P + 200 g·animal^{-1}·d^{-1} of bypass fat (**F**),with 6.7 Mcal ME/kg DM; and 3) P + a protein-energy mix (**PE**) with 45% CP and 3.25 Mcal ME/kg DM (1 kg·animal^{-1}·d^{-1}) as shown in Table 1. All animals had free access to a mineral mix (approximately 50 g·animal^{-1}·d^{-1}) and water from a natural source. The animals were kept in a rotation pasture system with 12 paddocks of *Brachiaria arrecta,* with an average stocking rate of 0.75 animals/ha. Animals were kept in three paddocks for 9 d and than rotated in the same paddock every 3 d to minimize pasture effect. Eighteen samples of forage were taken every 9 d, using a metallic frame of 0.375 m² (Paladines, 1992). Forage sample were dried to determine total DM present in the pastures. Crude protein was determined by Kjeldahl (AOAC, 1980) and NDF by the method described Van Soest y Wine (1967). The breeding period started 57 d after the beginning of the supplementation period and lasted for 45 d. Heifers were bred by AI using sexed semen at the first insemination and non sexed semen at the second one.

Animal BW was measured at 0, 28, 56, 84 and 102 days of the experimental period, with no access to grazing and water 16 h before weighing. At the same time, blood sample were taken by jugular puncture from six animals per treatment randomly selected at the beginning of the experiment. The same animals were sampled at the same time when BW was determined. Blood samples were centrifuged and serum was kept at -20°C to determine

cholesterol by colorimetric method and urea by an enzymatic procedure. Pregnancy was detected by transrectal palpation, at 45 days after the end of the breeding season.

Data for BW, ADG and blood chemistry were treated by ANOVA as a complete randomized design, with measurement repeated in time (Davis 2000). Pregnancy at the first and second insemination was analyzed by a contingency table (Verde, 2002).

Table 1. Supplement composition.

Ingredients	PE (%)
Urea	5
Ammonium sulfate	5
Bypass fat (Biolac®)	20
Molasses	5
Corn meal	35
Hydrolyzed feather meal	30
Nutrients	
Crude Protein[1]	45.0
ME, Mcal/kg[1]	3.25

[1]Calculated values.

Results and Discussion

The amount of biomass in the pastures (Table 2) showed variations during the experimental time (P<0.05) with lower values corresponding to periods 0-28 d (2,401.5 kg DM·ha^{-1}) and 84-102 d (2,248.8 kg DM·ha^{-1}). The lower value of biomass at the beginning of the experiment may be due to the fact that the pastures were overgrazed by other animals prior the experiment. However, biomass gradually increased and by the end of the experiment diminished again due to limited rainfall. In all cases forage DM was always higher than 2,000 kg MS·ha^{-1}, value suggested by Minson (1990) as sufficient for an adequate intake under grazing conditions. Nevertheless, available biomass along the experimental time was lower than 30 kg of DM·animal^{-1}·d^{-1}suggested by Lamela (1992) as the adequate amount required to express 90% of the animal potential production.

Crude protein and NDF of forage decreased along the experimental time (P<0.05), being CP higher than 7%, value indicated by Milford and Minson (1965) as the amount required for an adequate intake. This was not the same at the end of the experiment (85-102 d) when CP dropped to 6.16%.

Table 2. Amount of biomass in the pastures and forage chemical composition during the experiment.

Periods	Biomass		CP	NDF
	kg DM·ha^{-1}	kg DM·animal^{-1}·d^{-1}	%	
0-28 d (End of the rainy season)	2,401.5^{b}	21.32^{bc}	9.40^{a}	72.1^{a}
29-56 d (Dry season)	2,645.4^{ab}	26.99^{ab}	8.36^{ab}	69.8^{ab}
57-84 d (Dry season)	2,928.0^{a}	28.71^{a}	7.46^{ab}	67.7^{b}
85-102 d (Dry season)	2,248.8^{b}	18.47^{c}	6.16^{b}	66.6^{b}

$^{a, b, c}$ Means with different superscripts in the same column are different (P<0.05).

Intake of F supplement was complete (200 g·animal^{-1}·d^{-1}), while PE supplement progressively increased as the CP content of forage decreased, with values of de 87.7, 250 y 280.7 g·animal^{-1}·d^{-1} respectively for 0-28, 29-56 and 57-84 periods. Values for supplement intake were not recorded for the last period (85-102 d).

Heifers BW (Figure 1) showed an interaction treatment x time (P<0.01), being similar at day 84 of the experiment (335.6 ± 3.43 kg) and different at the end of the experiment (347.5; 338.4 and 331.1 kg, respectively, for P, F and PE). Body weights at the beginning of the breeding season (56 d) were 328.2; 335.7 y 329.4 kg for P, F y PE, respectively. These values are higher than the 280 kg suggested by Plasse et al. (1989) as the minimum weight for commercial Brahman heifers at mating time. On the other hand, ADG were influenced by treatments (P<0.01), with greater gains for F in relation to PE and lower for P (Table 3). Similar response in ADG with fat supplementation was reported by Zinn (1989) and Van Houtert et al. (1990). The last authors indicate that the addition of a protein meal improved even more animal response; this was not achieved in this research, probably due to a low intake of the PE supplement. Chilliard (1993) suggests that dietary fat tends to increase body fat deposition in growing cattle, if the supplement does not decrease DM intake, since protected fat might have this negative effect. On the other hand, protein supplementation also improved ADG of cattle when compared to a negative control (Godoy and Chicco, 1991).

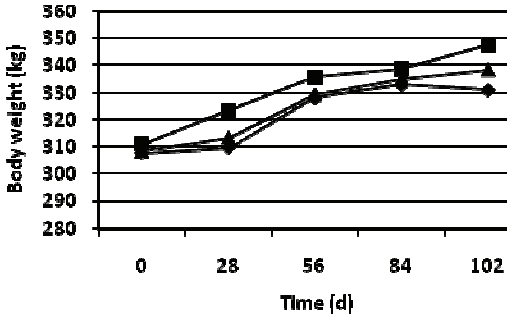

Figure 1. Effect of the interaction treatment x time on BW of Brahman heifers at mating. P (♦), F (■) and PE (▲).

Table 3. Average daily gain of Brahman heifers with energy and protein supplements.

Treatment	ADG (g/d) Day				Average (g/d)	SEM
	0-28	29-56	57-84	85-102		
P	59.6	686.1	179.3	-99.7	206.3[c]	19.8
F	407.0	452.2	113.0	459.8	358.0[a]	19.8
PE	164.9	594.5	210.5	171.7	285.4[b]	19.8
Average (g/d)	**210.5[b]**	**577.6[a]**	**167.6[b]**	**177.2[b]**	**283.3**	**11.5**
SEM	**27.2**	**23.4**	**22.1**	**25.1**	**11.5**	

[a, b, c] Means with different superscripts in the same column or row are different (P<0.01).

The overall pregnancy (%) was improved (P<0.05) by energy and protein supplementation with values of 35.3, 41.2, and 63.2 for P, F and PE supplements, respectively. The same tendency was registered at the first insemination with values of 23.5, 29.4 and 52.6 % for the same treatment order (Table 4), suggesting that CP requirements in PE treatment were met. Sasser et al. (1988) reported a better conception rate in bovine females when dietary protein was adequate to requirements. Lammoglia et al. (2000) did not register pregnancy difference when heifers were supplemented with sun flower seed oil.

Pregnancy corresponding to the second insemination was 50, 28.6, and 66.7% for P, F and PE, respectively, with no differences among treatments (P>0.05). The low number of animals at the second insemination could be limiting to detect differences among treatments (Rusche et al., 1993). Serum blood urea concentration (Table 5) was not influenced by supplements (P>0.05), with an overall average of 31.2 ± 0.76 mg/100 mL. However time influenced urea concentration (P<0.05), with lower values at day 56 (25.1 mg/100 mL) and higher at day 102 (39.7 mg/100 mL), being these values similar to the reported reference (15-42 mg/100mL; Wittwer, 2000).

Table 4. Percent pregnancy of Brahman heifers with energy and protein supplementation.

Treatments	Animals at first insemination (n)	Pregnant animals (n)	Pregnancy at first insemination (%)	Animals at second insemination (n)	Pregnant animals (n)	Pregnancy at second insemination (%)	Total pregnancy (%)
P	17	4	23.5[b]	4	2	50.0	35.3[b]
F	17	5	29.4[b]	7	2	28.6	41.2[b]
PE	19	10	52.6[a]	3	2	66.7	63.2[a]

[a, b] Means with different superscripts in the same column are different (P<0.05).

On the other hand, cholesterol was influenced by supplementation type (Table 5) with higher concentrations (P<0.05) for PE (139.3 mg/100 mL) and lower for P (117.4 mg/100 mL). In addition, there was a time effect (P< 0.05), being the lower values at 0 time and increasing progressively by days 56, 84 and 102. The increased lipid levels in blood of cattle have been associated with a better reproduction performance at the second insemination, with higher levels of blood progesterone (Sklan et al., 1991). Cholesterol concentration at 0 and 28 days were lower than the reference values (115-193 mg/100 mL; Wittwer, 2000).

Table 5. Urea and cholesterol in blood serum of heifers with energy and protein supplementation.

Metabolite	Treatment	Day 0	28	56	84	102	Average	SEM
Urea (mg/100 mL)	P	29.8	36.5	24.0	29.5	40.6	**32.1**	**1.31**
	F	29.4	30.5	21.5	31.0	38.4	**30.2**	**1.31**
	PE	27.1	28.0	29.8	31.5	40.2	**31.3**	**1.31**
	Average	**28.8[bc]**	**31.6[b]**	**25.1[c]**	**30.6[b]**	**39.7[a]**	**31.2**	**0.76**
	SEM	**1.68**	**1.50**	**1.07**	**1.50**	**1.66**	**0.76**	
Cholesterol (mg/100 mL)	P	94.3	94	152.7	128.5	117.3	**117.4[B]**	**5.68**
	F	90.7	110.5	159.1	159.0	142.0	**132.3[AB]**	**5.68**
	PE	72.5	127.8	153.5	170.8	171.7	**139.3[A]**	**5.68**
	Average	**85.8[c]**	**110.7[b]**	**155.1[a]**	**152.7[a]**	**143.6[a]**	**129.6**	**3.28**
	SEM	**3.71**	**5.04**	**6.74**	**4.05**	**5.16**	**3.28**	

[a, b, c] Means with different superscripts in the same row are different (P<0.05).
[A, B] Means with different superscript in the same column are different (P<0.05).

Implications

Energy and energy-protein supplementations improved BW and ADG of Brahman heifers at first mating. However, reproductive performance and cholesterol levels increased only with energy-protein supplementation, in spite of low consumption of the supplement.

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EFFECTS OF RUMINAL PROTEIN DEGRADABILITY ON SITE AND EXTENT OF DIGESTION IN BEEF COWS GRAZING SUMMER RANGELANDS AND FED FLAXSEED

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ABSTRACT [1] [2]: Metabolizable protein can be limiting in animals supplemented fat and grazing summer pasture. Therefore, our hypothesis was that the provision of supplements high in RUP would increase intestinal protein supply. Eight ruminally and duodenally cannulated cows (698 ± 25 kg) were used in a completely randomized design to evaluate the effects of ruminal protein degradability on site and extent of digestion when grazing summer rangelands and fed flaxseed. Starting on June 3, 2009 cows grazing a 15 ha native pasture were individually fed one of four treatments (DM basis): 1) ground flaxseed (2.5 kg, **FLX**); 2) ground flaxseed + soybean meal (2.5 kg flaxseed, 0.31 kg soybean meal, **SBM**); 3) ground flaxseed + dried distillers grains plus solubles (2.3 kg flaxseed, 0.20 kg soybean meal, 0.72 kg dried distillers grains plus solubles, **DDGS**); or 4) ground flaxseed + Soyplus (2.4 kg flaxseed, 0.52 kg Soyplus, **SOYPLS**). Diets containing protein sources were formulated to provide similar quantities of RDP (449 g/d) with the distillers dried grains plus solubles and Soyplus providing an additional 93 g/d RUP. There were three experimental periods that were 15 d in length. Dietary supplement did not influence (P = 0.29) forage OM intake. Likewise, duodenal flow of total OM did not differ between dietary treatments (P = 0.28). Microbial OM flow to the small intestine was greater (P = 0.02) for DDGS and SOYPLS compared to SBM. True ruminal OM digestibility (% of intake) was not different (P = 0.29). Total duodenal N flow was similar (P = 0.40) in spite of the fact that microbial N flows were greater for DDGS and SOYPLS (P = 0.03) compared to SBM. Non-microbial non-NH_3 flow tended (P = 0.08) to be greater for protein supplemented cows than FLX. True ruminal N digestibility was unchanged (P = 0.27) with protein supplementation. Ruminal molar proportions of acetate were greater (P = 0.02) for SBM than DDGS and SOYPLS. The provision of supplemental RUP fed at the levels reported herein did not increase intestinal metabolizable protein supply in beef cows grazing summer rangelands and supplemented flaxseed.

Key Words: Digestion, Flaxseed, Grazing

Introduction

Protein can often be limiting in dormant, low-quality forages, however, this can also be true in actively growing forages as well (Anderson et al., 1988; Karges et al., 1992; Klopfenstein, 1996). The type of protein that is limited differs between these two types of forage. Specifically, ruminally degradable protein is often limiting in dormant forages (Köster et al., 1996). Likewise, actively growing forages have been suggested to be deficient in ruminally undegradable protein (Anderson et al., 1988). Karges et al. (1992) reported a linear increase in beef steer performance when grazing native range and supplemented increasing levels of escape protein ranging from 0.07 to 0.21 kg/d, whereas, a quadratic response was observed for degradable protein up to 0.15 kg/d.

The provision of supplements high in omega-3 fatty acids for reproducing beef cows or production systems designed to increase carcass unsaturated fatty acid composition is desirable. Brokaw et al. (2001) who saw no differences in forage OM intake, however, intestinal supply of metabolizable protein was lower for the fat supplemented heifers. Likewise, Scholljegerdes and Kronberg (2010) reported that duodenal supply of non-ammonia, non-microbial N was numerically less in flax-fed cows than those consuming forage or a corn-based supplement and grazing summer pastures in the northern Great Plains. Therefore, feeding supplemental fats to cattle grazing actively growing forages may exacerbate the deficiency observed in metabolizable protein supply. Therefore, our hypothesis was that the provision of supplements high in RUP would increase intestinal protein supply. Our objectives were to evaluate the provision of supplemental ruminally undegraded protein on site and extent of digestion in beef cows fed ground flaxseed and grazing native summer rangelands.

Materials and Methods

Animals and diets

All experimental procedures were reviewed and approved by the Northern Great Plains Research Laboratory, Animal Care and Use Committee. Eight Angus cows (BW = 698 ± 25 kg) fitted with ruminal and duodenal cannulae were used in a completely randomized design. Typical rangeland at the USDA-ARS Northern Great Plains Research Laboratory (46° 46` N 100° 50`W) from June 3, 2009 until July 30, 2009. The study site was comprised of one 15 ha pasture containing 2,337 kg/ha of forage DM that consisted of predominately Kentucky bluegrass (*Poa pratensis* L.) and smooth brome (*Bromus inermus* Leyss.), along with some native species. Cattle were randomly assigned to one of four treatments (DM basis): 1) ground flaxseed (2.5 kg, **FLX**); 2) ground flaxseed + soybean meal (2.5 kg flaxseed, 0.31 kg soybean meal, **SBM**); 3) ground flaxseed + dried distillers grains plus solubles (2.3 kg flaxseed, 0.20 kg soybean meal, 0.72 kg dried distillers grains plus solubles, **DDGS**); or 4) ground flaxseed + Soyplus (2.4 kg flaxseed, 0.52 kg Soyplus, **SOYPLS**). Supplement formulated

[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.

[2] We wish to acknowledge the Western Central Soy Cooperative, Ralston, IA for their gracious donation of SoyPLUS.

chemical composition is presented in Table 1. In an effort to keep fat levels as similar as possible, the amount of DDGS, which is 10% fat, was limited, therefore, we formulated the diets containing two common RUP sources (DDGS and SoyPlus) to provide 100 g additional RUP. Cattle had free access to fresh water and trace mineralized blocks (American Stockman Trace Mineralized Salt, North American Salt Co., Overland Park, KS; NaCl >95.5%, Zn >3,500 mg/kg, Fe >2,000 mg/kg, Mn >1,800 mg/kg, Cu >280 mg/kg, I >100 mg/kg, Co >60 mg/kg).

Table 1. Formulated chemical composition of diets

	Flax[1]	SBM[2]	DDGS[2]	SOYPLS[3]
CP, %	2.8	25.5	27.5	25.9
EE, % DM	35	31	30	27
RDP, g/d	383.6	477.2	467.9	469.3
RUP, g/d	186.4	236.8	328.5	330.7

[1]Based published values (Lardy and Anderson, 2009; Mustafa et al., 2003).

[2]Based on published values (NRC, 2000).

[3]Based on published values (Anonymous, 2011).

Sampling and laboratory analysis

There were three experimental periods lasting 14 d. Cows were allotted to treatment 10 d prior to the start of the experiment so that they could be adapted to diet before masticate collection could take place on d 1 of each period. Ruminal masticate was collected and processed as described by Scholljegerdes and Kronberg (2010). Titanium dioxide was dosed twice daily (5g per dosage) from d 1 through d 11. Starting on d 10, duodenal and fecal samples were collected every 6 h. Then on d 11, sample times were advanced 3 h and again collected every 6 h such that every three hours in a 24-h theoretical clock was represented. On d 12, 800 mL of rumen fluid from each cow was collected for IVDMD analysis of masticate and feedstuffs. Immediately after rumen fluid was collected, duplicate Dacron in situ bags (50 µm pores) containing 5 g of each masticate and supplement ingredient was placed in tepid water for 1 min and then suspended in the rumen. Insitu bags were removed every 4 h for 24 and at 36 and 48 h. In situ bags were immediately placed in freezer at -20ºC until processing.

Finally, on d 14, prior to supplementing, 200 mL of Co-EDTA (5 g of Co) was dosed and whole ruminal contents were collected (0 h). Subsequent ruminal samples were collected every 3 h for 15 h. On d 20, prior to the 0700 feeding, 200mL of Co-EDTA (5g of Co; Uden et al., 1980) was dosed intraruminally and whole rumen contents were collected (0 h) and again at 3, 6, 9, 12, and 15 post dosing. Ruminal pH was immediately collected using a combination electrode (Orion Research Inc., Boston, MA) and samples were processed as described by Scholljegerdes and Kronberg (2010).

All feed, microbe, duodenal digesta, and fecal samples were analyzed for DM and ash (AOAC, 1990). Nitrogen content of feed, microbes, duodenal digesta, and feces were determined using a Carlo Erba Model NA 1500 Series 2 N/C/S analyzer (CE Elantech, Lakewood, NJ). Neutral detergent fiber of feed, duodenal digesta and feces were determined using an ANKOM 200 fiber analyzer (ANKOM Technology, Fairport, NY). Duodenal and fecal samples were analyzed for Titanium dioxide according to the procedures of Myers et al. (2004).

Ruminal fluid samples were centrifuged at 10,000 × *g* for 20 min at 4°C, and a 2.5-mL aliquot was added to 0.5 mL of 25% metaphosphoric acid containing 2 g/L of 2-ethyl-butyric acid (Goetsch and Galyean, 1983). These samples were analyzed for concentrations of VFA using a Varian 3800 GC equipped with a 15 m × 0.533 mm (i.d.) column (Nukol, Supelco, Bellefonte, PA). Approximately 100 mg of duodenal digesta were reconstituted to 3% DM using 0.1 *N* HCl for subsequent analysis of NH3 concentration (Hannah et al., 1991). Reconstituted duodenal digesta NH3 concentration was determined by the phenol-hypochlorite procedure (Broderick and Kang, 1980) using a spectrophotometer (DU-640, Beckman Instruments Inc., Fullerton, CA). Ruminal fluid Co concentrations were determined using an air-plus-acetylene flame by atomic absorption spectroscopy (model 3110, Perkin Elmer Inc., Norwalk, CT). Duodenal and isolated bacteria samples were analyzed for purine concentration as described by Zinn and Owens (1986) using a micro-plate reader (Synergy HT, Bio-Tek Instruments, Inc., Winooskie, VT)

Statistical analysis

All data were analyzed using the MIXED model of SAS (SAS Inst. Inc., Cary, NC) as a completely randomized design. The model included treatment, period and treatment x period. All time course data included the effects of h, period, and all possible interactions. Single degree of freedom contrasts were used to detect treatment differences. Specifically, Flax versus protein supplement, RUP versus RDP and DDGS versus SOYPLS. Autoregressive order one was determined to be the most desirable covariance structure according to the Akaike's information criterion. Treatment differences were considered significant at an alpha of $P < 0.05$. No treatment × period interactions observed ($P \geq 0.13$). Using the NLIN procedure of SAS using the model of Ørskov and McDonald (1970) was used to calculate protein Fractions A and B, as well as protein degradation rate (k_d = %/h). Effective ruminal degradation (**ERD**) was calculated using the equations of Broderick (1994).

Results and Discussion

Total and forage OM intake did not differ across treatments ($P \geq 0.29$). An unsupplemented control was not included in this experiment due to the fact that it has been established previously (Scholljegerdes and Kronberg, 2008, 2010) that supplemental flaxseed can depress forage intake therefore; we were focused on the effects of supplemental protein source in combination with flaxseed. Total duodenal OM flow was not different ($P = 0.28$) across dietary treatments. In addition, microbial OM flow was not improved ($P = 0.24$) with protein supplementation over that of FLX. Supplementing RUP increased microbial OM flow ($P = 0.02$) compared to RDP. This is surprising if one considers that RDP is reported to increase microbial flow (Köster et al., 1996; Bohnert et al., 2002). True ruminal, lower tract, and total tract OM digestibility did not differ with supplementation composition ($P \geq 0.27$).

Due to experimental design, N intake tended ($P = 0.08$) to be lower for FLX compared to protein supplements. In spite of

differences in N intake, total duodenal N flow did not differ (P = 0.40). This lack of difference in duodenal N flow between supplements high in RDP compared to high RUP has been observed previously by Salisbury et al. (2004) when wethers consumed low-quality grass hay and were fed increasing levels of RUP. Microbial N flow was greater (P = 0.03) for RUP compared to RDP. This is contrary to the report of MacDonald et al. (2007) who observed that high RDP supplements (distillers dried grains) did not affect microbial flow. Duodenal NH_3 was not different (P = 0.60) across treatment. Non-ammonia non-microbial N tended (P = 0.08) to be greater for protein supplemented cows compared to FLX. As discussed previously, the response observed between RDP and RUP for microbial N flow is likely due to changes in degradability of protein associated with each of the feedstuffs. True ruminal, lower tract (% of entering) and total tract N digestibility (% of intake) did not differ ($P \geq 0.11$) across treatments. In situ analysis (data not shown) indicates that masticate ERD values were 40.8, 41.2, 48.9, and 36.1% for FLX, SBM, DDGS, and SOYPL, respectively. These values are lower than what has been reported previously (Haugen et al., 2006). Nevertheless, the relatively low ERD value for forages would explain the relatively high non-ammonia non-microbial N flow. The pastures used in study had not been grazed for two years; therefore, there was a large amount of mature forage mixed in with the current year's growth.

Because forage intake did not differ it is not surprising that NDF intake also did not change (P = 0.48) with treatment. Likewise, duodenal flow was not affected by protein supplementation (P = 0.43). Although, supplemental RDP and RUP has been shown to increase ruminal NDF digestibility (Karges et al., 1992; Köster et al,. 1996; Mathis et al., 2000), forage CP in the current trial was greater than 7%, which is above the threshold, where additional protein has been reported to improve digestion. Therefore, ruminal and total tract NDF digestibility did not differ ($P \geq 0.29$).

Ruminal pH was lower for FLX than protein supplemented cows ($P < 0.001$). Ruminal fluid passage rate, %/h) increased (P = 0.01) with protein supplementation and tended to be greater (P = 0.09) for SOYPL (P = 0.09). Ruminal NH_3 was greater for protein supplemented cows compared to FLX (P = 0.05) and tended to be greater (P = 0.09) for RDP than RUP with SOYPL being greater than DDG (P = 0.06). Ruminal VFA were greater for FLX than protein supplemented cows (P = 0.002) with SBM being greater than RUP (P = 0.02). The higher concentration of total VFA was likely the cause of the lower ruminal pH for FLX. Although differences in acetate were observed for RDP versus RUP (P = 0.02) and DDGS versus SOYPL (P = 0.04), the biological significance is likely minimal based on no differences being observed for ruminal NDF digestibility. Molar proportions of propionate was greater (P = 0.04) for RUP and SOYPL was greater than DDGS (P = 0.04). No differences were observed for butyrate (P = 0.13), yet isobutyrate was greater (P = 0.001) in protein supplemented cows than FLX and SOYPL was greater (P = 0.01) than DDG.

Feeding an additional 100 g of RUP to grazing beef cows was not sufficient to provide a significant increase in intestinal supply of protein.

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Table 2. Effects of RDP or RUP and source of RUP on OM intake, flow and digestion in grazing beef cows fed flaxseed

	Treatments[1]					Contrasts[2]		
Item	FLX	SBM	DDGS	SOYPL	SEM	FLX vs PROT	RDP vs RUP	DDGS vs SOYPL
OM intake, g/d								
Forage	6851	7109	6081	6543	538.1	0.68	0.29	0.58
Total	9285	9829	9176	9361	528.1	0.80	0.44	0.82
Duodenal OM flow, g/d								
Total	4007	4034	4200	3715	273.1	0.94	0.83	0.28
Microbial	1395	1011	1404	1371	84.6	0.24	0.02	0.80
Fecal OM flow, g/d	2667	2710	2706	2424	176.4	0.80	0.54	0.32
OM digestibility								
True ruminal, % of intake	71.3	68.8	68.3	74.0	3.36	0.82	0.60	0.29
Lower tract, % of entering	32.6	31.7	34.6	34.0	7.29	0.93	0.78	0.96
Total tract, % of intake	70.7	72.2	70.1	73.7	1.98	0.61	0.91	0.27

[1]1) ground flaxseed (2.5 kg, **FLX**); 2) ground flaxseed + soybean meal (2.5 kg flaxseed, 0.31 kg soybean meal, **SBM**); 3) ground flaxseed + dried distillers grains plus solubles (2.3 kg flaxseed, 0.20 kg soybean meal, 0.72 kg dried distillers grains plus solubles, **DDGS**); or 4) ground flaxseed + Soyplus (2.4 kg flaxseed, 0.52 kg Soyplus, **SOYPLS**).
[2]Contrasts: FLX vs PROT = Flax versus SBM, DDGS, and SOYPL; RDP vs RUP = SBM vs DDGS and SOYPLS; DDGS vs SOYPL.

Table 3. Effects of RDP or RUP and source of RUP on N intake, flow, and digestibility (OM basis) in grazing beef cows fed flaxseed

	Treatments					Contrasts[2]		
Item	FLX	SBM	DDG	SOYPL	SEM	FLX vs PROT	RDP vs RUP	DDGS vs SOYPL
N intake, g/d	228	262	258	261	12.0	0.08	0.85	0.88
Duodenal N flow, g/d								
Total	162.3	168.4	186.8	171.6	12.29	0.40	0.51	0.43
Microbial	83.0	60.9	87.1	85.2	6.05	0.50	0.03	0.83
NH_3	18.9	19.2	19.7	18.6	1.40	0.90	0.99	0.60
NANM	60.4	88.4	80.0	67.8	6.76	0.08	0.16	0.27
Fecal N flow, g/d	56.0	60.8	63.2	54.0	3.21	0.42	0.61	0.11
N digestibility								
True ruminal, % of intake	72.8	65.9	68.8	73.3	3.31	0.41	0.27	0.39
Lower tract, % of entering	64.9	63.3	65.5	68.1	3.78	0.88	0.49	0.65
Total tract, % of intake	75.1	76.8	75.6	79.2	1.26	0.22	0.68	0.11
MOEFF	12.3	13.7	11.7	10.4	1.30	0.84	0.17	0.53

[1]1) ground flaxseed (2.5 kg, **FLX**); 2) ground flaxseed + soybean meal (2.5 kg flaxseed, 0.31 kg soybean meal, **SBM**); 3) ground flaxseed + dried distillers grains plus solubles (2.3 kg flaxseed, 0.20 kg soybean meal, 0.72 kg dried distillers grains plus solubles, **DDGS**); or 4) ground flaxseed + Soyplus (2.4 kg flaxseed, 0.52 kg Soyplus, **SOYPLS**).
[2]Contrasts: FLX vs PROT = Flax versus SBM, DDGS, and SOYPL; RDP vs RUP = SBM vs DDGS and SOYPLS; DDGS vs SOYPL.

Table 4. Effects of RDP or RUP and source of RUP on NDF intake, flow, and digestibility (OM basis) in grazing beef cows fed flaxseed

	Treatments					Contrasts		
Item	FLX	SBM	DDGS	SOYPL	SEM	FLX vs PROT	RDP vs RUP	DDGS vs SOYPL
Intake, g/d	5847	6123	5724	5775	393.6	0.96	0.48	0.93
Duodenal flow, g/d	2000	2120	2159	1915	198.1	0.79	0.75	0.43
Fecal flow, g/d	2157	2195	2163	1910	172.2	0.75	0.49	0.36
Digestibility, % of intake								
Ruminal	65.3	65.0	61.3	65.8	5.22	0.84	0.83	0.57
Total tract	62.4	63.7	61.3	66.1	2.78	0.71	0.99	0.29

[1]1) ground flaxseed (2.5 kg, **FLX**); 2) ground flaxseed + soybean meal (2.5 kg flaxseed, 0.31 kg soybean meal, **SBM**); 3) ground flaxseed + dried distillers grains plus solubles (2.3 kg flaxseed, 0.20 kg soybean meal, 0.72 kg dried distillers grains plus solubles, **DDGS**); or 4) ground flaxseed + Soyplus (2.4 kg flaxseed, 0.52 kg Soyplus, **SOYPLS**).
[2]Contrasts: FLX vs PROT = Flax versus SBM, DDGS, and SOYPL; RDP vs RUP = SBM vs DDGS and SOYPLS; DDGS vs SOYPL.

Table 5. Effects of RDP or RUP and source of RUP on ruminal pH, fluid passage rate, and VFA in grazing beef cows fed flaxseed

	Treatments					Contrasts		
Item	FLX	SBM	DDGS	SOYPL	SEM	FLX vs PROT	RDP vs RUP	DDGS vs SOYPL
Ruminal pH	5.7	6.1	6.3	6.4	0.10	<0.0001	0.06	0.40
Ruminal fluid passage rate, %/h	5.13	7.07	6.84	8.92	0.684	0.01	0.38	0.09
Ruminal NH_3, mM	7.1	9.6	7.3	9.1	0.61	0.05	0.09	0.06
Total VFA, mM	56.6	52.2	48.0	44.1	1.91	0.002	0.02	0.17
Ruminal VFA, mol/100mol								
Acetate	65.7	66.5	65.7	64.1	0.51	0.65	0.02	0.04
Propionate	19.1	18.1	18.6	19.8	0.40	0.57	0.04	0.04
Butyrate	12.0	12.1	12.4	12.5	0.202	0.19	0.13	0.71
Isobutyrate	0.99	1.08	1.08	1.18	0.0232	0.001	0.14	0.01
Isovalerate	1.38	1.42	1.37	1.49	0.045	0.40	0.95	0.09
Valerate	0.82	0.81	0.87	0.94	0.034	0.22	0.06	0.17

[1]1) ground flaxseed (2.5 kg, **FLX**); 2) ground flaxseed + soybean meal (2.5 kg flaxseed, 0.31 kg soybean meal, **SBM**); 3) ground flaxseed + dried distillers grains plus solubles (2.3 kg flaxseed, 0.20 kg soybean meal, 0.72 kg dried distillers grains plus solubles, **DDGS**); or 4) ground flaxseed + Soyplus (2.4 kg flaxseed, 0.52 kg Soyplus, **SOYPLS**).
[2]Contrasts: FLX vs PROT = Flax versus SBM, DDGS, and SOYPL; RDP vs RUP = SBM vs DDGS and SOYPLS; DDGS vs SOYPL.

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