Proceedings

Volume 58

Western Section
American Society of Animal Science

University of Idaho

Department of Animal & Veterinary Science

Moscow, Idaho
June 20-22, 2007

2007 WSASAS
Organizing Committee
University of Idaho
Dr. Richard Battaglia, Chair

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Western Section
American Society of Animal Science
2006-07 Committee Assignments
***Denote chairperson**

Executive
T. T. Ross, President (New Mexico State Univ.)*
K. C. Olson, President-Elect (S. Dakota State Univ.)
J. Thompson, Past-President (Oregon State Univ.)
R. Battaglia, Secretary-Treasurer (Univ. of Idaho)
D. H. Hallford, ASAS Board Director (New Mexico State Univ.)
S. Paisley, A&C, Chair (Univ. of Wyoming)
G. Tibbetts, Industry Director (Zinpro Corp.)

Awards
K. Olson (07, S. Dakota State University)*
T. W. Geary (07, USDA-ARS Miles City)
B. Hess (07, University of Wyoming)
J. Bowman (07, Montana State University)
T. Engle (08, Colorado State University)
C. Mathis (08, New Mexico State University)

Symposium
C. Mueller (07, Oregon State University)*
G. Moss (07, University of Wyoming)
G. Duff (07, University of Arizona)
B. Christensen (08, Virtus Nutrition)
R. Waterman (08, USDA-ARS Miles City)
J. B. Glaze (08, University of Idaho)

Advising and Coordinating
S. Paisley (07, University of Wyoming)*
J. B. Glaze (07, University of Idaho)
J. B. Lamb (07, BYU-Idaho)
J. Sprinkle (07, University of Arizona)
L. B. Bruce (07, University of Nevada)
S. Daugherty (07, Cal Poly)
J. Busboom (07, Washington State University)
D. Garrick (07, Colorado State University)
C. A. Loest (08, New Mexico State University)
D. Drake (08, University of California, Davis)
R. Wiedmeier (08, Utah State University)
T. Bodine (08, Western Feed Supplements)
J. Stellflug (08, USDA-ARS, Dubois, ID)
D. H. Crews (09, AAFC, Edmonton)
M. Salisbury (09, Angelo State University)
S. Ivey (09, New Mexico State University)

Paper Competition
J. Rumph (08, Montana State University)*
D. W. Bohnert (07, Oregon State University)
T. Bodine (07, Western Feed Supplements)
S. Soto-Navarro (08, New Mexico State University)
A. Ahmadzadeh (08, University of Idaho)
M. Shipka (09, University of Alaska)
B. Taylor (09, USDA-ARS, Dubois, ID)
L. Baumgard (09, University of Arizona)

Academic Quadrathalon
D. C. Rule (University of Wyoming)*
N. A. Irlbeck (Colorado State University)
J. B. Lamb (BYU – Idaho)
C. W. Hunt (University of Idaho)
L. M. Surber (Montana State University)
S. Soto-Navarro (New Mexico State University)
C. Mueller (Oregon State University)

Extension
M. Encinias (07, New Mexico State University)*
R. Hathaway (07, Oregon State University)
S. Paisley (08, University of Wyoming)
J. Ahola (08, University of Idaho)
J. Paterson (08, Montana State University)
B. Bruce (09, University of Nevada, Reno)
J. Sprinkle (09, University of Arizona)

Necrology
Elaine Grings (07, USDA-ARS, Miles City, MT)*

Nominating
J. Thompson, Past-President (07, Oregon State University)*
A. Roberts (07, USDA-ARS, Miles City, MT)
D. Hallford (07, New Mexico State University)

Minutes of the Western Section of the American Society of Animal Science
Business Meeting
June 23, 2006
Utah State University
Logan, UT

President Jim Thompson called the meeting to order at 8:00 am.

Acceptance of the minutes of the 2005 business meeting.
The minutes of the 2005 business meeting were approved as printed in the 2006 Proceedings of the Western Section of the American Society of Animal Science.

Advisory and Coordinating Committee Report
Benton Glaze, chair

2005–2006 A&C Committee Members:
J. B. Glaze Jr. (University of Idaho)
J. M. Rumph (Montana State University)
S. J. Filley (Oregon State University)
J. B. Lamb (BYU-Idaho)
J. Sprinkle (University of Arizona)
S. I. Paisley (University of Wyoming)
L. B. Bruce (University of Nevada)
J. Busboom (Washington State University)
D. Garrick (Colorado State University)
C. A. Loest (New Mexico State University
D. Drake (University of California, Davis)
R. Wiedmeier (Utah State University)
T. Bodine (Western Feed Supplements)
S. Daugherty (Cal Poly)

Committee report: On November 17, 2005, the WSASAS Executive committee requested input from the A&C committee regarding proposed guidelines for the Graduate Student Paper Competition. The proposed guidelines as presented by the Graduate Student Paper Competition Committee are:

- Competition papers should include an implications statement,
- Competition papers should be limited to four (4) pages,
- Students should be limited in the number of times they can enter the competition,

The A&C committee communicated via e-mail and phone to draft recommendations related to each of the proposed guidelines. The final recommendations were sent via e-mail to James M. Thompson, WSASAS President, and are presented below:

- 'Implications' statements should be a part of all competition papers.
- Graduate Student Competition papers should be limited to four (4) pages. Tables and figures may go beyond the four (4) page limit.
- Students should be able to enter as many times as they desire during their MS and PhD programs. If a student places first in the competition, they should not be allowed to enter again. Exception: If a student wins the competition during an MS program, he/she should be able to enter again during their PhD program.

Action: A motion was presented to accept all three recommendations. It was amended to consider each separately. The implications requirement and limitations for four pages were accepted. The limitation on competing after winning failed. It was amended to not allow first-place winners to compete again, regardless of degree program. It passed after amending it to read "Students should be able to enter as many times as they desire during their MS and PhD programs. If a student places first in the competition, they *will not be allowed* to enter again.

Academic Quadrathlon Report
A report was not available. The low level of participation (only 4 teams) at the 2006 Quadrathlon was discussed. The membership recommended that WSASAS communicate with department heads to garner support for faculty and student involvement. This will be addressed by the Executive committee as a first step to improve participation. Other suggestions that will be considered later were to change the Quadrathlon to reflect the interests of the current student profile and to hold the Quadrathlon during the annual meeting. These were tabled for later consideration.

Awards Committee Report
Tim Ross, chair

Committee Members:
- Tim Delcurto (Oregon State University)
- Mark Petersen (New Mexico State Univ.)
- Tom Geary (USDA-ARS, Miles City)
- Bret Hess (University of Wyoming)
- Jan Bowman (Montana State University)
- Tim Ross (New Mexico State University)

Distinguished Teacher (2 nominations):

Recipient:	Dr. Anita Oberbauer University of California, Davis
Sponsor:	Elanco Animal Health
Nominator:	Dr. Gary Anderson

Young Scientist (3 nominations):

Recipient:	Dr. Terry Engle Colorado State University
Sponsor:	Ridley Block Operations c/o Dr. Dan Dhuyvetter 424 N. Riverfront Drive Mankato, MN 56002 ddhuyvetter@ridleyinc.com
Co-nominators:	Dr. David Anderson Dr. David Ames

Extension Award (1 nomination):

Recipient:	Dr. Jim Sprinkle University of Arizona
Sponsor:	Fort Dodge Animal Health c/o Dr. Frank Prouty 9401 Indian Creed Parkway Overland Park, KS 66225 FPROUTY@fdah.com
Co-nominators:	Dr. Glenn Duff Dr. Bill Schurg

No nominations were received for the Service Award.

Applied Animal Science Award Report
Bret Christensen, chair

This year, the Applied Animal Science Award committee had to go back to each industry judge sponsoring $50 due to loss of sponsorship from Alltech. This year we are going to be splitting the money we collect among the top three papers. There are nine papers submitted for judging this year. Next year, I would like to change submission rules to not require a paper copy. This would ease the submission process and the paper copy is not needed because they are sent to the judges via e-mail.

I want to ask all people on the Executive committee who are aware of the people conducting applied research to be pushed to submit their paper for this award. This allows recognition for their work, as well as exposing industry judges to the work that is being conducted at the land-grant universities.

Graduate Student Paper Competition Committee Report
R. Mark Enns, chair

Committee Members:
- Amin Ahmadzadeh (University of Idaho)
- Tim Bodine (Western Feed Supplements, Washington)
- Dave Bohnert (Oregon State University)
- Denny Crews (Agriculture and Agri-Food Canada)
- R. Mark Enns (Colorado State University)
- Paul A. Ludden (University of Wyoming)
- Janice Rumph (Montana State University)
- Sergio A. Soto-Navarro (New Mexico State University)

The current committee would like to thank Jim Berardinelli and Clint Loëst for their service to the committee through the 2005 competition. We would also like to thank Ken Olson and Paula Schultz for their efforts in helping to conduct a successful competition.

The 2006 Graduate Student Paper Competition was a great success with 13 papers representing faculty and students from 11 universities and 3 USDA facilities. After an extremely competitive morning, the overall placings were as follows:

1. Waggoner, Justin W., New Mexico State Univ.
2. Murrieta, Charles M., Univ. of Wyoming
3. Atkinson, Rebecca L., Univ. of Wyoming

The institutional award went to the University of Wyoming.

Denny Crews and Paul Ludden will be ending their terms on the committee. Mark Enns has volunteered for a second term if deemed appropriate.

The committee appreciates the efforts of those mentors who encourage their students to participate in this competition.

Extension Committee Report
Dale ZoBell, chair

Committee Members:
- D. ZoBell (06, Utah State University)
- R. Zinn (06, University of California, Davis)
- M. Encinias (07, New Mexico State University)
- R. Hathaway (07, Oregon State University)
- S. Paisley (08, University of Wyoming)
- J. Ahola (08, University of Idaho)
- J. Paterson (08, Montana State University)

The Extension committee was solicited for ideas for the 2006 Extension Symposium via e-mail and telephone. There were many ideas and it was decided that the theme or central idea for 2006 would be for speakers to address the topic of collaboration among agencies in applied research studies or extension activities.

WSASAS membership was invited to present on this topic. The list of presenters was as follows:

1. Working together to achieve natural resource sustainability in central Oregon. C. T. Parsons, G. Hudspeth, and J. Dedrick, Oregon State University, Baker City, OR; Crook County Soil and Water Conservation District, Prineville, OR; and Crooked River Watershed Council, Prineville, OR.

2. Dutchman Butte revisited: Examining paradigms for livestock grazing exclusion. J. Sprinkle, M. Holder, C. Erickson, A. Medina, D. Robinett, G. Ruyle, J. Maynard, S. Tuttle, J. Hays, Jr., W. Meyer, S. Stratton, A. Rogstad, K. Eldredge, J. Harris, L. Howery, and W. Sprinkle. University of Arizona, Tucson, AZ; Gila County Cattle Growers, Tonto Basin, AZ; Rocky Mountain Research Station, Flagstaff, AZ; NRCS, Tucson and Chandler, AZ; Southwest Resource Consultants, LLC, Las Cruces, NM; Arizona State Land Department, Pinetop, AZ.

3. Digital imagery and landscape-scale rangeland monitoring. J. B. Taylor, D. T. Booth, and C. M. Moffet. USDA-ARS US Sheep Experiment Station, Dubois, ID; USDA-ARS, High Plains Grasslands Research Station, Cheyenne, WY.

4. Vegetative management using controlled sheep grazing—The Montana Sheep Institute. L. M. M. Surber, R. W. Kott, J. D. Moore, B. L. Roeder, G. Hewitt, J. Smith, and K. Williams. Montana State University, Bozeman, MT; Powell County Weed Supervisor, Deer Lodge, MT; Custer County Extension, Miles City, MT.

5. Using pre-weaning and post-weaning variables to predict carcass quality. J. S. Davy, J. W. Oltjen, D. J. Drake, and A. L. Van Eenennaam. Department of Animal Science, University of California, Davis, CA.

6. Survey provides information on cow-calf handbook use and value. J. B. Glaze Jr., J. W. Oltjen, and D. J. Drake. University of Idaho, Twin Falls, ID; University of California, Davis, CA.

Nominating Committee Report

Committee Members:
- Elaine Grings, chair
- Bret Christensen
- Jack Whittier

Nominations for the 2006 WSASAS elections were:

- President-Elect: Ken Olson, Utah State Univ.
- Secretary-Treasurer: Richard Battaglia, Univ. of Idaho
- Industry Director: Gary Tibbetts, Zinpro Corp.

All nominees were elected to office.

Western Section Symposium Report
Terry Engle, chair

I. 2006 Symposium Committee Members:
Terry Engle (Chair; Colorado State Univ.)

Mark Wise (New Mexico State University)
Mike MacNeil (USDA-ARS, Miles City, MT)

II. Program

"What to do with all the omics - genomics, proteomics, etc.?"
Western Section, American Society of Animal Science
Utah State University, Logan, Utah
Wednesday, June 21, 2006

7:00–10:00 Registration

9:00–10:00 MIXER

10:00–10:15 Introductions & Welcome

James Thompson, Oregon State University, Dept. of Animal Sciences; WSASAS President
Mark Healey, Utah State University, Animal, Dairy, and Veterinary Sciences Department Chair
Terry Engle, Dept. of Animal Sciences, Colorado State University, CO; Symposium Chair
Dorian Garrick, Dept. of Animal Sciences, Colorado State University, CO; Moderator.

10:15–11:00 *Visioning the future of animal sciences in the world of ...omics*
Keynote speaker: Noelle Cockett, Utah State University

"Statistical" genomics

11:00–11:45 *Experimental approaches for discovery and refinement of quantitative trait loci*
Speaker: Mike MacNeil, ARS Miles City, MT
11:45–12:00 Introduction of sponsors
12:00–1:30 LUNCH

Use of functional genomics tools to tackle problems in animal production

1:30–2:15 *An introduction to microarrays and their use in evaluating gene expression*
Speaker: Andy Roberts, ARS Miles City, MT
2:15–3:00 *Use of gene expression arrays in identifying new research targets in animal science*
Speaker: Bob Collier, University of Arizona
3:00–3:30 BREAK

Use of omics tools by industry and summary of symposium
3:30–4:15 *Exploiting "omics" for animal improvement*
Speaker: Dorian Garrick, Colorado State University
4:15 Panel of speakers – Questions

III. List of challenges/questions
   a. Symposium topics are becoming more difficult to develop.
   b. Budgeting guidelines need to be clearly outlined.
   c. Should at least one person on the committee be from the hosting institution to aid with the preparation of the symposium?
   d. Should at least one industry representative serve on the committee to assist with topic development?

IV. Budget
   a. Income
      i. Registration
         1. Approximately 100 people @ $40.00/person (early registration) = $4,000.00
      ii. National Office
         1. $1,500.00
      iii. Total projected income = $5,500.00
   b. Expenses
      i. Conference room
         1. $1,000
      ii. Symposium breaks
         1. Two breaks @ $800.00/break = $1,600.00
      iii. Hotel for speakers
         1. $800 total
      iv. Travel for one speaker
         1. $1,000
      v. Total projected expenses = $4,200.00
   c. Total revenue = $1,100.00

Necrology Report
A necrology report was not available. Names of lost members over the last year were requested from the membership in attendance.

2006 Meeting Report
Ken Olson
Attendance was 160 registered participants; 98 abstracts were presented. Faculty and staff at Utah State University were thanked for their help in organizing the meeting.

Financial Report
Ken Olson

American Society of Animal Science Western Section
Financial Report as of December 31, 2005

Balance as of December 31, 2004		**44,692.37**
Revenue and Support		
Donations - General	3,000.00	
Donations - Awards	500.00	
Meeting Registrations	32,980.00	
Ticketed Events		
Proceedings	9,850.00	
ASAS-Symposium Support	1,500.00	
ASAS-Dues	1,142.50	
Interest Income	2,242.57	
Miscellaneous Income		
Total Revenue and Support		**51,215.07**
Expense		
Program	358.48	
Call for Papers/Abstracts	228.38	
Awards/Plaques	5,051.22	
Quadrathlon	5,850.10	
Convention Fees	13,767.82	
Proceedings	4,877.17	
Postage/Supplies	188.34	
Symposium Expense	3,611.87	
Travel-Speaker	874.72	
Travel	988.84	
Telephone	3.44	
Miscellaneous	927.00	
Staff Support	3,870.98	
Total Expenses		**40,598.36**
Net Revenue over Expense		**10,616.71**
Balance as of December 31, 2005		**55,309.08**

ASAS Reports

Dr. Dave Buchanan, President ASAS, and Dr. Meghan Wulster-Radcliffe, Executive Director ASAS, reported on the state of ASAS and FASS.

New Business
Jim Thompson passed the gavel to Tim Ross and Tim thanked Jim for serving as president and presented him with the past president's plaque. Doug Hixon suggested that department heads be asked to contact the leadership of local Block and Bridle Clubs to find better times to hold the Academic Quadrathlon.

Meeting was adjourned at 8:55 am.

The Western Section of the American Society of Animal Science gratefully acknowledges the financial contributions of the following organizations

Awards Donors

Young Scientist Award

Ridley Block Operations
Mankato, MN

Distinguished Teacher Award

Elanco Animal Health

Extension Award

Fort Dodge Animal Health

Distinguished Service

DSM Nutritional Products, Inc.

Proceeding, Western Section, American Society of Animal Science

Vol. 58, 2007

A META-ANALYSIS EVALUATION OF FEEDING MGA® TO FEEDLOT HEIFERS IMPLANTED WITH TBA

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Department of Animal Sciences, Colorado State University, Fort Collins 80521

ABSTRACT: A mixed models approach was used to study the use of MGA in the diet of feedlot heifers implanted with TBA. One hundred and one treatment means from 18 research trials were included in the analyses. Interactions between MGA and implant treatment were important ($P < 0.10$) for finished weight, average daily gain (ADG), feed efficiency (FG), hot carcass weight (HCW, $P < 0.12$), and ribeye area (REA), suggesting that the effect of MGA on these measurements depended upon how the heifers were implanted. For non-implanted heifers, MGA improved FG and increased finished weight, ADG, dry matter intake (DMI), HCW, yield grade (YG), and the percentage of YG 4 and 5 carcasses. Feeding MGA appeared less effective in heifers implanted with multiple implants, especially combinations of estrogen and TBA, compared with non-implanted heifers or heifers implanted with a single implant.

Keywords: MGA, Heifers, Implants, Trenbolone acetate, Meta-analysis

Introduction

Melengestrol acetate (MGA) is an estrus suppressant available for feeding to feedlot heifers which increases circulating estrogen concentrations (Henricks et al., 1997). A review of early studies examining the use of MGA in implanted heifers demonstrated no growth response but an increase in carcass rib fat depth and in the percentage yield grade 4 and 5 carcasses (Hutcheson et al., 1993). Furthermore, no interaction between implant type and MGA use was found, suggesting that heifers implanted with TBA or estradiol responded similarly to MGA. In a review of the effects of implants on performance and carcass traits in steers and heifers, Ducket et al. (1997) found positive performance responses for MGA and interactions between MGA feeding and implant type. These authors suggested that fed MGA replaced the benefit from including estrogen in the implant program.

The objective of this review was to use mixed models statistical procedures to conduct a meta-analysis of several research trials evaluating the use of MGA in the diet of feedlot heifers implanted with both TBA and estrogenic compounds.

Materials and Methods

An extensive review of the literature was completed to compile research reports and journal articles that compared diets with and without MGA for feedlot heifers. Only studies that included at least one TBA implant treatment were included in the evaluation. A mixed models approach as described by St.-Pierre (2001), using PROC MIXED in SAS (2003), was used to evaluate the data. The various initial and terminal implant strategies outlined in the research articles were categorized into seven implant treatment categories including: a no implant negative control (NI); an initial estrogen implant followed by another estrogen implant or no re-implant (EST); an initial estrogen implant followed by a TBA or estrogen plus TBA re-implant (EST-TBA); an initial[1] TBA implant followed by no re-implant (TBA); an initial TBA implant followed by a TBA re-implant (TBA-TBA); an initial estrogen and TBA implant followed by no re-implant (ET); and an estrogen and TBA initial implant followed by a TBA or estrogen plus TBA re-implant (ET-TBA).

Several different products, some no longer available for use, were used as the sources of estrogen and TBA for the various implant treatments. The estrogen implants included Synovex-C, Ralgro, Synovex-H, Heiferoid, Implus-H, and Compudose. TBA implants included Finaplix-H, Revalor-H, and Synovex-Plus. Implant treatment categories requiring simultaneous administration of estrogen and TBA resulted from the use of Revalor-H, Synovex-Plus, or the simultaneous administration of Finaplix-H and one of the estrogen implants.

Implant treatment category (IMP), MGA treatment, and study (STUDY) were considered in the analysis as class variables. Model fixed effects were IMP, MGA, and IMP*MGA. Days on feed for each observation were used as a covariate in the analysis. STUDY was included in the models as a random effect and the number of replicates per observation was selected as a weighting factor for the analysis. Treatment means were separated using the PDIFF option in the LSMEANS statement of PROC MIXED.

[1] One of the treatment groups in a single trial actually received a single TBA implant on d37 rather than d0. No other implants were administered to that treatment so it was categorized with the d0 TBA treatments.

Results and Discussion

One hundred and one treatment means from 18 research trials were included in the analyses (Table 1). Total number of heifers represented in the data set was 11,168. Heifers averaged 329 kg at the start of each trial and were harvested after an average of 130 days at a mean weight of 508 kg. Average HCW, 12th rib fat depth, USDA YG, and the percentage of carcasses grading USDA Choice or higher were 318 kg, 1.19 cm, 2.45 units, and 65.5%, respectively.

Least squares means showing the effect of implant treatment and MGA on growth performance and carcass merit are displayed in table 2. Implant treatment (IMP) had a significant ($P < 0.01$) impact on finished weight, ADG, FG, HCW, and fat depth at the 12th rib. The percentage of USDA Choice carcasses also tended ($P < 0.13$) to differ among implant treatments. In general, performance increases were greater with multiple implant use, especially for treatments involving combinations of estrogen and TBA. Feeding MGA improved ($P < 0.03$) FG, reduced REA, and increased finished weight, ADG, HCW, fat depth, YG, and the percentage of YG 4 and 5 carcasses ($P < 0.09$).

Interactions between MGA and IMP were important ($P < 0.10$) for finished weight, ADG, FG, HCW ($P < 0.12$), and REA, suggesting that the effect of MGA on these measurements depended upon IMP. For non-implanted heifers, MGA improved ($P < 0.0001$) FG and increased finished weight, ADG, DMI ($P = 0.0183$), HCW, YG ($P = 0.0615$), and the percentage of YG 4 and 5 carcasses ($P = 0.0444$). For heifers implanted with EST, MGA tended to improve FG ($P = 0.1421$). For heifers implanted with EST-TBA, MGA increased ($P < 0.07$) fat depth, YG, and the percentage of USDA Choice carcasses. Ribeye area was reduced ($P = 0.0004$). Feeding MGA improved ($P < 0.07$) ADG and FG in heifers implanted with a single TBA implant. The effects of MGA on performance and carcass merit were not statistically significant for heifers implanted with TBA-TBA. MGA tended to increase YG ($P = 0.1548$) and increased the percentage of YG 4 and 5 carcasses ($P = 0.0101$) for the ET implant treatment. For heifers implanted with ET-TBA, MGA tended to increase fat depth ($P = 0.1674$), reduce REA ($P = 0.0246$), increase YG ($P = 0.0988$), and tended to improve the percentage of USDA Choice carcasses ($P = 0.1320$).

The finding of an interaction between IMP and MGA is in contrast to the findings of previous studies summarized by Hutcheson et al. (1993) but in agreement with the review by Ducket et al. (1997). Both of these previous reviews appeared to include study in the model as a fixed rather than a random effect. Treating study effects as fixed generates considerable bias in the regression coefficients that are estimated due to the fact that observations within a given study have more in common than observations across studies and differences in the accuracy of measurements within and across studies are ignored (St-Pierre, 2001).

Implications

The results of this analysis suggests that the impact of MGA on performance is reduced in heifers implanted with multiple implants especially combinations of estrogen and TBA. However, use of MGA increased fat depth, reduced REA, and increased USDA yield grade. Feeding MGA appeared to increase the percentage Choice carcasses when multiple implants of estrogen and TBA are used. However, very few observations are included for the most aggressive implant strategy examined in this review.

Acknowledgements: The authors would like to thank Dr. Gary Sides of Pfizer Animal Health for his help in locating several of the references that the data were obtained from. In addition, the advice and counsel of Dr. Normand St-Pierre of the Ohio State University in setting up the statistical models is greatly appreciated.

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Table 1. Description of the data used in the implant program and MGA analyses.

Variable	N	Mean[a]	Std. dev.	Min.	Max.
Replicates	101	5.57	2.23	3	12
Days fed	101	130	18	105	172
Initial weight, kg	101	328	28	276	386
Finish weight, kg	101	508	26	458	558
Average daily gain, kg	101	1.39	0.13	1.02	1.72
Dry matter intake, kg	101	8.70	0.60	7.02	10.15
Gain/Feed	101	0.16	0.22	0.14	0.17
Hot carcass weight, kg	101	318	14	289	349
Fat depth, cm	82	1.19	0.20	0.76	1.91
Ribeye area, sq. cm	82	85.36	6.45	65.62	98.59
Yield grade, units	101	2.45	0.36	1.58	3.55
Percentage YG4&5	72	4.7	5.32	0	30
Percentage Choice & Prime	101	65.54	15.97	24.93	90
Percentage Standard	18	2.11	2.69	0	10.6
Percentage dark cutters	65	5.76	12.33	0	52.38

[a]Raw mean not weighted for the number of replicates.

Table 2. Least squared means showing the effect of implant treatment[a] and MGA on feedyard performance and carcass merit.

Item[b]	NI		EST		EST-TBA		TBA		TBA-TBA		ET		ET-TBA			Probability <		
MGA:	0	+	0	+	0	+	0	+	0	+	0	+	0	+	SEM[c]	Imp[d]	MGA	I*M[e]
N	11	12	5	3	8	14	2	13	2	4	12	10	2	3				
Inwt	325	325	324	324	323	323	325	324	325	324	325	324	328	325	5.99	0.95	0.49	0.99
Finwt	484	497	495	498	497	499	496	502	499	507.26	506	508	516	515	6.80	0.01	0.01	0.05
ADG	1.22	1.33	1.31	1.33	1.36	1.37	1.30	1.37	1.35	1.41	1.40	1.42	1.44	1.47	0.04	0.01	0.01	0.07
DMI	8.44	8.74	8.77	8.73	8.49	8.64	8.64	8.58	8.32	8.58	8.81	8.80	8.45	8.82	0.23	0.21	0.12	0.48
FE	0.14	0.15	0.15	0.15	0.16	0.16	0.15	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.18	0.01	0.02	0.01
HCW	305	313	313	315	315	316	313	316	314	318	318	321	326	325	4.41	0.01	0.03	0.12
FAT	1.17	1.24	1.19	1.32	0.99	1.17	1.19	1.24	1.09	1.22	1.17	1.24	0.89	1.22	0.10	0.56	0.02	0.86
REA	81.49	81.68	84.07	82.71	86.07	81.88	84.46	83.17	87.62	84.39	85.55	84.78	95.62	86.33	2.58	0.01	0.01	0.07
YG	2.47	2.66	2.43	2.69	2.25	2.54	2.39	2.53	2.22	2.46	2.34	2.48	2.05	2.45	0.15	0.21	0.01	0.91
YG4	3.19	7.67	3.33	8.65	2.79	4.49	1.09	5.69	4.23	2.39	2.05	7.50	1.09	4.23	3.24	0.95	0.09	0.90
CH	64.91	67.37	66.48	68.14	52.58	59.30	69.39	63.34	68.50	68.51	62.38	61.00	51.74	64.16	5.73	0.13	0.34	0.39

[a]NI = No implant negative control. EST = Estrogen initial implant followed by no implant or another estrogen implant. EST-TBA = Estrogen initial implant followed by a TBA or estrogen plus TBA re-implant. TBA = A single TBA implant. TBA-TBA = TBA initial implant followed by a TBA or an estrogen plus TBA re-implant. ET = Estrogen plus TBA initial implant. ET-TBA = Estrogen plus TBA initial implant followed by a TBA or an estrogen plus TBA re-implant.
[b]Inwt = Initial weight, kg. Finwt = Finished weight, kg. ADG = Average daily gain, kg. DMI = Daily dry matter intake, kg. Fe = Gain/Feed. HCW = Hot carcass weight, kg. FAT = Fat depth at 12th rib, cm. REA = Ribeye area, square cm. YG = Average yield grade, units. YG4 = Percentage USDA yield grade 4 carcasses. CH = Percentage USDA Choice carcasses.
[c]Average standard error for the least square means.
[d]Implant treatment by MGA interaction.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

REPRODUCTIVE PERFORMANCE IN EARLY POSTPARTUM RAMBOUILLET EWES AND PREPUBERTAL EWE LAMBS TREATED WITH INTRAVAGINAL PROGESTERONE

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ABSTRACT: Two experiments were conducted to examine effects of a controlled internal drug-releasing (CIDR; 0.3 g progesterone, P4) device on return to estrus in early postpartum ewes and onset of puberty in ewe lambs. In Exp. 1, 18 Rambouillet ewes (72.4 ± 3.9 kg) were stratified by age and number of lambs and randomly assigned to 1 of 3 treatments (n = 6/group) on d 10 (d 0 = parturition). Treatments were no CIDR (control), CIDR for 5 d (d 10 to 15), and CIDR for 12 d (d 10 to 22). Blood samples were collected daily from d 10 to 60 after lambing and BW of ewes and lambs were recorded every 10 d through weaning (d 60). Before CIDR insertion on d 10, serum P4 was less than 1 ng/mL in all ewes. On d 11, serum P4 was 0.3, 2.3, and 2.8 (± 0.2) ng/mL in control ewes and those in the 5- and 10-d CIDR groups, respectively ($P < 0.01$). Serum P4 declined to control values 1 d after CIDR removal in both CIDR-treated groups. Ewe BW did not differ among treatments ($P > 0.66$) and ewes remained anestrus after CIDR removal (based on serum P4 concentrations). On d 20, lambs born to ewes in the 5-d CIDR group (11.4 ± 0.3 kg) tended to weigh less ($P = 0.07$) than lambs born to control ewes (12.4 ± 0.3 kg) or ewes receiving CIDR for 12 d (12.0 ± 0.3 kg). Lambs born to control ewes (16.3 ± 0.5 kg) tended to weigh more ($P = 0.07$) on d 30 and were heavier ($P = 0.02$) on d 60 (26.7 ± 0.7 kg) than lambs born to ewes in the 5-d (14.6 ± 0.5 kg d 30, 23.8 ± 0.7 kg d 60) or 12-d (15.0 ± 0.5 kg d 30, 24.0 ± 0.7 kg d 60) CIDR groups. In Exp. 2, 20 prepubertal spring-born Rambouillet ewe lambs (193 ± 1.6 d of age; 38.8 ± 1.0 kg) were stratified by BW and type of birth and treated with either no CIDR (control, n = 12) or CIDR (n = 8) for 7 d. Serum samples were collected daily for 66 d and BW were recorded every 20 d. Puberty was determined by serum P4 values greater than 1 ng/mL for 3 or more days. Serum P4 was elevated ($P < 0.01$) in CIDR-treated ewe lambs compared with controls on all days when the CIDR was in place (P4 ranged from 2.9 to 5.2 ng/mL in CIDR-treated ewes and were 0.3 ng/mL or less in controls). One of 8 (12.5%) CIDR-treated ewe lambs exhibited puberty after treatment whereas 4 of 12 (33%) control ewe lambs exhibited puberty. Treatment with a CIDR elevated serum P4 concentrations in both postpartum and prepubertal ewes and resulted in decreased lamb weights, but failed to induce estrus in early postpartum ewes or in prepubertal ewe lambs.

Keywords: sheep, puberty, CIDR

INTRODUCTION

Controlled internal drug-releasing (**CIDR**) devices containing progesterone (**P4**) have the potential to become convenient tools for sheep producers and researchers. The efficacy of CIDR for synchronizing estrus in ewes during a fall breeding season has been demonstrated (Dixon et al., 2006). Likewise, their ability to influence reproduction during the non-breeding season has been examined (Daniel et al., 2001; Knights et al., 2001b). However, effects of CIDR application during the early postpartum period in lactating ewes have not been extensively examined.

Because of their short gestation length, sheep could potentially produce 2 lamb crops a year if fertile estrus could be induced shortly after parturition. Hoefler and Hallford (1987) demonstrated that serum P4 concentrations were low in early postpartum, spring-lambing Debouillet ewes and that weaning spring-born lambs 2 d after birth did not allow onset of postpartum estrus in dams. Fertile estrus can be induced in seasonally anestrous ewes through CIDR use and is comparable to other P4 delivery systems (Daniel et al., 2001; Iida et al., 2003). Knights et al. (2001a) demonstrated that CIDR insertion for 5 d was as effective as insertion for 12 d in producing a fertile estrus. Lambing can be accelerated and the breeding season advanced through use of a CIDR and the ram effect (Wheaton et al., 1992) but the advancement is not enough to produce 2 lamb crops in a single year. To achieve 2 lamb crops a year, ewes must be bred within 30 d after lambing.

Improved reproduction can be accomplished by decreasing age at puberty. Ryan et al. (1991) observed a short rise in P4 a few days before puberty in ewe lambs, the source of which appears to be luteal-like tissue on the ovary of pubertal ewe lambs (Berardinelli et al., 1980). Robinson (1954) demonstrated that P4 priming is necessary for a pubertal ewe to display estrus. These previous studies suggest that a preceding rise in P4 is necessary for ewe lambs to become fully pubertal. Therefore, the objectives of this study were to evaluate the ability of exogenous intravaginal progesterone to induce cyclicity in early postpartum ewes and prepubertal ewe lambs.

MATERIALS AND METHODS

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

Experiment 1

Animals and Treatments. Eighteen spring-lambing Rambouillet ewes (72.4 ± 3.9 kg BW) were used to examine the effectiveness of intravaginal P4 on return to estrus. Ewes were maintained in a single pen (8 x 18 m) with ad libitum access to salt, water, and shade. Alfalfa hay was fed at 2.7 kg·ewe^{-1}·d^{-1} and cracked corn was fed at 0.45 kg·ewe^{-1}·d^{-1}. Ten days after lambing (d 0 = parturition), ewes were stratified by age (yearling or mature) and number of lambs (single or twins) and randomly assigned to 1 of 3 treatments (n = 6/treatment): control, CIDR for 5 d, or CIDR for 12 d. Treated ewes had CIDR (0.3 g P4, Pharmacia and Upjohn LTD. Co., Auckland, NZ) inserted according to package instructions on d 10 for either 5 d (CIDR removed on d 15) or 12 d (CIDR removed on d 22). Body weight measurements were taken every 10 d after lambing for 60 d. Ewes were checked 4 times daily for CIDR loss.

Blood Collection and Analysis. Beginning on d 10 and continuing through d 60, blood was collected daily from ewes 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 P4 was quantified by RIA (Schneider and Hallford, 1996) using components of a commercial kit (Diagnostic Products Corp., Los Angeles, CA). Within and between assay CV were less than 15%. Return to estrus was determined by serum P4 concentrations greater than 1 ng/mL for at least 3 consecutive days.

Lamb Management. At parturition, ewes were separated from the main herd and lambs were weighed and tagged. One day after birth, lambs received (i. m.) 1 mg of Se and 68 USP of vitamin E (BO-SE, Schering-Plough Animal Health, Union, NJ) and tails were docked. On d 10, lambs were given access to creep feed including ad libitum alfalfa hay and limited amounts of cracked corn. On d 30, lambs were immunized against *Clostridium perfringens* type C and D and *Clostridium tetani* (Bar Vac CD/T, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) and male lambs were castrated with elastrator bands. The amount of cracked corn was increased to 0.25 kg·lamb^{-1}·d^{-1} after immunization. Lamb BW was recorded at 10, 20, 30, and 60 d of age. All lambs were weaned on d 60.

Statistical Analysis. Effects of CIDR on serum P4 during the treatment period were evaluated using split-plot analysis of variance. Treatment, day, and interaction effects were included in the model and were analyzed using the mixed procedure of SAS (SAS Inst. Inc, Cary, NC). Ewe and lamb BW at each day were evaluated using ANOVA for a completely random design and analyses were computed using the GLM procedure of SAS. When significant treatment effects were detected, means were separated using PDIFF of SAS.

Experiment 2

Twenty prepubertal spring-born Rambouillet ewe lambs (193 ± 1.6 d of age; 38.8 ± 1.0 kg) were maintained in a single pen (4 x 18 m) with other replacement ewe lambs and given ad libitum access to salt, water, and shade. Ewe lambs were fed alfalfa hay at 1.8 kg·ewe^{-1}·d^{-1}. Beginning on d 0 (approximately 6.5 mo of age), ewe lambs were stratified by BW and type of birth and received either no CIDR (control, n = 12) or CIDR treatment for 7 d (n = 8) as described in Exp. 1. Body weights were recorded every 20 d. Blood was collected daily for 20 d and then 3 times weekly thereafter to measure serum P4. Blood was collected, handled, and assayed for P4 as described for Exp. 1 (assay CV < 15%). Puberty was determined by serum P4 concentrations greater than 1 ng/mL for 3 or more days. Serum P4 profiles during CIDR treatment and ewe lamb BW were analyzed statistically as described previously for Exp. 1.

RESULTS AND DISCUSSION

Experiment 1

Before CIDR insertion, serum P4 concentrations in all ewes were below 1 ng/mL. Serum P4 concentrations rose rapidly after CIDR insertion and remained elevated ($P < 0.01$) until CIDR removal after which serum P4 returned to control values within 1 d of removal in both CIDR-treated groups (Figure 1). Because serum samples were collected daily, exogenous P4 clearance rate was not established for this study, but Gifford et al. (2003) showed that serum P4 concentrations declined to basal levels within 1 h of CIDR removal in ovariectomized ewes. Ewe BW did not differ among treatments ($P > 0.66$) and were 72.5, 72.6, and 70.6 ± 2.0 kg on d 30 for control ewes and those receiving CIDR for 5 and 12 d, respectively. After CIDR removal, no change ($P > 0.10$) in serum P4 concentration was observed among treatments and concentrations remained at control values, suggesting that ewes did not begin cycling after CIDR removal. This failure to cycle could have been due to lactation, which has been shown to extend the postpartum interval (Edgerton, 1980). However, Pope et al. (1989) showed that lactation did not inhibit rebreeding in fall-lambing ewes, suggesting that season is more important to sheep cyclicity. Hoefler and Hallford (1987) weaned spring-born lambs at 2 d of age and observed no onset of cyclicity in the dams within 30 d after parturition.

Before CIDR treatment of ewes on d 10, lambs weighed 5.4 ± 0.2 kg and lamb BW was similar ($P = 0.34$) among the 3 groups of dams. On d 20, however, lambs produced by ewes in the 5-d CIDR group (11.4 ± 0.3 kg) tended ($P = 0.07$) to weigh less than lambs born to control ewes (12.4 ± 0.3 kg) or ewes treated with CIDR for 12 d (12.0 ± 0.3 kg). At 30 d of age, lambs born to control dams (16.3 ± 0.5 kg) tended ($P = 0.08$) to weigh more than offspring of ewes treated with CIDR for 5 (14.6 ± 0.5 kg) or 12 d (15.0 ± 0.5 kg). Likewise, at weaning (60 d of age), control offspring (26.7 ± 0.7 kg) were heavier ($P = 0.02$) than those from ewes in the 5- (23.8 ± 0.7 kg) or 12-d (24.0 ± 0.7 kg) CIDR groups. Decreased lamb weights may be related to decreased milk production by treated ewes. In an earlier experiment conducted in our laboratory, progestogen-impregnated ear implants decreased milk

production in early postpartum ewes (Miller et al., 1996). Endogenous progesterone inhibits initial lactogenesis in dairy cows but is not generally considered to affect lactation once established (Tucker, 2000). Our data and those of Miller et al. (1996) suggest that exogenous P4 may alter milk production in early postpartum Rambouillet ewes.

Experiment 2

Serum P4 concentrations were similar ($P = 0.33$) between control and CIDR-treated prepubertal ewe lambs and were less than 1 ng/mL before CIDR insertion. Beginning on the day after CIDR insertion, CIDR-treated ewe lambs had elevated serum P4 concentrations ($P < 0.01$) throughout treatment compared with controls. Serum P4 concentrations during CIDR placement are shown in Figure 2. After CIDR removal, serum P4 concentrations declined to control values within 1 d. Of control ewe lambs, 4 exhibited puberty during the experiment. Of the CIDR-treated ewe lambs, only 1 exhibited puberty after treatment. Onset of puberty in ewe lambs is regulated by an increased tonic secretion of LH pulses in response to decreased negative regulation of the hypothalamus by estradiol (Kinder et al., 1995). Previous research has shown that P4 administration can induce puberty in heifers (Gonzalez-Padilla et al., 1975) and Anderson et al. (1996) suggested that administration of progestogen induced puberty by decreasing the negative feedback exerted by estradiol on LH secretion. However, Foster and Karsch (1976) showed that exogenous P4 suppressed LH secretion in prepubertal ewe lambs, which supports our findings. Administration of P4 in cycling ewes can block the LH surge even after a surge-generating system has been activated (Harris et al., 1999); therefore, P4-impregnated CIDR could have inhibited onset of puberty in our study by blocking the required LH surge. Additional studies are needed to examine effects of age at CIDR insertion on puberty in ewe lambs.

IMPLICATIONS

Intravaginal progesterone failed to induce cyclicity in early postpartum, seasonally anestrous ewes and may result in decreased offspring weights. Progesterone treatment for 7 days failed to induce estrus in prepubertal ewe lambs.

ACKNOWLEDGEMENTS

Research was supported by the New Mexico Agricultural Experiment Station Department of Animal and Range Sciences.

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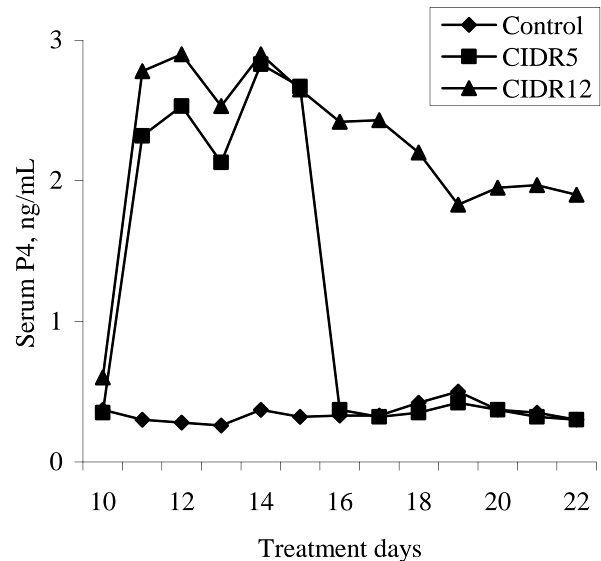

Figure 1. Serum progesterone (P4) in Rambouillet ewes (n = 6 ewes/treatment) receiving intravaginal progesterone (CIDR) for 5 (CIDR5) or 12 (CIDR12) d beginning 10 d after parturition. During the time when CIDR were in place (d 10 to 15 in CIDR5 and d 10 to 22 in CIDR12), serum P4 in CIDR-treated ewe lambs was greater ($P < 0.01$) than in controls. Serum P4 declined rapidly to control values after CIDR removal. The SE ranged from 0.1 to 0.3 ng/mL.

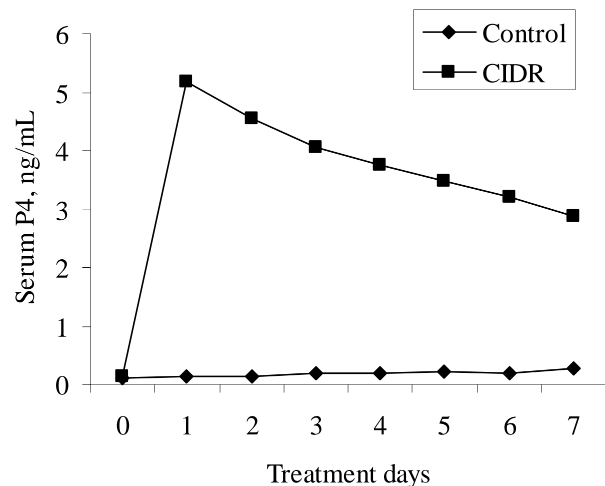

Figure 2. Serum progesterone (P4) in prepubertal Rambouillet ewe lambs receiving intravaginal progesterone (CIDR) for 7 d. Ewe lambs were 193 ± 1.6 d of age and weighed 38.8 ± 1.0 kg when treatment began (12 control and 8 CIDR-treated ewe lambs). During the time when CIDR were in place (d 0 to 7), serum P4 in CIDR-treated ewe lambs was greater ($P < 0.01$) than in controls. Serum P4 declined rapidly to control values after CIDR removal. The SE ranged from 0.03 to 0.24 ng/mL.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

HETEROGENEOUS VARIANCE OF DOCILITY SCORES IN LIMOUSIN CATTLE

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ABSTRACT: Analyses of docility in Limousin cattle have shown models including maternal or sire by herd interactions as random effects fit significantly better than a model limited to direct genetic and residual random effects. Variance of docility scores between herds is not homogeneous due to the subjective nature of scoring, with some breeders avoiding use of undesirable scores. Most sires have very few progeny and are represented in only a single herd, whereas a very small proportion of sires are widely used across herds. Sire effects that contribute to the estimation of variance components may therefore exhibit heterogeneity, biasing the apparent fit of models that assume homogeneity. The objective of this study was to determine whether maternal or sire by herd interaction effects are appropriate, or an artifact of the nature of this data. Heterogeneous variance was examined in a two-step process. First, absolute estimated residuals were obtained from a model with direct genetic, maternal genetic, sire by herd interaction and residual random effects. Second, these were analyzed in a fixed effects model using SAS. Results indicated sire by herd (P = 0.027) was significant. However, sums of squares for herd effects (F = 4.62) was nearly twice that of sire effects (F = 2.68), and over four times that of the sire by herd interaction (F = 1.09), implying herd effects account for most of the heterogeneity observed in docility scores. Absolute residuals were further analyzed in a random effects model using ASReml. As expected, there was no genetic variation in direct or maternal genetic effects. However, the proportion of phenotypic variance accounted for by the interaction between sire and herd was 0.02 ± 0.01. These results imply significance of maternal and sire by herd interaction effects inferred by previous research is, in fact, an artifact of the data. Heterogeneous variance due to herd effects is likely a result of the subjective method used to allocate docility scores.

Key Words: beef cattle, heterogeneous variance, temperament

Introduction

Recent work examining maternal effects on docility in Limousin cattle (Beckman et al., 2007) showed a model containing maternal effects fit significantly better than a reduced model with direct genetic and residual random effects. A negative direct-maternal correlation estimate of -0.55 ± 0.09 suggested sires' with genes that result in docile daughters will tend to produce grand progeny with unfavorable temperaments.

Negative estimates of direct-maternal correlations observed in weaning weight in beef cattle have been attributed to sire by herd (**SH**) (Notter et al., 1992) or sire by year (**SY**) (Robinson, 1996; Lee and Pollak, 1997) interactions. Using simulated weaning weight data, Robinson (1996) found SY effects explained 6% of phenotypic variation, and produced negative direct-maternal correlation estimates of approximately -0.5 when ignored. Lee and Pollak (1997) reported SY interaction represented only 3% of phenotypic variance but explained 62% of the covariance between direct and maternal genetic effects in weaning weight of Simmental cattle.

Similar to Notter et al. (1992), additional analysis of docility was conducted to assess the strong negative relationship between direct and maternal genetic effects reported by Beckman et al. (2007). Models incorporating SH interaction as a random effect revealed the interaction was a significant source of variation (unpublished data).

Models including maternal or SH interaction effects used to analyze docility assumed homogeneous variance of residuals, which may be inappropriate considering a skewed distribution of scores within the data (Beckman et al., 2007).

The objective of this study was to determine whether maternal or sire by herd interaction effects appropriately describe docility in Limousin cattle, or whether these effects are artifacts of the nature of this data.

Materials and Methods

Animal Care

Data for this study were obtained from an existing historical database (North American Limousin Foundation; **NALF**) and were not subject to Animal Care and Use Committee approval.

Description of Data

Docility scores, pedigrees, and other relevant performance information were obtained from NALF. Producers were assumed to have used NALF guidelines for determining temperament (NALF, 2004) and allocating docility scores at weaning while calves were restrained in a chute. Individuals with scores of 1 or 2 were considered docile or mildly restless and were handled with little trouble, encompassing the most desirable behavior. A score of 3 was assigned if the animal was nervous, impatient, or exhibited a moderate amount of struggle. Animals scored 4, 5, or 6 were very

nervous and difficult to handle, possibly exhibiting attack behavior when handled individually.

Uninformative data were eliminated based on several criteria. Weaning contemporary groups (**WCG**) with less than 10 observations, single-sire WCG, and WCG with no variation in docility scores were removed from the analyses. Individuals were classified as having age of dam (**AOD**) 2, or >2. Animals with unknown AOD were removed. Detailed characteristics of fixed effects are described by Beckman et al. (2007).

Variation in the relative performance of sires across herds contributes to SH interaction. Previous analyses of docility in Limousin cattle indicated models including maternal or SH interactions as random effects fit significantly better than a basic model (i.e., with direct genetic and residual random effects). Further investigation of the data revealed approximately 83% of sires had progeny in only one herd. These progeny accounted for 63% of all docility observations. Additional data edits based on ≥5 offspring per sire and sire parentage of contemporaries (similar to Notter et al., 1992), were employed to obtain a balanced representation of SH interaction effects. Final data contained 7,670 animals with docility observations in 893 contemporary groups.

A 2-generation pedigree was compiled for animals with docility observations and contained 19,672 animals. An average of 43 animals per herd, 29 offspring per sire, and 1.4 offspring per dam were represented. Five percent (416) of the 7,670 individuals with docility scores went on to become dams with docility scores on their offspring. Table 1 summarizes the number of docility observations in the final data file and corresponding pedigree file.

Table 1. Summary of docility observations[1], means, levels of fixed effects[2], and pedigree information

Data File	Count/Value
Observations	7,670
Mean Docility Score	1.97
SD	0.83
Sires	263
Dams	5,546
Herds	186
WCG[2]	893
AOD[2]	2
Pedigree File	**Count/Value**
Sires	2,146
Dams	10,292
Total	19,672

[1]Means and SD of raw docility scores.
[2]Fixed effects included weaning contemporary group (WCG) and age of dam (AOD).

Assessment of Heterogeneous Variance

Heterogeneous variance was examined in a two-step process. First, residuals were estimated with a mixed model using ASReml (Gilmour et al., 2002). The first analysis (**BASE**) served as the comparison for additional analyses. Second, absolute values of estimated residuals were analyzed in a fixed effects model using SAS (SAS Inst., Inc., Cary, NC).

Mixed Model. Direct genetic, maternal genetic, and SH interaction were random factors in the mixed model

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_D\mathbf{u}_D + \mathbf{Z}_M\mathbf{u}_M + \mathbf{Z}_{SH}\mathbf{u}_{SH} + \mathbf{e}. \qquad [1]$$

Observed docility scores were contained within $\mathbf{y}$; known incidence matrix $\mathbf{X}$ related fixed effects in $\mathbf{b}$ (WCG, AOD) to observations in $\mathbf{y}$; $\mathbf{Z}_D$, $\mathbf{Z}_M$, and $\mathbf{Z}_{SH}$ were known incidence matrices for random effects $\mathbf{u}_D$, $\mathbf{u}_M$, and $\mathbf{u}_{SH}$ (direct, maternal, and SH interaction effects, respectively). Random residual effects were $\mathbf{e}$.

The (co)variance structure of random effects was

$$V\begin{bmatrix}\mathbf{u}_D\\ \mathbf{u}_M\\ \mathbf{u}_{SH}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{A}\sigma_D^2 & \mathbf{A}\sigma_{DM} & 0 & 0\\ \mathbf{A}\sigma_{DM} & \mathbf{A}\sigma_M^2 & 0 & 0\\ 0 & 0 & \mathbf{I}_{SH}\sigma_{SH}^2 & 0\\ 0 & 0 & 0 & \mathbf{I}_N\sigma_e^2\end{bmatrix}.$$

Wright's numerator relationship matrix was represented by $\mathbf{A}$; $\mathbf{I}_{SH}$, and $\mathbf{I}_N$ were identity matrices of order equal to number of SH subclasses and total number of docility observations, respectively. Additive direct genetic variance, additive maternal genetic variance, and variance due to SH interaction effects were σ^2_D, σ^2_M, and σ^2_{SH}, respectively. Direct-maternal genetic covariance was σ_{DM}. Remaining environmental (residual) variance was σ^2_e.

Fixed Effects Model. Absolute values of estimated residuals from the BASE analysis substituted for docility scores to characterize heterogeneous variance. Sire, herd, and SH interaction components formed the fixed effects model

$$|\hat{\mathbf{e}}| = \mathbf{X}^*\boldsymbol{\beta} + \boldsymbol{\varepsilon}. \qquad [2]$$

Absolute values of estimated residuals were $|\hat{\mathbf{e}}|$; known incidence matrix $\mathbf{X}^*$ related fixed effects in $\boldsymbol{\beta}$ (sire, herd, SH interaction) to values in $|\hat{\mathbf{e}}|$. Remaining residual variance was $\boldsymbol{\varepsilon}$. Ratio of mean squares for sire, herd, and SH interaction relative to mean square error were used to identify sources of heterogeneous variance. Significant effects were detected with significance level set at $P < 0.05$.

Variance Component Estimation With |ê|

Further analysis of *|ê|* with mixed model [1] (**RESID**) was used to determine whether *|ê|* effectively accounted for heterogeneous variance. Variance components were estimated using ASReml which fits linear mixed models using Residual Maximum Likelihood (**REML**). Convergence was presumed when the REML log-likelihood changed less than 0.002 in successive iterations and individual variance parameter estimates changed less than 1% (Gilmour et al., 2002).

Estimates were used to calculate heritabilities for direct and maternal genetic effects, and the correlation between direct and maternal effects. A variance component ratio was used to calculate SH interaction as a proportion of phenotypic variance (i.e., SH^2).

Results and Discussion

Fixed Effects Model

Average magnitude of residuals is expected to equal 0 (i.e., homogeneous residual variance) when fixed effects models utilize |**ê**| in place of observed data (i.e., **y**). Fixed effects model [2] results (Table 2) revealed SH interaction ($P = 0.027$) as a significant source of variation, indicating |**ê**| did not account for all heterogeneous variance in docility.

Sums of squares (Table 2) for herd effects ($F = 4.62$) was nearly twice that of sire effects ($F = 2.68$), and over four times that of SH interaction ($F = 1.09$). Herd effects accounted for most of the heterogeneity observed in docility scores. These results may reflect different scales of scoring being implemented across herds.

Table 2. Ratio of mean squares for fixed effects model analysis of *|ê|*[1] using SAS

Source[2]	Source df	Mean Square	*F*-value[3]	*P*-value[4]
Sire	262	0.251	2.68	< 0.0001
Herd	185	0.433	4.62	< 0.0001
SH	1160	0.102	1.09	0.027

[1]Absolute values of estimated residuals.
[2]Sire, herd, and sire by herd (SH) interaction.
[3]Mean square relative to mean square error (0.094).
[4]Level of significance set at $P < 0.05$.

Variance Components Estimated With |ê|

Results from RESID analysis indicated there was no variation in direct or maternal genetic effects (Table 3). Parameter estimates in Table 3 illustrated analysis of mixed model [1] using *|ê|* (RESID) in place of **y** (BASE) reduced but did not eliminate heterogeneity, as the proportion of phenotypic variance accounted for by SH interaction was 0.02 ± 0.01.

Similarly, power transformations utilized by Garrick et al. (1989) to generate homogeneous genetic and residual variance reduced, but did not completely remove heterogeneous variance in birth weight and weaning weight in Simmental cattle.

Notter et al. (1992) standardized data, accounted for sire relationships, and included dam effects when analyzing weaning weight in Australian Angus to remove heterogeneous herd variance. Significant ($P < .05$) SH interactions were reported in all cases, including the most complete model (i.e., with direct, maternal, and SH interaction as random effects). The interaction accounted for 3.3% of phenotypic variance. Estimates of SH interaction (Notter et al., 1992) were attributed not only to genotype by environment interaction, but also to common unreported environmental effects.

Although a moderate direct heritability estimate (0.34 ± 0.01) reported by Beckman et al. (2007) indicated selection of cattle with favorable docility scores would be effective in producing cattle with desirable temperaments, not accounting for heterogeneous variance associated with herd effects may greatly reduce selection efficiency.

Genetic evaluations that assume homogeneous variances reduce selection efficiency when heterogeneity exists and is not accounted for (Garrick and Van Vleck, 1987). Ideally, each sire would have progeny with docility scores evenly distributed in each herd. However, the majority of Limousin sires have progeny in only one herd (approximately 83%). Evaluation of docility assuming homogeneous variances may result in selection bias associated with these sires if their progeny are within more variable herds.

Implications

Significance of maternal and SH interaction effects inferred by previous research was determined to be an artifact of this data. Most of the heterogeneous variance observed in docility was due to herd effects, resulting from the subjective method breeders use to allocate scores. Breeders are not obligated to report all performance information on each individual within the herd. Consequently, animals' allocated scores for unacceptable temperament are typically not registered.

Further investigation of heterogeneous variance resulting from herd effects is necessary to ensure genetic evaluation of docility accurately describes the underlying nature of the trait.

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Table 3. Estimates of mixed model[1] parameters and phenotypic variance for docility in Limousin cattle using ASReml

		Parameters[2]			
Analysis[3]	σ^2_{PHEN}[4]	h^2_D	h^2_M	r_{DM}	SH^2
BASE	0.088 ± 0.002	0.18 ± 0.05	0.03 ± 0.02	0.13 ± 0.36	0.04 ± 0.01
RESID	0.459 ± 0.009	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01

[1]Direct genetic, maternal genetic, sire by herd interaction, and residual random parameters.
[2]h^2_D = direct heritability; h^2_M = maternal heritability; r_{DM} = direct-maternal correlation; SH^2 = sire by herd interaction as a proportion of phenotypic variance.
[3]BASE = original analysis used to obtain absolute estimated residuals; RESID = absolute values of estimated residuals used as data.
[4]σ^2_{PHEN} = phenotypic variance.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECTS OF MELENGESTROL ACETATE AND PG600 ON FERTILITY IN RAMBOUILLET EWES OUTSIDE THE NORMAL BREEDING SEASON

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ABSTRACT: The effects of melengestrol acetate (MGA) and PG-600 on ewe fertility outside the normal breeding season were evaluated. In April, Rambouillet ewes at the Hettinger Research Extension Center (46˚N) were assigned to one of four groups: 1) control (C, n=98); 2) PG600 (n=98); 3) MGA (n=100); 4) MGA+PG600 (n=100). A commercially prepared pellet with or without MGA was fed at 0.15 kg (0.3 mg of MGA) ewe^{-1} d^{-1} for 7 d. On the last d of pellet feeding ewes received a 5-mL injection of PG600 (400 IU PMSG and 200 IU hCG) or saline. Thereafter, ewes were exposed to intact rams for a 31-d breeding period (1 ram:15 ewes). Transrectal ultrasonography was performed between d 20 and 25 of gestation for ewes marked during the first 6 d of the breeding period, and the numbers of corpora lutea and embryos were counted. During the first 6 d of the breeding period, MGA increased ($P < 0.10$) the percentage of ewes mated and conceived when compared to C and PG600. Relative to MGA, ovulation rate was enhanced ($P < 0.03$) in MGA+PG600 (1.53 +/- 0.13 vs. 2.38 +/- 0.42 corpora lutea, respectively), however as gestation progressed the number of embryos (1.5 +/- 0.13 vs. 1.8 +/- 0.16, respectively) and lambs born (1.3 +/-0.15 vs. 1.5 +/- 0.27, respectively) remained similar. During the entire 31 d breeding period, all groups achieved high rates of pregnancy (77 to 80%). MGA treatment reduced ($P < 0.01$) the interval from ram introduction to lambing when compared to groups that did not receive MGA (168 +/- 0.8 vs. 171 +/- 0.6 d, respectively). The total number of ewes conceiving, lambing rate, and mean lamb birth weight were not affected by treatment. In conclusion, MGA was a useful tool for shortening length of the lambing season, and although PG600 enhanced ovulation rate, it had no beneficial effects on ewe productivity.

Key Words: Melengestrol Acetate, PG-600, Embryo Survival

Introduction

As seasonal breeders, sheep naturally come into estrus as day length decreases in the fall. This creates a seasonal supply of fresh lamb to marketing organizations and consumers alike. Synchronization of breeding activity and the ability to breed ewes outside the normal breeding season would allow production of a more consistent supply of fresh lamb throughout the entire year.

The ram-effect, which involves spontaneous introduction of rams to a group of anestrous ewes, has long been known to elicit estrous activity and result in fertile matings (Underwood et al., 1944). Although adequate lambing rates can be achieved (75-90%) in certain breeds, the immediate efficacy of the ram-effect is low and a longer breeding period is generally needed.

As a result, the introduction of rams is often used in combination with progestogen pre-treatments. An intravaginal progesterone treatment for 5- to 12-d will stimulate estrus at the first ram-induced ovulation(s), resulting in an increased opportunity for fertile matings during the first 5 d of the breeding period (Dutt, 1953; Knights et al., 2001). This combination has also been reported to decrease the interval from ram introduction to lambing (Carlson et al., 1989; Knights et al., 2001).

Unfortunately, intravaginal progestogen inserts are commercially unavailable in the United States. This has led to investigation of commercially available, orally active, melengestrol acetate (MGA) for induction of estrus and fertile mating in ewes outside the normal breeding season. The effects of MGA pre-treatment on ewe fertility are highly variable; the percentage of ewes lambing after treatment with MGA has ranged from 25 to 85% (Safranski et al., 1992; Jabbar et al., 1994; Morrical et al., 1995; Powell et al., 1996; Daniel et al., 2001), and appears to be dependent upon ewe breed (Safranski et al., 1992), length of MGA treatment (Powell et al., 1996), or use in combination with gonadotropins (Morrical et al., 1995; Powell et al., 1996).

Commercially available PG-600, which contains 400 IU of eCG and 200 IU of hCG per 5 ml dose, has been used in combination with MGA pre-treatments to enhance fertility in ewes. Morrical et al. (1995) reported a 20% increase in conception rate when a single 5-mL dose of PG600 was injected at the end of a 10-d MGA treatment. In contrast, Safranski et al. (1992) reported no beneficial effects of a 5-mL PG600 dose on ewe fertility when used in combination with a 10-d MGA treatment.

The objectives of the current study were to: 1) evaluate the effects of MGA and / or PG-600 on ewe fertility outside the normal breeding season; and 2) determine the impacts of PG600 on ovulation rate and embryo survival in MGA-treated ewes.

Materials and Methods

In April, nonlactating, multiparous Rambouillet ewes at the Hettinger Research Extension Center (46˚N) were assigned to one of four groups: 1) Control (C, n=98); 2)

PG600 (n=98); 3) MGA (n=100); 4) MGA+PG600 (n=100). A commercially prepared pellet with or without melengestrol acetate (MGA) was fed at 0.15 kg (0.3 mg of MGA) ewe^{-1} d^{-1} for 7 d. Linear feed-trough space was at least 25 cm/ewe to ensure each ewe had access to the appropriate amount of MGA. On the last d of pellet feeding ewes received a 5-mL i.m. injection of PG600 (containing 400 IU of equine chorionic gonadotropin and 200 IU of human chorionic gonadotropin) or saline. Thereafter, ewes were exposed to intact rams fitted with a marking harness for a 31-d breeding period (1 ram:15 ewes). All rams passed a standard breeding soundness examination as previously described by Kimberling and Marsh (1991). Transrectal ultrasonography was performed (using an Aloka 500 with a 7.5 MHz probe attached) between d 20 and 25 of gestation for MGA and MGA+PG600 ewes marked during the first 6 d of the breeding period, and the number of corpora lutea and embryos were counted as previously described by Schrick and Inskeep (1993). Corpora lutea and embryos were counted to determine ovulation rate and indicate the incidence of fertilization failure or embryonic loss. In May of 2006, a group of ewes crowded into the corner of a dry-lot during extreme weather conditions, and unexpected death losses from trampling totaled 27 ewes. The number of ewes per treatment group after death losses was reduced to: C, n=92; PG600, n=86; MGA, n=99; MGA+PG600, n=92. Lamb birth weight and the number of lambs born per ewe was determined at term.

Data regarding the number of ewes in a synchronized estrus and lambing in the initial 6-d, and entire breeding period, were analyzed by the chi-square procedure of SAS. Data regarding the interval from ram introduction to lambing, lamb birth weight, and the no. of ovulations, embryos, and lambs born per ewe were analyzed using the general linear model procedure of SAS and presented as means ± SEM. When the F test was significant ($P < 0.10$), differences between specific means were evaluated by using the least significant differences test.

Results and Discussion

Ewe fertility and lamb birth weight in C, PG600, MGA and MGA+PG600 groups are presented in Table 1. During the first 6-d of the breeding period the overall percentage of ewes mated was low (13%). However, the percentage of ewes mated was increased ($P < 0.01$) in PG600, MGA, and MGA+PG600 groups when compared to the C group. The increased proportion of ewes exhibiting an estrous response to MGA was anticipated based on previous studies (Safranski et al., 1992; Jabbar et al., 1994; Powell et al., 1996), although the increase reported by Safranski et al. (1992) was of a much greater magnitude (45% increase) when compared to the current study (20% increase). The lower ($P < 0.01$) percentage of ewes exhibiting an estrous response in the MGA+PG600 versus MGA only groups was not expected. The dose of PG600 given on the last day of MGA feeding contained 200 IU of hCG and may have caused ovulation or luteinization of large hCG responsive follicles, thereby decreasing the estrous response. Safranski et al. (1992) fed MGA twice per d (morning and evening) for 10-d, and observed a similar estrous response in MGA versus MGA+PG600 ewes.

A greater ($P < 0.10$) proportion of MGA only ewes lambed to a synchronized estrus when compared to the PG600 and Control ewes (18% vs. 5 and 2%, respectively). During the first 10-d of the breeding period, Jabbar et al. (1994) reported similar conception rates of 26% after feeding MGA for 10-d. In contrast, 40% of anestrous ewes lambed following a MGA-synchronized estrus in the study of Safranski et al. (1992). The latter was probably the result of a greater estrous response after feeding MGA twice per d (morning and evening).

The number of lambs born per ewe lambing to a synchronized estrus during the first 6-d of the breeding period was similar among all groups and thus, was not enhanced by PG600 treatment. Safranski et al. (1992), Jabbar et al. (1994), and Morrical et al. (1995) also reported a similar number of lambs born per ewe lambing when ewes were treated with MGA versus MGA+PG600. Future studies may reveal that a different treatment time or dose can be used to enhance the number of lambs born per ewe.

For the entire 31-d breeding period, the number of ewes lambing, number of lambs born per ewe, and mean lamb birth weight were similar among groups. Overall conception rates during the 31-d breeding period (77 to 80%) were similar to previous studies using MGA outside the normal breeding season (Jabbar et al., 1994; Morrical et al., 1995; Powell et al., 1996). This population of Rambouillet ewes and those utilized by Jabbar et al. (1994) were derived from a traditional fall lambing flock. In addition to the ram effect, Rambouillet ewes have been shown to conceive more successfully out-of-season than other breeds, such as the Hampshire (Safranski et al., 1992; Notter, 2002). Furthermore, ewes born naturally during the fall will generally demonstrate higher fertility outside the normal breeding season when compared to ewes born during the spring (Notter, 2002). The Rambouillet ewes in this study were from a traditional fall lambing flock and were selected to breed successfully outside the normal breeding season. Breeding period length is another possible explanation for the high percentage of ewes lambing. The current study used a 31-d breeding period, while others with a 21-d period had lower rates of conception (Daniel et al., 2001).

Figure 1 illustrates the mean interval in d between ram introduction and lambing for Control, PG600, MGA, and MGA+PG600 groups. Both progestogen groups, MGA only and MGA+PG600, had a significantly shorter ($P < 0.005$) interval when compared to Control and PG600 groups. MGA decreased the mean interval to lambing by increasing the proportion of fertile matings during the first 6-d of the breeding period. Safranski et al. (1992) reported that 40% of MGA treated ewes lambed during the first 10-d of the breeding season as apposed to 10% of the ewes not treated with MGA. Jabbar et al. (1994) also demonstrated a decrease in d from ram introduction to lambing for the MGA (154 d) versus Control (166 d) groups. Relative to the MGA+PG600 group, MGA treatment alone unexpectedly reduced ($P < 0.005$) the interval from ram introduction to lambing. This finding was largely due to the higher ($P < 0.01$) proportion of ewes exhibiting an

estrous response in the MGA versus MGA+PG600 groups during the first 6-d of the breeding period.

The number of corpora lutea (CL), embryos and lambs born per ewe in selected MGA and MGA+PG600 ewes conceiving to a synchronized estrus is illustrated in Figure 2. Ovulation rate, indicated by the number of CL present at ultrasonography, was significantly increased ($P < 0.03$) with the use of PG600. A 5 mL dose of PG600 has been shown to increase ovulation rate at the end of MGA treatment (Safranski et al. 1992). Ovulation during anestrous is low (Hulet et al., 1974; Hall et al., 1986), but has been enhanced with PMSG (Ainsworth and Shrestha, 1985; Hamra et al., 1989). The number of embryos between MGA and MGA+PG600 groups was similar ($P > 0.10$) at d 20 to 25 of gestation, and resulted in a similar ($P > 0.10$) number of lambs being born per ewe. The risks of embryonic loss have long been known to be enhanced with increasing ovulation rate (Quinlivan et al., 1966; Knights et al., 2003), but did not vary with the method of synchronization of estrus (Dixon et al., 2007).

Implications

Treatment with MGA once per d for 7-d resulted in a low rate of synchronized estrus, however the overall percentage of ewes lambing (78 to 80%) after the 31-d breeding period was high in this population of Rambouillet ewes. MGA could potentially be used to shorten the fall lambing period which would allow for better management of labor resources and lambing facilities. Although PG600 did increase ovulation rate, it had no beneficial impact on the number of lambs born per ewe. Current studies are evaluating methods to enhance embryonic survival in Rambouillet ewes treated with PG600 outside the normal breeding season.

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Table 1. Ewe fertility and lamb birth weight in C, PG600, MGA, and MGA+PG600 groups outside the normal breeding season. Values are means ± SEM.

	Treatment			
Item	C	PG600	MGA	MGA+PG600
No. of ewes exposed during the breeding period	92	86	99	92
d 0 to 6 of the breeding period				
No. of ewes mated (%)	3 (3.3)[a]	9 (10.5)[b]	24 (24.2)[c]	13 (14.1)[b]
No. of ewes lambing (%)	2 (2.2)[a]	4 (4.7)[a]	18 (18.2)[b]	9 (9.8)[ab]
Lambs born per ewe lambing*	1.5 ± 0.50	1.5 ± 0.29	1.6 ± 0.16	1.7 ± 0.17
Lamb birth weight (kg)*	4.2 ± 0.00	4.3 ± 0.50	4.3 ± 0.17	4.5 ± 0.17
Days 0 to 31 of the breeding period				
No. of ewes lambing (%)	74 (80.4)	68 (79.1)	77 (77.8)	74 (80.4)
Lambs born per ewe lambing*	1.5 ± 0.07	1.6 ± 0.07	1.6 ± 0.06	1.6 ± 0.06
Lamb birth weight (kg)*	4.6 ± 0.07	4.5 ± 0.08	4.6 ± 0.08	4.7 ± 0.08

[a,b,c]Means in the same row with no superscripts in common are significantly different ($P < 0.01$).

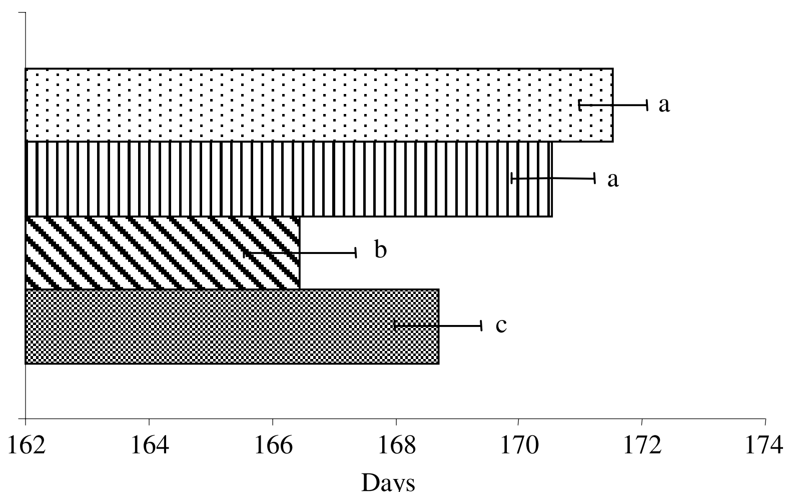

Figure 1. Interval in days from ram introduction to lambing for Control (⛶, n=92), PG600 (◫, n=86), MGA (◪, n-99), and MGA+PG600 (▩, n=92) ewes conceiving from Days 0 to 31 of the breeding season. Values are means ± SEM. Different superscripts are statistically significant ($P < 0.10$).

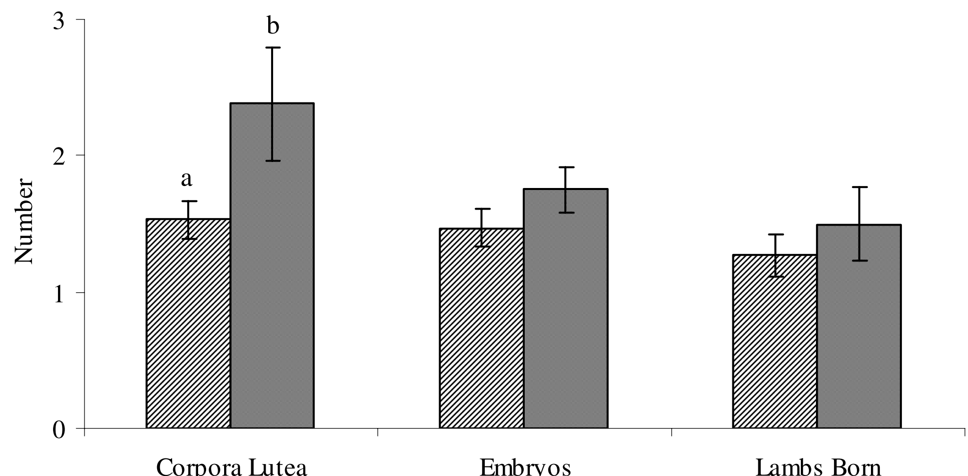

Figure 2. Number of corpora lutea, embryos, and lambs born per ewe for MGA (◪, n=24) and MGA+PG600 (▩, n=13) ewes conceiving from Days 0 to 6 of the breeding season. Values are means ± SEM. Different superscripts are statistically significant ($P < 0.03$).

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECTS OF BUNK SCORING ON FEEDLOT STEER INTAKE

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Abstract : One hundred twenty seven crossbred yearling steers (497.42 kg ± 34.82) were utilized to determine the impact of bunk score on DMI of steers. The steers were randomly sorted into pens and placed into one of three groups. Groups were then randomly assigned to one of three bunk scores based on the amount of daily orts left over from the previous days feeding; a score of 0 was a bunk devoid of all feed particles, a score of ½ represented a bunk with trace to 2.26 kg of feed, and a score of 1 was 2.27 to 9.08 kg of feed remaining. A 3 X 3 Latin square design was utilized to determine the effects of bunk score on DMI. An adaptation period of 9 days was implemented for each period prior to the four days of data collection. During the data collection phase all bunks were observed at 0630, 1630, 2200, and 0200 the next morning. Each morning bunk scores were assigned and feed intake was determined by weighing the orts in each bunk. Dry matter intake was greatest (*P* < 0.0001) for steers which received a bunk score of 1 (2.27 to 9.08 kg of orts; DMI of 11.21 kg ± 0.16) each morning. By allowing steers ad libitum access to feed daily intake was increased which could result in increased performance and decreased days on feed. Additionally, when feed remains in the bunk throughout the night it can be assumed that all steers have access to the feed in a time frame that supports the steers' diurnal eating patterns.

Key Words: Bunk Management, Feedlot, Feed Intake, Steers

Introduction

Bunk management is directly correlated to the health and profitability of feedlot cattle as digestive upsets, which can be caused by overeating and infrequent eating patterns, are a major cause of death in the feedlot industry (Vogel, 2003). The U.S. Department of Agriculture (USDA, 2000) reported that 1.9 ± 0.3 percent all feedlot cattle develop some type of digestive disorder, excluding those cattle that were considered to be non-eaters (cattle that refuse to come to the bunk and eat). Cattle in large feedlots (≥ 8,000 head) are more likely to have digestive problems as 2.0 percent ± 0.3 of all cattle develop digestive disorders (USDA, 2000). The USDA estimates the average cost of treating a single animal diagnosed with a digestive disorder based on medicine and re-treatment costs (veterinary and labor charges were not included in this cost) was $6.19 ± 0.56 (USDA, 2000). Galyean et al. (1998) developed a list of possible factors and interrelationships among nutritional diseases in feedlot cattle which consisted of environment, management, diet type, intake, rumen metabolism, feeding behavior, social behavior, and cattle type. It can therefore be assumed that there are multiple places in the production cycle where metabolic disorders can occur.

Bunk management systems are needed in the feedlot industry to ensure that diseases such as acidosis are decreased to avoid a decreased profit (Schwartzkopf-Genswein, 2003). Slick bunk or limit fed bunk management is a system which aims for all feed delivered to a pen to be consumed on a daily basis with a slick bunk for a certain duration of the time prior to the next day's feed delivery (Erickson, 2003). Research has shown that ADG will not be reduced by DMI restricted by 10% to 15% of maximum DMI (Pritchard, 1998). However, by restricting DMI cattle will consume feed at a more rapid rate which can lead to metabolic disorders (Schwartzkopf-Genswein, 2003). Ad libitum bunk management describes a feed delivery system which allows for feed to be in the bunk from one feeding period to the next at a given level. If cattle have ad libitum access to feed and intake variation remains less than 1.8 kg/d performance levels and incidence of acidosis will not be increased (Cooper, 1999). Therefore, the objective of the present study was to determine what bunk score yielded the greatest DMI of steers at the Southeastern Colorado Research Center, in Lamar Colorado.

Materials and Methods

One hundred twenty seven crossbred steers (497.42 kg ± 34.82) were used in this experiment. Steers were of varied days on feed and varied weights (Table 1). The bunk score system utilized was developed based upon ease

of implementation into a production feedlot setting and clarity of the system (Table 2). Pens were fed at 0700 and 1100 to ensure that the steers were kept on a regular daily eating pattern to avoid data collection errors due to bunk reading as well as to prevent metabolic digestive disturbances caused by large daily shifts in feeding times. Upon initiation of the trial, steers were pen weighed and assigned to a treatment group. Treatment one was each period's group on the slick bunk management system (0.0 to trace feed left in bunk) with a daily bunk score of 0. Trace amounts of feed were determined to be the feed dust and inedible feed products left in the bunk. Treatment 2 groups of steers per period were fed enough feed so that there was between trace to 2.26 kg left in the bunk each morning with a daily bunk score of ½. Treatment 3 was the group of steers per period fed enough feed so there was between 2.27 to 9.08 kg of feed left in the bunk each morning with a daily bunk score of 1.

An adaptation period of 9 days was implemented for each period prior to the four days of data collection. The adaptation period was utilized to ensure the cattle were transferred from their previous bunk score feed delivery to their new bunk score feed delivery, gradually, to avoid metabolic disorders and excess orts During the data collection phase of each of the three periods, all bunks were read at 0630, 1630, 2200, and 0200 the next morning. Each morning after the bunk scores were assigned, ADFI was determined by weighing the orts in each bunk. This number was subtracted from the total amount delivered from the previous day. If the amount of left over feed was less than 1.1 kg it was discarded, if the amount of leftover feed was greater than 1.1 kg it was placed back in the bunk. After three days of a bunk scoring feed was discarded after it was weighed. In the event of inclement weather (i.e. rainfall), all bunks were scooped, weighed, and a sample was collected for DM analysis.

All steers were fed a finishing ration of steam flaked corn grain, a roughage source, and a urea and limestone based vitamin and mineral supplement (Table 3). Diets were formulated to meet or exceed all nutrient requirements for finishing steers (NRC, 1996).

Steers were housed in pens measuring 6.10 x 18.29 m and a single automatic waterer was shared between every two pens. Steers were fed in fence-line 3.66 m long concrete bunks (0.31 m/hd) which had a 3.66 m wide 6.10 m long concrete apron adjacent to the bunk to provide a solid area to stand on while eating. Steers were pen weighed at the beginning of each period; d 0, d 14, and d 28.

Statistical Analysis. Data were analyzed as a 3 x 3 Latin Square using the GLM model procedure of SAS (2003). The model included the effects of treatment, treatment x period interactions, metabolic BW, average bunk score, minimum and maximum temperature for each period, rainfall per period, and wind speed per period. Interactions were considered to be significant if $P < 0.05$.

Results and Discussion.

Intake. Dry matter intake was greater ($P < 0.0001$) for steers which consistently received enough feed to ensure 2.27 to 9.05 kg of feed was left in the bunk on a daily basis (11.21 kg ± 0.16). Steers which received enough feed to ensure that between trace and 2.26 kg were left in the bunk at all times had an average daily DMI of 10.37 kg ± 0.16. Steers kept on a slick bunk management system had an average daily DMI of 9.74 kg ± 0.16. In a preliminary study conducted at the Southeastern Colorado Research Center in Lamar, CO cattle with an average bunk score of 1 had a greater average weight and a greater DMI than steers who averaged a bunk score of 0 or 1/2 (Wagner, 2006). By maximizing intake, steers will potentially have an increase in performance and a reduction in the duration of the feeding period (Anderson, 1990). Additionally, research conducted at the University of Saskatchewan has shown that major periods of eating are around sunrise, sunset, and midnight (Gonyou, 1984). It can be hypothesized that if cattle are fed enough feed to last throughout the night, that all of the cattle in the pen will have increased access to a complete ration for a 24 hour period of time.

Bunk scores were collected four times daily and averaged to ensure that each treatment was receiving feed in amounts to yield different ort amounts (Figure 1). Additionally, intake per period was analyzed to ensure that intake was not varying across periods. Furthermore, all animals remained healthy throughout the experimental period.

Ambient temperature effects were also analyzed to ensure that intake variation was not due to climatic changes. Average minimum and maximum temperature for each period, rainfall per period, and wind speed per period did not interact with treatment.

Implications

Results of this study suggest that bunk management strategy has a significant effect on DMI characteristics of feedlot steers. Feeding enough feed to last through the night and into the next feeding encouraged greater DMI. This increased intake could result in decreased days on feed, increased performance traits, and a decrease in morbidity as all cattle would have a greater access to a more complete diet. However, if producers were to implement a bunk management practice such as this, they would need to make sure that consistent feed delivery, bunk readings, and cattle management practices were in place.

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Table 1. Group background data and allotment to Latin square design.

Group	**A**	**B**	**C**
# of Hd	42	42	43
Avg DOF	78.25	71.00	71.00
Bunk Score→ Period↓	**0**	**1/2**	**1**
1	B	C	A
2	A	B	C
3	C	A	B

Table 2. Southeastern Colorado Research Center Feed Call Score Sheet

Call [1]	**kgs in Bunk**	**Feed Bumps**
0	0	↑ feed by 0.23 kg every third morning
½	Trace-2.26 kg	Remains Same
1	2.27-9.08 kg	↓ feed by 0.91 kg; on the third morning (SCOOP) [2]
2	9.09-18.18 kg	↓ feed by 1.82 kg; on the third morning (SCOOP) [2]
3 [2]	> 18.19 kg	↓ feed by 2.27 kg; on the third morning (SCOOP) [2]

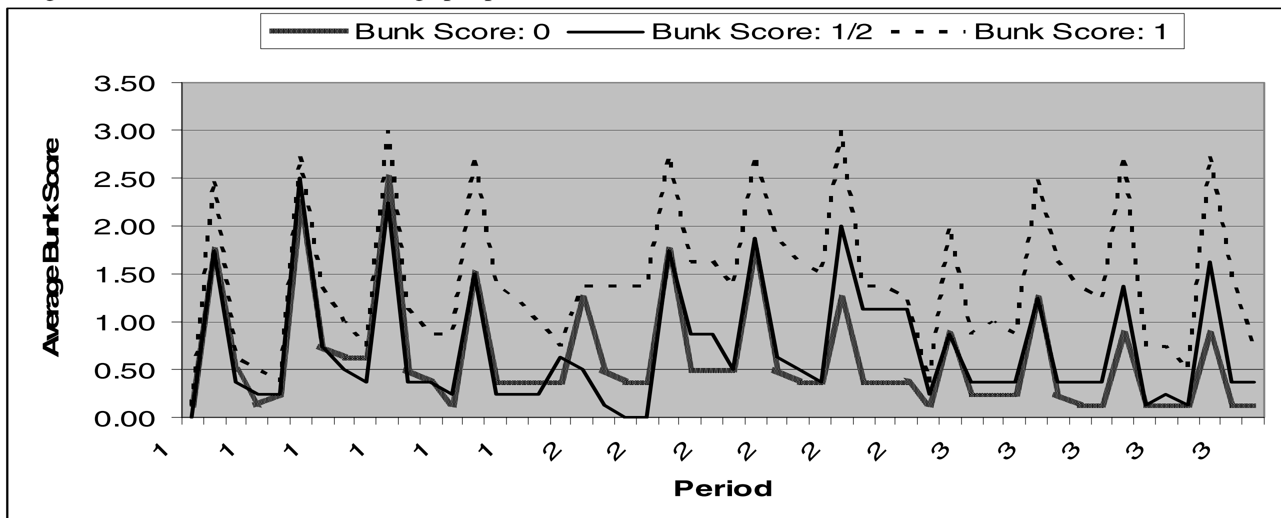
[1]Due to rain or a feed call of 1 or 2 for three days or a call of 3 for one day bunks will be scooped. Orts will be weighed and recorded and a sample will be collected for dry matter analysis.
[2] On the FIRST day that the bunk is slick (0) the pen will get HALF of TOTAL pounds CUT back.

Table 3. Nutrient Composition of basal diet (% DM)

Ingredients	
Corn Silage	15.35
Steam flaked corn	71.93
Soybean meal	03.08
CCDS [a]	03.00
Yellow Grease	03.50
Supplement [b]	03.14

[a] Condensed Corn Distillers Soluble
[b] Supplement consisted of: Calcium, Salt, Urea, Min Ad, ATM 398, Vitamin A, Vitamin E, Rumensin 80, Tylan 100, and Mineral Oil.

Figure 1. Overall bunk score [a] readings per period



[a] Bunk score 0=Treatment 1=0 to trace kg of feed left, bunk score ½=Treatment 2=trace to 2.26 kg of feed left, bunk score 1=Treatment 3=2.27 to 9.08 kg of feed left over

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

RECEPTOR TRANSPORTING PROTEIN-4 (RTP4) EXPRESSION AND LOCALIZATION IN THE OVARY AND ENDOMETRIUM OF CYCLIC AND EARLY PREGNANT EWES

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ABSTRACT: Interferon-tau (IFNT) is a type I interferon secreted by the trophoblast that is the signal for maternal recognition of pregnancy in ruminants. Pregnancy/IFNT regulates expression of genes in the endometrium, peripheral blood leukocytes (PBL) and corpus luteum (CL). Expression of a novel G-protein coupled receptor transporter (RTP4) increased in the endometrium and PBL during early pregnancy. Here we quantify expression of RTP4 in CL of cyclic and early pregnant ewes and determine localization and size of the RTP4 transcripts in the ovary and endometrium. Ewes were randomly assigned to be bred to a vasectomized (cyclic) or intact ram (pregnant). Tissues were collected at 11, 13, 15 (n = 4/status/day), 17 and 19 (pregnant only; n = 4/day) days after mating. Total RNA was isolated and analyzed for levels of *RTP4* mRNA in CL and transcript size in endometrium and CL. Northern blot analysis revealed the expected 1.6 kB mRNA and an unexpected 2.6 kB mRNA. Levels of *RTP4* mRNA were not different ($P > 0.10$) to day 15; however, *RTP4* mRNA levels were elevated over 14- and 6-fold on days 17 and 19 of pregnancy, respectively. *In situ* hybridization localized expression of *RTP4* mRNA to the glandular epithelium, stratum compactum and caruncular stroma. *RTP4* mRNA was ubiquitously expressed in luteal cells and in an uncharacterized population of immune cells. Findings suggest that an alternate form of *RTP4* mRNA may exist in sheep and that *RTP4* mRNA levels are regulated in the ovine CL during early pregnancy. Regulation of RTP4 during early pregnancy may contribute to the complex signaling cascade associated with pregnancy recognition in this species.
Keywords: Ovine, Pregnancy, RTP4, interferon, uterus

Introduction

It is estimated that early embryonic loss costs the livestock industries more than a billion dollars annually. During early embryo development, it is critical that the trophoblast cells of the blastocyst produce Interferon-τ (IFNT) which is the signal for maternal recognition of pregnancy in ruminants (Spencer and Bazer, 2004). Interferon-τ acts indirectly to rescue the CL from luteolysis (Spencer & Bazer 2004) and also increases expression of a host of IFN-stimulated genes (ISG) in the endometrium including *2', 5' oligoadenylate synthetase* (Johnson et al., 2001), *β2-microglobulin* (Vallet et al., 1991), *interferon stimulated gene-15* (ISG15; Johnson et al., 2000), and *MX* (Ott et al., 1998; Hicks et al., 2003). Some ISGs are upregulated in peripheral blood leukocytes (PBL) during early pregnancy (Yankey et al., 2001; Gifford et al., 2007) and in the CL after intrauterine infusions of IFNT (Spencer et al., 1999) indicating that systemic responses to IFNT can occur in ruminants.

Recently, ovine *RTP4* was cloned and shown to be upregulated in endometrium, PBL, and IFNT-treated immortalized ovine glandular epithelial cells (oGE; Gifford et al., 2006). However, *RTP4* transcript size is larger in cattle (BC105539) and sheep (Gifford et al., 2006) compared with human *RTP4* (NM_022147).

Therefore, the first objective of the current study was to determine ovine *RTP4* transcript size in the endometrium, CL, and immortalized oGE cells. Because several ISGs were shown to be temporally and spatially expressed in the endometrium and ISGs are up-regulated in the CL in response to IFNT, the second objective of the current study was to determine *RTP4* mRNA levels in the CL and localization of *RTP4* transcript in the endometrium and ovary from early pregnant and cyclic ewes. We hypothesize that *RTP4* is an IFNT-regulated gene in the ovary and endometrium involved in the process of establishment and maintenance of pregnancy in ruminants.

Materials and Methods

Animals

Mature cross bred ewes were housed with a vasectomized ram and observed for estrus. At estrus ewes were randomly assigned to be bred to either a vasectomized ram (cyclic) or an intact ram (pregnant). Ewes were also randomly assigned to collection day, and blood and tissue samples were collected at 11, 13, 15 (n = 4/status/day), 17 and 19 (pregnant only; n = 4/day) days after breeding. Tissues were snap frozen and PBL were isolated from blood samples as previously described (Gifford et al., 2007). Ewes were also randomly assigned to be hysterectomized on Days 10, 12, 14 and 16 of the estrous cycle and pregnancy as well as on Days 18 and 20 of pregnancy (n=5 ewes/day/status). All procedures were in compliance with the Guide for the Care and Use of

Agriculture Animals in Research and Teaching and with approval of the Institutional Animal Care and Use Committees at the University of Idaho and Texas A&M University.

Cell Culture

Immortalized oGE cells (Johnson et al., 1999) were plated at 80% confluency and cultured in 6-well plates (Costar® 3516; Corning Inc., Corning, NY, USA) for 4 h in Dulbecco's Modified Eagle's Medium (15.63 g/L DMEM; Sigma, St Louis, MO, USA) with 10% fetal bovine serum (FBS) under 5% CO2 at 38.5^o C prior to 24 h treatment. Treatments of either IFNT (n = 3 wells; 10,000 U/mL; provided by Dr. Fuller Bazer, Texas A&M University) in media or media alone (n = 3 wells; no treatment) were applied at 24, 12, 6, 3, 1.5, and 0 h prior to harvest. Cells were harvested at the same time for all time points using Trizol reagent (Invitrogen, Carlsbad, CA) according to manufacturer's recommendations and were approximately 90% confluent at harvest. The experiment was conducted 2 independent times.

RNA Isolation and cDNA Synthesis

Total RNA was isolated from CL, endometrium, and oGE samples from pregnant and non-pregnant ewes using Trizol reagent (Invitrogen, Carlsbad, CA). For quantitative real time PCR of *RTP4* mRNA in CL, the RNA was cleaned using RNeasy (Qiagen) spin columns prior to cDNA synthesis, and cDNA was synthesized as previously described (Gifford et al., 2007).

Quantitative Real Time RT-PCR

Steady state levels of *RTP4* mRNA in the CL were quantified as previously described (Gifford et al., 2007) with the following minor modifications: Power SYBR Green master mix (Applied Biosystems) was used, quantification was done using Applied Biosystem's 7500 fast real time RT-PCR machine, and 18s ribosomal RNA served as an internal control. Annealing temperature for RTP4 primers (forward: CACATGTACCTGGAGAACCAGA; reverse: AGGTAGCTCTGAAACCTTCCTG) and 18s primers (forward: AAACGGCTACCACATCCAAG; reverse: CGCTCCAAGATCCAACTA) was 56.6^o C.

Northern Blot Analysis

Northern blot analysis was conducted as previously described (Hicks et al., 2003). Ten µg total RNA from CL, endometrium, and oGE cells was used for gel electrophoresis and transfer, and blots were incubated with a biotin-labeled 513 bp ovine *RTP4* antisense cRNA probe. All other steps were completed as described (Hicks et al., 2003) with the exception of RNase A treatment.

In Situ Hybridization Analysis

Location of *RTP4* mRNA in the uterus and ovary was analyzed using a 513 bp radiolabeled antisense cRNA probe for *RTP4* using methods described previously (Satterfield et al. 2006). The cRNA probe used for *in situ* analysis was the same sequence as the probe used for Northern blot analysis and therefore likely recognizes both forms of *RTP4* (see Results).

Statistical Analysis

Data for *RTP4* mRNA levels in the CL are presented as least squares mean ± standard error of the mean of relative fold change from d 11 cyclic values calculated by the ΔΔCt method (Kubista et al., 2006), with 18s rRNA serving as the internal control. Data were analyzed using the MIXED procedure in SAS (Version 9.1; SAS Institute, Cary, NC). Animal was the experimental unit and fold change was tested against status, day, and status x day.

Results and Discussion

Ovine RTP4 Transcript Size

Northern blot analysis detected an approximately 1.6 kB mRNA in the endometrium, CL, and oGE cells (Figure 1). An additional mRNA of 2.6 kB was also detected in all samples tested (Figure 1).

Some G-protein coupled receptors (GPCR) require accessory proteins to transport the receptor from the *trans*-Golgi to the cell surface (Brady and Limbird, 2002). Receptor transporting protein-4 belongs to a gene family consisting of members *RTP1, RTP2, RTP3*, and *RTP4* (Saito et al., 2004). *RTP1* and *RTP2* are specifically expressed in olfactory neurons and facilitate functional expression of odorant receptors (Saito et al., 2004). Behrens et al. (2006) found that *RTP4* is expressed in a broad range of tissues and has recently been identified as a cofactor for functional expression of some bitter taste receptors. Results from the current experiment clearly indicate that ovine *RTP4* transcript is larger than the human form, and an alternate 2.6 kB transcript is present in the CL and endometrium of sheep (Figure 1). Further studies are needed to determine if this discrepancy in transcript size leads to different functional properties of *RTP4* in domestic ruminants. Although the sequence for the 2.6 kB form of *RTP4* has not been determined, the 513 bp probe was complimentary to the 5' end of the 1.6 kB ovine *RTP4* transcript yet also hybridized to the 2.6 kB form of *RTP4* indicating the two forms share homology in this region.

RTP4 Expression and Localization

Previously, steady-state mRNA levels of *RTP4* were shown to be elevated in the endometrium during early pregnancy (Gifford et al., 2006). The type I interferon receptor is expressed in all cell types in the endometrium with highest expression in luminal epithelium (LE; Rosenfeld et al., 2002). The majority of ISG expression in response to IFNT is in the GE and stromal compartments (Spencer and Bazer, 2004). Results from the current experiment show that *RTP4* mRNA is localized to the glandular epithelium, stratum compactum and caruncular stroma (Figure 2). However, variation in *RTP4* expression was observed within day and status (Figure 2). It may be that *RTP4* is upregulated for a short time period from days 15 to 19 of pregnancy, and individual animals vary in expression of *RTP4* between these days. Alternatively, *RTP4* expression may exhibit spatial regulation in the pregnant endometrium, and the cross sections used in the current study were from areas of the endometrium with lower/higher expression of *RTP4*. Regulation of gene expression by IFNT in the endometrium is hypothesized to be necessary for establishment of pregnancy. Choi et al. (2001) showed that interferon regulatory factor-2 (IRF2) increased in the

endometrial LE during early pregnancy, and IRF2 is potent repressor of transcription of ISG in the LE (Choi et al., 2001). In addition, IRF2 may be involved in suppressing expression of estrogen receptor α, which in turns prevents expression of the oxytocin receptor, thereby indirectly blocking luteolytic pulses of prostaglandin (Choi et al., 2001). Recently, Dunlap et al. (2006) showed that progesterone regulated endogenous Jaagsiekte sheep retroviruses (enJSRV) are important for conceptus growth and differentiation, and *in vivo* knockdown of enJSRV envelope mRNA reduced pregnancy rates on day 20 of pregnancy. Collectively, these results highlight the complex gene regulation in the endometrium during early pregnancy and indicate that additional studies are needed to determine possible function(s) of RTP4 during this period.

A status x day interaction was not detected ($P > 0.10$) for *RTP4* levels in CL from pregnant and cyclic ewes. However, steady state mRNA levels for *RTP4* increased in CL from pregnant ewes over 7-fold and 14-fold on Days 15 and 17, respectively (Figure 3). Spencer et al. (1999) found that either s.c. or intrauterine injections of IFNT up-regulated expression of *ISG15* and *MX* in the CL. Results from the current experiment localized the message for *RTP4* to luteal cells specifically (data not shown). This is interesting because most tissues and cell types express the type I interferon receptor (Constantinescu, et al., 1995), yet expression of *RTP4* was not detected in the ovarian stroma. Interestingly, IFNT has not been detected in the circulation or uterine venous drainage in pregnant ewes (Bazer et al., 1996); thus, the mechanism by which ISG are activated in the CL is not understood. Although, IFNT acts as an antiluteolytic mechanism by blocking pulsatile release of prostaglandin from the endometrium, it is also possible that IFNT/pregnancy affects CL function through regulation of ISG including RTP4 in luteal cells resulting in changes supporting establishment of pregnancy in domestic ruminants.

Implications

Two forms of receptor transporting protein-4 were identified, and are up-regulated and localized in the corpus luteum during early pregnancy. In the endometrium, receptor transporting protein-4 was localized to the glandular epithelium, stratum compactum and caruncular stroma. Receptor transporting protein-4 belongs to a class of G-protein coupled receptor transporters and is required for functional expression of some G-protein coupled receptors. The function of receptor transporting protein-4 in domestic ruminants is unknown. However, because receptor transporting protein-4 exhibits spatial and temporal regulation during early pregnancy in the uterus and ovary, it may play a role in the complex signaling cascade between mother and conceptus during maternal recognition of pregnancy. It is estimated that early embryonic loss costs livestock producers over a billion dollars annually; therefore, it is important to understand events that occur during a successful pregnancy.

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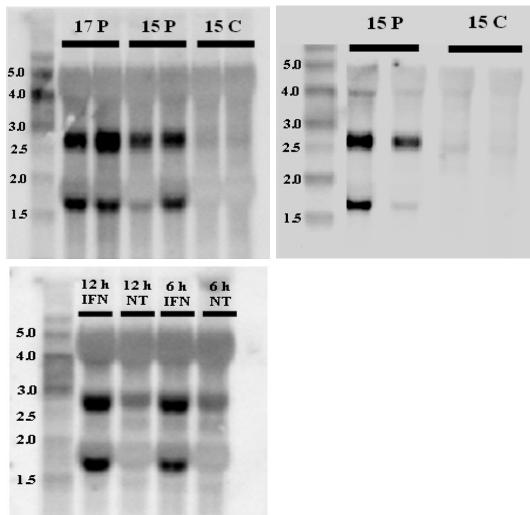

Figure 1. Northern Blot analysis of receptor transporting protein-4 (RTP4) in total RNA from corpus luteum (top left) in cyclic ewes 15 days after breeding (15C) and in pregnant ewes on 15 (15P) and 17 (17P) days after breeding. Top right figure represents Northern Blot analysis of RTP4 in total RNA from endometrium in pregnant (15P) and cyclic (15C) 15 days after breeding. Bottom figure represents Northern Blot analysis of RTP4 in total RNA from immortalized ovine glandular epithelial cells treated with 10,000 U/mL interferon tau for 12 h (12h IFN) or 6 h (6 h IFN) and control cells that were not treated (12h NT and 6h NT).

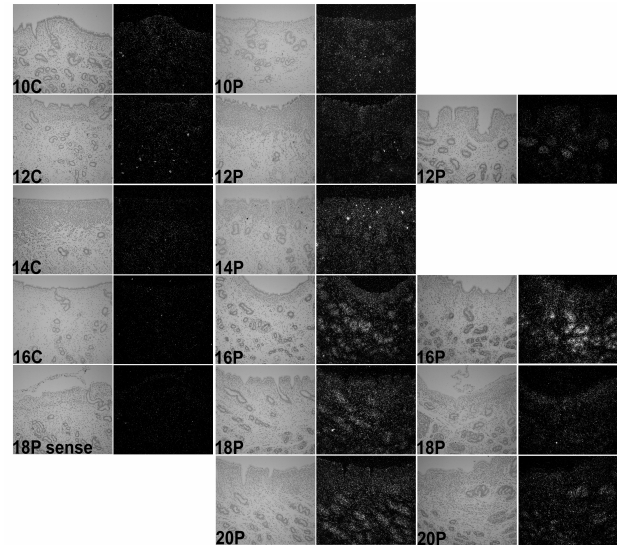

Figure 2. In situ hybridization analyses of receptor transporting protein-4 (RTP4) mRNA in cyclic and pregnant ewes. Cross sections of the uterine wall from cyclic (C) and pregnant (P) ewes were hybridized with radiolabeled antisense or sense ovine RTP4 cRNA probe. RTP4 mRNA was detected in glandular epithelium, stratum compactum and caruncular stroma.

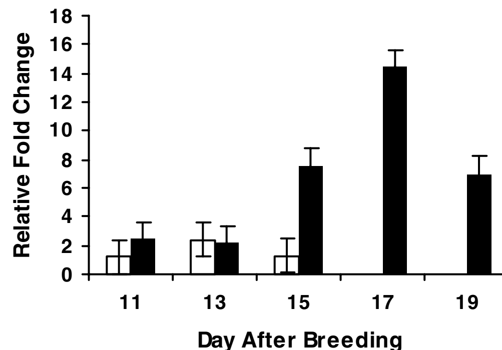

Figure 3. Steady-state mRNA levels for receptor transporting protein-4 (RTP4) in CL from cyclic (open bars; n = 4/day) or pregnant (closed bars; n = 4/day) ewes. Results are presented as relative fold compared to day 11 cyclic; error bars represent standard error of the mean. No status x day interaction was detected between days 11 and 15 ($P > 0.10$), but levels of RTP4 increased over 14-fold in pregnant ewes on day 17 and over 4-fold on day 19 of pregnancy.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

STAYABILITY TO ALTERNATE AGES

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ABSTRACT: Beef cattle stayability is traditionally defined as the probability a cow will remain in the herd until 6 yr of age given she has calved once. Genetic evaluation is based solely on the success/failure of females reaching age 6. With current methodology, no account is made for daughters not yet 6 yr or extra reward for staying beyond 6 yr. This approach has been contested as inadequate by researchers and cattlemen because sires have low accuracy until at least 8 yr of age. An approach that utilizes information from earlier and later ages would improve accuracy of sire evaluations. Failure to stay in the herd can be caused by voluntary or involuntary culling. Accordingly, genetic and environmental factors influencing stayability may vary with cow age. This likely will alter heritabilities and genetic correlations between stayability at different ages. The objective of this study was to estimate heritability of stayability at alternate age endpoints of 3, 4, 5 or 6 yr of age and to compare EPD from earlier ages with the current standard 6 yr of age. Data from the American Gelbvieh Association, Red Angus Association of America and American Simmental Association, was used separately to compare estimates of stayability at different ages. Variance components were estimated from a sire model using a probit threshold model including contemporary group as a fixed effect. Estimates of stayability heritability were similar across breeds and averaged 0.16, 0.17, 0.18, and 0.18 for 3, 4, 5 and 6 yr, respectively. Stayability calculated at earlier ages was nearly as heritable as the current definition. These heritabilities were then used in a threshold animal model to obtain EPD. Independent data sets were used for each age definition so animals eligible for older age definitions were not considered in younger age definitions. Correlations of EPDs for sires with daughters at different age definitions ranged from 0.18 to 0.47. Accordingly sire merit likely changes depending on the age definition of stayability. A method to combine information from all these independent predictions of stayability would be desirable. It would provide more accurate evaluation at earlier ages but retain the current interpretation of published EPD.

Key words: beef cattle, threshold, longevity, stayability

Introduction

Sustained cow fertility and long term production are important contributors to the profitability of the cow herd (Enns et al., 2005) and therefore should be considered when selecting bulls. Stayability benefits herd productivity in two ways, a decreased need for replacements and higher average weaning weights because of the greater number of older cows in production (Garrick, 2006). Stayability in beef cattle is defined as the probability a cow will remain in production until 6 yr old given she calved as a 2 yr old.

A female must reach the defined benchmark age of 6 yr to receive a stayability observation. At that age, the sire of that female will be at least 8 yr old resulting in very low prediction accuracy for sires less than 8 yr old. Most sires are no longer actively in service at 8 yr of age unless available through AI. This lag between accurate prediction of stayability and the need for young replacement sires has constantly received criticism (e.g., Hudson and Van Vleck, 1981).

Research supports a high probability that females in production at 4 yr will remain in production to 6 yr or more, suggesting that earlier measures could be used as indicator traits in the genetic prediction of stayability to 6 yr of age. Martinez et al., (2005) reported correlations between 4 and5 yr or 4 and 6 yr stayabilities of 0.85 or 0.86 respectively. Anecdotally, cattle producers support the premise that a female conceiving at 4 yr of age will remain in the herd until 6 yr of age. Cows culled after conceiving as a 4 yr old are most likely culled for reasons unrelated to reproductive ability.

Female reproductive efficiency directly affects economics in beef and dairy cattle production systems. Hudson and Van Vleck (1981) showed positive correlations between stayability and milk production in Holstein cattle. Females with low milk production were typically culled young thus never having the opportunity to reach later parities. In beef production systems, development or purchase of replacement females is a substantial cost. Estimates of beef cattle phenotypic stayability to 6 yr range from 30% to 40% (Snelling et al., 1995; Martinez et al., 2005; Westhuizen et al., 2001). Enns et al., (2005) determined a 1 unit increase in overall herd stayability resulted in an increase in profit of $2500 for herds with 40% of cows remaining in the herd to age 6 yr.

The objective of this study was to assess the heritabilities of indicators of stayability and the relationship between EPD for stayability to these earlier ages and EPD for stayability to 6 years of age.

Material and Methods

Herd book data was supplied from American Gelbvieh Association (**AGA**), American Simmental Association (**ASA**), and Red Angus Association of America (**RAAA**). The AGA data included animal performance records from 1961 to 2005, ASA data included animal performance records from 1959 to 2005 and RAAA data included animal performance records from 1944 to 2005. Variance component estimation via a sire model and breeding value estimation from an animal model were calculated for each set of records.

Variance component estimation

Data. The 3 data sets were prepared in similar manner. Stayability observations were assigned to dams based on their age in days at each calving. Variance components were estimated separately for the 4 different stayability definitions, 3 yr of age, 4 yr of age, 5 yr of age and 6 yr of age. In order for a dam to be eligible to receive a stayability observation she was required to be at least as old as the defined stayability definition. Dams that calved after the defined cut off date for the particular stayability age received a favorable designation of '1', while dams that did not calve at that age received an unfavorable designation of '0'. Counts of each data set, percentage of dams receiving a favorable stayability observations and pedigree sizes are summarized in Table 1.

Contemporary groups were formed based upon the breeder code of the dam and calf for each stayability definition. Contemporary groups were required to have at least 5 individuals in order to be included in the dataset used to estimate variance components.

Model. Variance component estimation was performed using ASREML (Gilmour et al., 2002). A residual maximum likelihood linear model was fitted using the PROBIT option for categorical data. A sire model was used with contemporary group as a fixed effect.

Breeding value estimation

Data. Data and stayability definitions were the same used for the previously described variance component estimation. However, independent data sets were formed for each stayability age, where animals with an older stayability definition were not considered in younger categories. For example if a female was 5 yr old she would receive a favorable or unfavorable 5 yr stayability designation only and not be considered in the 3 yr, 4 yr or 6 yr stayability data. Consequently stayability to ages 3, 4, and 5 yr were limited to single birth yr groups. A three generation pedigree including all animals from all age definitions was constructed and this one pedigree was used in each analysis.

Contemporary group formation was the same as previously described except contemporary groups were not sifted for minimum group size except that single animal contemporary groups were removed from the data set. Counts each data set, percentage of dams successfully staying to the age designation and pedigree sizes of data used to calculate EPD are summarized in Table 2.

Model. The calculation of EPD was conducted using the maximum a posteriori (MAP) probit threshold model (Gianola and Foulley, 1983; Harville and Mee, 1984). Stayability was analyzed in a univariate model on the underlying scale, using the model:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

where

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

and **y** was a vector of transformed observations on the underlying scale and **X** was a known design matrix relating fixed effects to those individuals in vector **y**. The only fixed effect included was stayability contemporary group formed as previously described and contained in vector $\boldsymbol{\beta}$ (p x 1). The design matrix Z related the random additive genetic effects in $\boldsymbol{u}$ (n x 1) to the individuals in vector **y**, and $\boldsymbol{e}$ was a vector of random residual errors. The matrix **A** represents Wright's additive numerator relationship matrix, **I** is an identity matrix and σ_a^2 and σ_e^2 are additive and residual variances, respectively. The additive genetic variance (σ_a^2) were unique for all stayability age definition within breed association data set for all of EPD set calculated. In accordance with the MAP model the residual variance (σ_e^2) was constrained to be equal to 1.

Results and Discussions

Heritability for each age definition and data set are in Table 3. In general heritabilities were similar across breeds and trended upward as the age definition increased averaging 0.16, 0.17, 0.18 and 0.18 at 3, 4, 5, and 6 yr stayability respectively. Snelling et al., (1995) reported average stayability heritability for 2 herds of 0.12, 0.18, and 0.18 with stayability definitions of 3, 6, and 9 yr respectively. Other estimates of heritability for 3, 4, 5 and 6 yr reported have varied in magnitude such as the 0.39, 0.38, 0.29, and 0.35 in a population of Hereford cows (Martinez et al., 2005) and the 0.06, 0.10, 0.06 and 0.03 in a population of composite beef cattle (Westhuizen et al., 2001).

The EPD were calculated using variance components estimated for each breed and age separately. Comparisons between age definitions within breed were limited to only those sires with daughters at each definition to avoid comparing animals with no progeny observations. Meaning to be included in the correlation between two age definitions a sire was required to have at least 1 daughter with a stayability record in both age definition data sets beginning compared. Correlations between EPD estimated at two different age definitions were low to moderate as summarized in Table 4. Rank correlations of the same sires show nearly identical results indicating sires EPD do change as EPD definition changes.

These results show heritabilities of earlier ages are in the same range as currently used in national genetic evaluations. EPD diagnostics show different age definitions result in different ranking of animals.

Implications

The ability to use younger age stayability information would benefit beef cow-calf producers by increasing the accuracy of sire EPD prior to 8 yr old. A method to combine data from all ages into a single prediction would be an advantage because it would reduce the problem of low accuracy sires by taking advantage of the high association between ages. The ability to accurately predict stayability of a bull's daughters earlier would be economically beneficial to cow-calf producers.

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Table 1. Total observations, percentage of dams successfully staying and pedigree sizes for each age definition of stayability by breed association used in variance component estimation.

		Breed Association[1]		
		AGA	ASA	RAAA
3 yr	Total	74,885	365,907	180,584
	% Success	0.82	0.71	0.77
	Pedigree	17,143	56,577	41,808
4 yr	Total	74,536	367,886	180,125
	% Success	0.74	0.62	0.67
	Pedigree	16,657	55,351	40,556
5 yr	Total	65,182	365,047	168,177
	% Success	0.68	0.52	0.58
	Pedigree	14,998	53,303	37,952
6 yr	Total	54,459	262,663	153,203
	% Success	0.62	0.43	0.50
	Pedigree	12,909	42,826	34,924

[1]AGA=American Gelbvieh Association; ASA=American Simmental Association; RAAA=Red Angus Association of America

Table 2. Total number of observations, percentage of dams successfully staying and pedigree sizes for each age definition of stayability by breed association used in EPD estimation.

		Breed Association[1]		
		AGA	ASA	RAAA
3 yr	Total	4,362	6,311	15,514
	% Success	0.77	0.51	0.49
4 yr	Total	5,151	5,912	14,657
	% Success	0.70	0.45	0.40
5 yr	Total	4,924	6,146	14,269
	% Success	0.61	0.35	0.33
6 yr	Total	68,401	373,303	253,047
	% Success	0.60	0.45	0.51
	Pedigree	152,352	681,045	404,352

[1]AGA=American Gelbvieh Association; ASA=American Simmental Association; RAAA=Red Angus Association of America

Table 3. Estimates of heritability and standard error for each stayability age for each breed association

Breed Association[1]	Stayability Age Definition			
	3 yr	4 yr	5 yr	6 yr
AGA	0.15 (0.017)	0.17 (0.016)	0.18 (0.017)	0.17 (0.018)
ASA	0.17 (0.009)	0.18 (0.008)	0.20 (0.008)	0.21 (0.010)
RAAA	0.17 (0.010)	0.18 (0.009)	0.16 (0.009)	0.15 (0.009)

[1]AGA=American Gelbvieh Association; ASA=American Simmental Association; RAAA=Red Angus Association of America

Table 4. EPD correlations of sires with daughters at each respective stayability age definition for each breed association

Breed Association[1]	Stayability Age Definition			
		4 yr	5 yr	6 yr
AGA	3 yr	0.18	0.24	0.39
	4 yr		0.30	0.38
	5 yr			0.37
ASA	3 yr	0.40	0.31	0.36
	4 yr		0.34	0.30
	5 yr			0.47
RAAA	3 yr	0.42	0.36	0.38
	4 yr		0.39	0.30
	5 yr			0.30

[1]AGA=American Gelbvieh Association; ASA=American Simmental Association; RAAA=Red Angus Association of America

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

DUODENAL FLOW AND INTESTINAL DISAPPEARANCE OF FATTY ACIDS IN LAMBS FED SAFFLOWER FATTY ACIDS IN THE FORM OF WHOLE SEEDS, CRACKED SEEDS, OR OIL EXTRACTED FROM SEEDS

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ABSTRACT: Four wether lambs (45.5 ± 3.4 kg BW) fitted with ruminal, duodenal, and ileal cannulas were used in a 4 x 4 Latin square experiment to determine effects of safflower seed processing on site and extent of fatty acid digestion. Isonitrogenous diets were 33% ground (2.54 cm) hay, 67% concentrate (Control), with safflower lipid replacing enough of the concentrate to provide 3% added fat from either high-linoleate whole or cracked safflower seeds or oil extracted from the seeds. Orthogonal contrasts included Control vs. fat-supplemented diets, and linear and quadratic effects of degree of safflower seed processing (whole, cracked, and oil). Duodenal flow of 18:1*c*9 was greatest (P = 0.03) for Control, but flow of biohydrogenation intermediates 18:1*t*9 (P = 0.08), 18:1*t*11 (P = 0.07), 18:1*t*12 (P = 0.02), 18:1*t*13 (P = 0.02), 18:1*c*11 (P = 0.03), and 18:1*c*12 (P = 0.11) were least for Control lambs. Duodenal flow of 18:0 in Control lambs was nearly half ($P < 0.001$) that of lambs supplemented fat. Quadratic responses for duodenal flow of 18:2*c*9*t*11 (P = 0.01), 18:2*t*10*c*12 (P = 0.07) and 18:2*c*9*c12* (P = 0.09) were due to greater flow of those fatty acids in lambs fed oil. Apparent small intestinal disappearance (g/d entering the duodenum) of 16:0 (P = 0.02), 18:0 (P = 0.002), 18:2*c*9*t*11 (P = 0.01), and 18:2*t*10*c*12 (P = 0.09) were greater for fat-supplemented lambs than Control. Percentage of 16:0 (P = 0.05) and 18:0 (P = 0.01) disappearing from the small intestine demonstrated a quadratic response because lambs fed oil had the lowest disappearance values for those fatty acids. Percentage of 18:2*c*9*c12* digested in the small intestine, however, was greater (quadratic, P = 0.08) in lambs fed oil. We conclude that duodenal flow of biohydrogenation intermediates increased when lambs were fed fatty acids from safflower seeds. Supplementing fatty acids in the form of extracted oil seems to be the most effective strategy to increase status of linoleic acid and biohydrogenation intermediates in lambs fed diets containing 3% added fat.

Key Words: lambs, supplementation, safflower seeds

Introduction

Adding fat to the diets of livestock is often practiced to increase dietary energy. Fats are also fed to alter the composition of different food products derived from ruminant animals (NRC, 2007). Fat from oilseeds can be supplemented as whole seeds, processed seeds, or as the oil extracted from the seed. Lammoglia et al. (1999) reported that ruminal disappearance of DM after 48-h of incubation in situ increased from 12.0 to 56.5% by cracking safflower seeds. The seed sheaths will presumably limit lipolysis and hydrogenation of fatty acids in the lipid by restricting access by the ruminal microorganisms (Chilliard and Ferlay, 2004). However, milk produced by goats had a greater percentage of 18:2*c*9*c12* when fat was supplemented as sunflower oil as opposed to whole sunflower seeds (Chilliard and Ferlay, 2004). In their extensive review of the literature, Chilliard and Ferlay (2004) noted that few experiments have been conducted to compare compositional changes of food products derived from ruminants fed fat in the form of seeds vs. oil extracted from the seeds. The process of ruminal biohydrogenation and subsequent effects on intestinal digestion complicate predicting changes in composition of food products derived from ruminants based on profile and form of dietary fatty acids (NRC, 2007). Evaluation of site and extent of fatty acid digestion is necessary to understand how dietary fatty acids may be altered and made available for metabolism by the ruminant animal. We are unaware of any studies that have been conducted to compare fatty acid digestion of oil seeds processed to different degrees. Our hypothesis was that whole safflower seeds will provide partial protection from ruminal biohydrogenation of fatty acids, but may also reduce digestibility in the small intestine. Our objective was to determine site and extent of digestion of safflower seed fatty acids fed as whole or cracked seeds, or top-dressed oil.

Materials and Methods

General

All procedures for the following experiment were approved by the University of Wyoming Animal Care and Use Committee. Four wether lambs (45.5 ± 3.4 kg BW) were fitted with ruminal, duodenal, and ileal cannulas. Lambs were placed in metabolism crates (1.4 × 0.6 m) in a climate controlled room under continuous lighting where they had free access to water.

Diets and Sampling

Following the design of a 4 x 4 Latin square experiment, lambs were assigned to 1 of 4 dietary treatments with 13-d collection periods. The **Control** diet contained no supplemental fat and consisted of 33% ground (2.54 cm) hay and 67% concentrate (as-fed basis; Table 1). Safflower fatty acids from either high-linoleate **whole** (76.2% 18:2*c*9*c12*) or **cracked** (76.8% 18:2*c*9*c12*) safflower seeds or **oil** (76.8% 18:2*c*9*c12*) extracted from the seeds were supplemented to the Control diet at the expense of the safflower seed meal so that fatty acid-supplemented diets contained 3% added fat. All dietary treatments were formulated to be isonitrogenous (actual dietary CP = 13.3% of DM) and to meet the requirements of a 45 kg growing lamb gaining 300 g/d (NRC, 1985). Lambs were offered their daily ration at 0530 and 1730. As an external marker of digesta flow, 2.5 g of TiO_2 was dosed intraruminally immediately before each feeding (Myers et al., 2006). Each

10-d adaptation period was followed by 3 d of duodenal and ileal sampling. Sampling began at 0500 on d 11 of each period with the collection of 150 mL of duodenal and ileal digesta repeated every 6 h. Collection times were advanced by 2 h on both d 12 and 13 so that digesta was collected every other h of a theoretical 24-h clock.

Table 1. Ingredient composition of diets fed to lambs

	Dietary treatment[1]			
	Control	Whole	Cracked	Oil
Ingredients, % of DM				
Bromegrass hay	32.73	32.29	32.58	31.98
Cracked corn	44.43	44.38	44.40	44.40
Soybean meal	3.90	3.90	3.90	3.90
Safflower seed meal	14.81	5.39	6.38	12.20
Whole safflower seeds	-	9.48	-	-
Cracked safflower seeds	-	-	8.47	-
Safflower oil	-	-	-	3.32
Salt	1.07	1.07	1.07	1.07
Urea	0.07	0.49	0.21	0.56
Limestone	1.92	1.92	1.92	1.92
Sodium bicarbonate	1.07	1.07	1.07	1.07

[1]Safflower fat was added at 3% of the diet (as-fed) in the form of whole or cracked seeds or oil extracted from the seeds.

Laboratory Analysis

Beginning 2 d before and continuing through the 3-d collection of digesta, samples of all feedstuffs were taken on a daily basis for laboratory analysis. Feed refusals were collected 2 d before and throughout the sampling period. Refusals were weighed back and collected for laboratory analysis. Feed and refusal samples were analyzed for DM (AOAC, 1990) and fatty acid content (Whitney et al., 1999 as modified by Kucuk et. al., 2001) to determine DMI and fatty acid intake (Table 2). Duodenal and ileal samples were frozen at -20° C, lyophilized (Genesis 25 freeze dryer, The VirTis Co., Gardiner, NY), and composited within lamb for each collection period for analysis of TiO_2 (Myers et al., 2004) and fatty acids (Kucuk et al., 2001).

Calculations and Statistical Analysis

Digesta flow was calculated by dividing the amount of TiO_2 dosed by the concentration of TiO_2 in duodenal and ileal samples. Fatty acid flow was calculated by multiplying fatty acid concentration by digesta flow. Data were analyzed using the GLM procedures of SAS (Version 8.0, 1998, SAS Inst., Inc., Cary, NC) for a Latin square. Orthogonal contrasts (Steel and Torrie, 1980) included Control vs. fat-supplemented diets, and linear and quadratic effects of degree of safflower seed processing (whole, cracked, and oil).

Results and Discussion

Duodenal Flow of Fatty Acids

Unlike previous experiments published by our laboratory in which heifers were fed a high-forage diet plus cracked high-linoleate safflower seeds (Scholljegerdes et al., 2004) or lambs were fed a high-concentrate diet plus safflower oil (Atkinson et al., 2006), Control lambs in the present experiment had greater ($P = 0.03$) duodenal flow of 18:1*c*9 (Table 2). Because intake of 18:1*c*9 was least for Control lambs, greater flow of 18:1*c*9 may be attributable to less ruminal biohydrogenation in lambs fed the Control diet. Scholljegerdes et al. (2004) noted that ruminal biohydrogenation of C18 unsaturated fatty acids in heifers fed a no added fat control supplement was 87% of that for heifers fed high-linoleate safflower seeds formulated to provide 3% added dietary fat. Support for greater biohydrogenation in lambs fed safflower lipids is provided by greater ($P < 0.001$) duodenal flow of 18:0, as this fatty acid is the product of complete biohydrogenation (Jenkins, 1993). Consistent with increased duodenal supply of ruminal biohydrogenation intermediates in heifers (Scholljegerdes et al., 2004) or lambs (Atkinson et al., 2006) fed high-linoleate safflower lipids to provide 3% added dietary fat, we observed greater flow of the biohydrogenation intermediates 18:1*t*9 ($P = 0.08$), 18:1*t*11 ($P = 0.07$), 18:1*t*12 ($P = 0.02$), 18:1*t*13 ($P = 0.02$), 18:1*c*11 ($P = 0.03$), and 18:1*c*12 ($P = 0.11$) in lambs fed fat.

Among the fat-supplemented lambs, duodenal flow of 18:1*c*9 increased linearly ($P = 0.04$) as safflower seeds were processed more extensively. Quadratic responses were also noted for 18:2*c*9*t*11 ($P = 0.01$), 18:2*t*10*c*12 ($P = 0.07$), and 18:2*c*9*c*12 ($P = 0.009$) flowing to the duodenum because of greater flow of these fatty acids in lambs fed oil. Greater flow of biohydrogenation intermediates in lambs fed oil may be due to accumulation of lipid in the rumen, which would reduce the extent of biohydrogenation (O'Kelly and Spiers, 1991). Triacylglycerol (**TAG**) from oil would presumably be more available than TAG from the other two sources. Lipolysis of TAG in the rumen of lambs fed oil should occur within 2 h (Batmen and Jenkins, 1998), whereas lipolysis of TAG in the seed would be delayed because the TAG would not be as readily accessible for ruminal enzyme attack as the TAG in oil. Subsequently, free 18:2*c*9*c*12 would be liberated more rapidly in the rumen of lambs fed oil. Accumulation of free 18:2*c*9*c*12 inhibits complete biohydrogenation (Noble et. al., 1974) because of inhibition of bacterial growth (Harfoot et al., 1973) and inhibition of bacterial hydrogenases (Griinari and Bauman, 1999).

Intestinal Disappearance

Apparent small intestinal disappearance (g/d) of 16:0 ($P = 0.02$), 18:0 ($P = 0.002$), 18:1*t*9 ($P = 0.07$), 18:1*t*11 ($P = 0.07$), 18:2*c*9*t*11 ($P = 0.008$), and 18:2*t*10*c*12 ($P = 0.09$) were greater for the diets supplemented with safflower fatty acids (Table 3). An increase in absorption of these fatty acids is expected to increase concentration in bodily tissues (Bolte et al., 2002) and milk (Lake et al., 2007) of ruminant animals fed these lipid sources.

Among the fat supplemented lambs there was a linear increase for 18:1*t*10 ($P = 0.03$) and 18:1*c*9 ($P = 0.05$), and a quadratic response for apparent small intestinal disappearance of 18:2*c*9*t*11 ($P = 0.005$) and 18:2*t*10*c*12 ($P = 0.06$) primarily because of an increase in oil-fed lambs.

Expressed as a percentage of fatty acids entering the duodenum, a quadratic response was noted for 16:0 ($P = 0.05$) because of an increase for lambs fed cracked seeds (Table 3). A quadratic response for intestinal disappearance was noted for 18:0 ($P = 0.01$) because the oil treatment had

the lowest digestibility coefficient for this fatty acid. A linear increase (P = 0.08), however, was noted for percentage of 18:2*c*9*c*12 digested in the small intestine because of greater digestion of this fatty acid by lambs fed oil. Our observations were consistent with Kucuk et al. (2004) who reported that intestinal digestibility of 18:2*c*9*c*12 was greater than intestinal digestibility of 18:0 in lambs that had an increase in 18-carbon PUFA entering the small intestine.

Contrary to the original hypothesis, feeding lambs whole seeds did not protect safflower fatty acids from ruminal biohydrogenation. Furthermore, amount of total fatty acids disappearing from the small intestine was not affected ($P \geq 0.48$) by form of supplemental safflower lipid (Table 3). Supplementing fatty acids in the form of oil extracted from high-linoleate safflower seeds enhanced intestinal digestibility of 18:2*c*9*c*12, but decreased intestinal digestibility of 18:0. Supplementing fatty acids in the form of oil seems to be the most effective strategy to increase status of 18:1*c*9 and 18-carbon PUFA in lambs fed diets containing 3% added fat.

Implications

Total energy available from fatty acids digested in the small intestine would be comparable regardless of how safflower seeds were processed. Nevertheless, producers are more likely to change composition of food products derived from ruminants fed oil vs. whole or cracked seeds when diets are supplemented to provide 3% added fat.

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Table 2. Dry matter intake (g/d), fatty acid intake (g/d), and duodenal flow of fatty acid (g/d) in lambs fed a basal diet (Con) or diets with safflower fat in the form of whole (Wh) or cracked (Cr) seeds or oil extracted from the seeds

	Dietary treatment					Contrasts[1]		
Item	Con	Wh	Cr	Oil	SEM[2]	Con vs. Fat-suppl	L	Q
DMI, g/d	1163.7	1083.2	1134.4	1162.6	50.4	0.55	0.50	0.42
	Fatty acid intake, g/d							
16:0	2.82	4.42	4.64	4.99	0.12	<0.001	0.01	0.64
18:0	0.62	1.31	1.37	2.16	0.15	0.001	0.01	0.09
18:1*c*9	8.01	8.78	9.12	11.50	0.34	0.004	0.001	0.05
18:2*c*9*c*12	9.64	30.93	32.00	37.13	0.84	<0.001	0.002	0.10
18:3*c*9*c*12*c*15	0.92	0.65	0.88	0.78	0.12	0.33	0.45	0.32
Unidentified	0.30	1.53	1.53	0.79	0.11	<0.001	0.003	0.03
Total	22.31	48.16	49.54	57.36	1.32	<0.001	0.003	0.09
	Duodenal flow, g/d							
16:0	4.45	6.51	5.60	6.18	0.42	0.02	0.60	0.20
18:0	19.37	41.69	36.11	44.20	2.66	<0.001	0.53	0.08
18:1*t*9	0.14	0.25	0.20	0.31	0.05	0.08	0.43	0.19
18:1*t*10	0.09	0.12	0.10	1.06	0.24	0.26	0.03	0.14
18:1*t*11	0.88	1.87	1.46	1.52	0.30	0.07	0.44	0.53
18:1*t*12	0.12	0.33	0.28	0.33	0.05	0.02	0.98	0.46
18:1*t*13	0.28	0.75	0.64	0.68	0.11	0.02	0.68	0.61
18:1*c*9	2.73	1.82	1.76	2.57	0.20	0.03	0.04	0.13
18:1*c*11	0.24	0.40	0.40	0.39	0.05	0.03	0.97	0.85
18:1*c*12	0.05	0.34	0.18	0.15	0.08	0.11	0.14	0.55
18:2*c*9*t*11	0.02	0.02	0.02	0.04	0.003	0.02	0.003	0.009
18:2*t*10*c*12	0.000	0.005	0.003	0.04	0.007	0.10	0.01	0.07
18:2*c*9*c*12	1.68	3.03	2.54	3.44	0.28	0.007	0.34	0.09
18:3*c*9*c*12*c*15	0.25	0.21	0.23	0.24	0.02	0.27	0.24	0.77
Total	33.47	61.73	53.48	65.07	4.03	0.001	0.20	0.18

1Orthogonal contrasts included Control (Con) vs. Fat-supplemented (Fat-suppl) diets and linear (L) and quadratic (Q) effects of degree of safflower seed processing (whole, cracked, and oil).
[2]n = 4.

Table 3. Apparent disappearance from the small intestine (g/d) and digestibility coefficients (% entering the duodenum) in lambs fed a basal diet (Con) or diets with safflower fat in the form of whole (Wh) or cracked (Cr) seeds or oil extracted from the seeds

	Dietary treatment					Contrasts[1]		
Item	Con	Wh	Cr	Oil	SEM[2]	Con vs. Fat-suppl	L	Q
	Intestinal disappearance, g/d							
16:0	3.80	5.39	4.80	5.02	0.36	0.02	0.48	0.39
18:0	17.05	31.10	29.92	30.38	2.23	0.002	0.83	0.78
18:1*t*9	0.13	0.24	0.19	0.29	0.04	0.07	0.48	0.21
18:1*t*10	0.08	0.12	0.10	0.91	0.21	0.26	0.03	0.15
18:1*t*11	0.80	1.70	1.33	1.35	0.26	0.07	0.38	0.57
18:1*t*12	0.11	0.26	0.25	0.28	0.04	0.02	0.99	0.52
18:1*t*13	0.26	0.66	0.59	0.59	0.10	0.02	0.62	0.73
18:1*c*9	1.98	1.31	1.29	1.98	0.19	0.08	0.05	0.18
18:1*c*11	0.21	0.35	0.37	0.36	0.05	0.03	0.91	0.81
18:1*c*12	0.05	0.33	0.18	0.11	0.08	0.13	0.09	0.70
18:2*c*9*t*11	0.01	0.02	0.01	0.04	0.002	0.008	0.002	0.005
18:2*t*10*c*12	0.000	0.005	0.003	0.04	0.007	0.09	0.01	0.06
18:2*c*9*c*12	1.24	2.25	1.91	2.93	0.29	0.02	0.15	0.11
18:3*c*9*c*12*c*15	0.19	0.14	0.08	0.18	0.02	0.39	0.18	0.60
Total	28.36	47.32	44.33	47.32	3.26	0.003	0.98	0.48
	Intestinal digestibility coefficient, %							
16:0	85.68	82.79	86.01	81.38	1.34	0.19	0.48	0.05
18:0	88.20	74.64	83.32	68.72	2.67	0.006	0.17	0.01
18:1*t*9	95.87	97.49	98.80	92.40	2.43	0.90	0.19	0.24
18:1*t*10	92.93	96.85	97.49	82.42	3.60	0.88	0.03	0.13
18:1*t*11	91.52	91.12	92.16	88.01	1.37	0.52	0.16	0.17
18:1*t*12	90.66	87.70	90.54	87.09	2.54	0.48	0.87	0.35
18:1*t*13	94.69	88.43	92.00	86.97	1.52	0.02	0.52	0.06
18:1*c*9	73.17	71.68	72.47	76.70	3.42	0.91	0.34	0.70
18:1*c*11	86.09	89.98	91.72	92.59	0.81	0.001	0.06	0.68
18:1*c*12	100.00	99.44	100.00	73.85	7.92	0.37	0.06	0.22
18:2*c*9*t*11	57.78	67.01	63.00	80.64	5.13	0.08	0.11	0.14
18:2*t*10*c*12[3]	-	-	-	-				
18:2*c*9*c*12	74.30	74.70	74.53	84.43	3.26	0.38	0.08	0.25
18:3*c*9*c*12*c*15	74.99	68.73	77.22	76.72	3.55	0.86	0.16	0.34
Total	84.98	76.62	83.26	72.95	1.95	0.02	0.23	0.01

[1]Orthogonal contrasts included Control (Con) vs. Fat-supplemented (Fat-suppl) diets and linear (L) and quadratic (Q) effects of degree of safflower seed processing (whole, cracked, and oil).
[2]n = 4.
[3]Insufficient observations for statistical analysis.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

THE CORTICOTROPHIN RELEASING HORMONE AS A STRONG CANDIDATE GENE FOR MARBLING AND SUBCUTANEOUS FAT DEPTH IN BEEF CATTLE

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ABSTRACT: The corticotropin releasing hormone (*CRH*) gene is mapped on bovine chromosome 14 (BTA14), where more than 30 fat-related quantitative trait loci QTL) have been reported in dairy and beef cattle. The gene regulates secretion of adrenocortocotrophin hormone, the hypothalamic-pituitary-adrenal axis and multiple hypothalamic functions, therefore, we hypothesize that *CRH* is a strong candidate gene for beef marbling score (BMS) and subcutaneous fat depth (SFD) in a Wagyu x Limousin F_2 population. A total of five single nucleotide polymorphisms (SNPs) were identified, including AAFC03076794.1: *g.9657C>T*, *c.10718G>C*, *c.10841G>A*, *c.10893A>C* and *c.10936G>C*. Among these four cSNPs, *c.10718G>C*, *c.10841G>A*, and *c.10936G>C* are missense mutations, leading to amino acid changes from arginine to proline, from serine to asparagine and from aspartic acid to histidine, respectively. These five SNPs were genotyped on ~250 F_2 progeny, but four were selected as tagging SNPs for association analysis due to no historical recombination observed between *c.10718G>C* and *c.10893A>C*. Statistical analysis showed *g.9657C>T*, *c.10718G>C* and *c.10936G>C* as well as their haplotypes had significant effects on SFD, but only *c.10936G>C* was significantly associated with BMS. An SNP in the promoter led to a gain/loss of a CpG site and four tentative regulatory binding sites. Different haplotypes among four cSNPs had significant impact on the mRNA secondary structures, but no associations with phenotypes. Overall, our results provide further evidence that *CRH* is a strong candidate gene for a concordant QTL related to lipid metabolism in mammals.
Key word: CRH, SNPs, haplotypes, beef marbling score, subcutaneous fat depth.

Introduction

Genome wide screenings using microsatellite markers have shown that bovine chromosome 14 (BTA14) harbors quantitative trait loci (QTL) for both beef marbling score (BMS) and subcutaneous fat depth (SFD) in beef cattle. In a half-sib family produced by mating a Brahman x Hereford sire to Hereford, Angus, Hereford x Angus and MARCIII dams a fat-depth QTL was identified at 16 cM, while a marbling QTL was located at 47 cM on BTA14 (Casas et al., 2003). QTL for marbling on BTA14 was also detected in a half-sib family of 348 purebred Japanese Wagyu steers, but it is located at 53 cM (Mizoshita et al., 2004).

Both diacylglycerol O-acyltransferase 1 (*DGAT1*) and thyroglobulin (*TG*) have been proposed as potential candidate genes for marbling and fat depth QTLs on BTA14, because they affect lipid metabolism. The DGAT1 enzyme utilizes diacylglycerol and fatty acyl CoA as substrates in order to catalyze the final stage of triacylglycerol synthesis, while thyroglobulin is the glycoprotein precursor to the thyroid hormones whose metabolism is important for energy expenditure and dissipation of heat in tissues. However, associations of these two genes with both marbling and fat depth have been reported inconsistently among different populations. Hence, it indicates that genes underlying QTLs for both marbling and fat depth on BTA14 remain unclear.

Corticotrophin releasing hormone (CRH) is involved in many biological and physiological actions and functions. CRH plays an important role as the major hypothalamic releasing factor for pituitary ACTH secretion (Seasholtz et al., 2002), which regulates glucocorticoid and catecolamines to mediate stress response. Interestingly, there is increasing evidence supporting the involvement of CRH peptide in the regulation of energy balance and body weight, influencing both food intake and sympathetically mediated thermogenesis. Furthermore, ACTH secretion under a stress environment stimulates glucocorticoids, which help return the stress system to homeostasis and mediate many metabolic changes, such as increases of leptin production (Buchanan et al., 2005). As the *CRH* gene is located on BTA14, we decided to validate its candidacy for BMS and SFD in a Wagyu x Limousin F_2 cross.

Materials and Methods

Animals. A Wagyu x Limousin reference population was generated as described previously (Jiang et al., 2005). However, DNA extraction was conducted in the USDA laboratory. BMS was a subjective measure of the amount of intramuscular fat in the *longissimus* muscle based on USDA standards (http://www.ams.usda.gov/). SFD was measured at the 12-13th rib interface perpendicular to the outside surface at a point three-fourths the length of the longissimus muscle from its chine bone end.

DNA sequences and primer design. The cDNA sequence (CO895988) and genomic DNA contig (AAFC03076794) of the bovine *CRH* gene were retrieved from GenBank databases and were used to determine genomic organization by sequence alignment. Two pairs

of primers were designed to target both of the proximal promoter and exon 1, as well as exon 2 region of the bovine *CRH* gene.

Mutation detection and genotyping. Approximately 50 ng of genomic DNA each from six Wagyu x Limousin F_1 bulls was amplified in a final volume of 10 μL that contained 12.5 ng of each primer, 150 μM dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl and 0.25U of Platinum Taq polymerase (Invitrogen, Carlsbad, CA). A single nucleotide polymorphism (SNP) was detected in the promoter/exon 1 product, which was genotyped with restriction enzyme *Hha*I. Four SNPs were identified in exon 2 region and were genotyped with direct sequencing.

Data analysis. The degrees of Hardy-Weinberg equilibrium within each marker and linkage disequilibrium among different markers in the bovine *CRH* gene were estimated using the HAPLOVIEW program (Barrett et al. 2005). The adjusted phenotypes were then used in a subsequent association analysis using the GLM (general linear model) procedure of SAS v9.1 and quantitative transmission disequilibrium test (QTDT) with *P* value <0.05. Pair-wise comparisons of least squares means were performed using a protected t-test.

Results

Annotation of the bovine CRH gene. Although a sequence (AF340152) with a complete coding sequence for the bovine gene was submitted to the GenBank in 2001, it represents the exon 2 region only. In the present study, therefore, we did a BLAST search against the bovine EST (expressed sequence tags) database using the sequence AF340152 as a reference and retrieved a full-length cDNA sequence (CO895988) for the bovine CRH gene. Alignment between the cDNA sequence and the genomic sequence determined the genomic organization of bovine CRH gene (Figure 1).

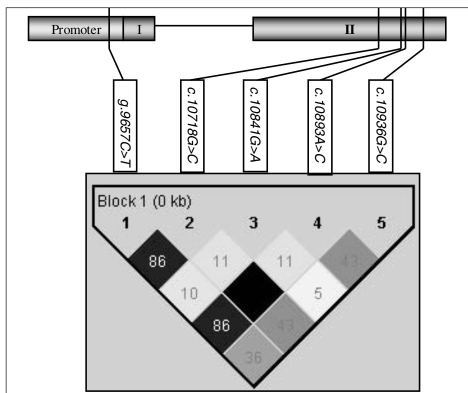

Figure 1. Genomic organization and haplotype analysis of the bovine *CRH* gene. Blue bar: exon I and II; straight line: intron. Pairwise linkage disequilibrium relationship for 5 mutations (g.9657 C>T, c.10718G>C, c10841 G>A, c. 10893 A>C and c.10936G>C) is illustrated based on r^2 measurements.

SNPs and haplotypes. A total of five SNPs were identified, including one SNP (*g.9657C>T*) in the proximal promoter region and four SNPs (*c.10718G>C*, *c.10841G>A*, *c.10893A>C* and *c.10936G>C*) in exon 2 region. Genotyping on ~250 F_2 progeny indicated that all five SNPs fall into the Hardy-Weinberg equilibrium (P>0.05). HAPLOVIEW analysis indicated that among these five SNPs, two SNPs *c.10718G>C* and *c.10893A>C* have no-historical recombination by forming two haplotypes of *CC* and *GA* (Figure 1).

Promoter SNP and potential regulatory binding sites. Screening the proximal promoter region using MatInspector (Quandt et al. 1995) revealed that mutation *g.9657C*>T eliminates four possible transcription factor binding sites, including neuron restrictive silencer factor (**NRSF**), E2F transcription factor, CDE-CHR binding factor-1 (**CDF-1**) and transcription factor CP2 (Figure 2). The promoter region flanking the polymorphic site in cattle is highly conserved in other mammals, such as human (NT_008183.18), chimpanzee (XM_519792.2), rhesus monkey (XM_001094433.1), mouse (NW_001030719.1), rat (M54987.1), sheep (M22853.1), dog (AB162117) and pig (DQ358705.1) (Figure 3).

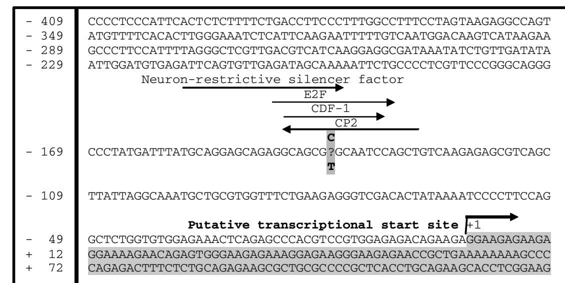

Figure 2. Nucleotide sequence of the proximal promoter region of the bovine *CRH* gene. The putative transcription start site is numbered as +1. The polymorphic site is bold and shadowed by green and exon 1 is highlighted in turquoise.

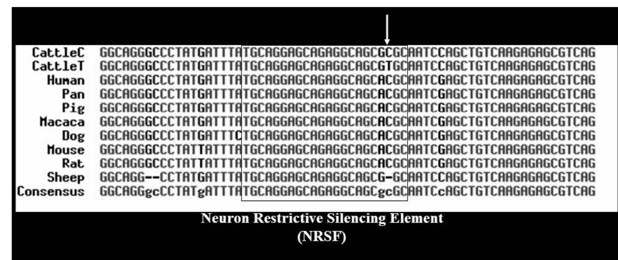

Figure 3. Sequence alignment of the CRH proximal promoter region among nine species with a conserved binding site for NRSF (boxed). The polymorphic site in the promoter of bovine CRH was detected (see arrow) with the allele T eliminating the conserved binding site.

Coding SNPs and the mRNA secondary structure. HAPLOVIEW analysis indicated that four cSNP form five haplotypes: *GGAG*, *CGCG*, *CGCC*, *CACG* and

GAAG. The Mfold program was used to predict how these haplotypes affect mRNA secondary structure involving a complete coding sequence of 573 bp for the preprohormone of the bovine *CRH* gene. Figure 4a shows that the first three haplotypes (*GGAG*, *CGCG* and *CGCC*) gave the same secondary structures. The secondary structures of the last two haplotypes are illustrated in Figures 4b and 4c, respectively, but they just slightly differ from each other (see arrows inside the boxes). However, there was a remarkable difference in the secondary structure between the first three haplotypes and the last two haplotypes. Obviously, polymorphic site *c.10841G>A* plays a critical role in determining the secondary structure of the *CRH* mRNA in cattle.

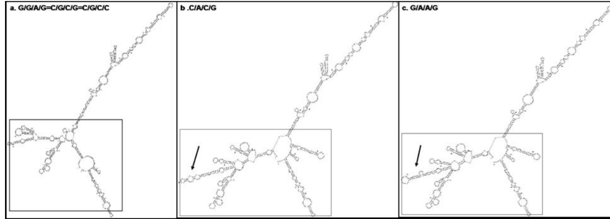

Figure 4. Predicted secondary mRNA structure of bovine *CRH* gene based on five haplotypes. a) represents *GGAG, CGCG* and *CGCC*, b) represents *CACG* and c) represents *GAAG* haplotype. The arrow shows the slightly different structure discovered between *CACG* and *GAAG* haplotypes.

Association analysis of SNPs with SFD and marbling. As both SNPs - *c.10718G>C* and *c.10893A>C* have no-historical recombination events in the population, four tagging SNPs - *g.9657C>T*, *c.10718G>C*, *c.10841G>A* and *c.10936G>C* were used in the association analysis. Except the SNP *c.10841G>A*, all other three SNPs were significantly associated with SFD (Table 1). The difference in SFD between two homozygotes reached 0.12 inches at *g.9657C>T* (P<0.01), 0.10 inches at *c.10718G>C* (P<0.001) and 0.11 inches at *c.10936G>C* (P<0.005), respectively, which account for 0.56 – 0.67 standard deviation of the trait in the population. However, only one SNP *c.10936G>C* had a significant effect on BMS (Table 1). Animals with *CC* genotypes had marbling scores that were 0.549 (P<0.05) and 0.399 (P<0.05) lower than animals with *GG* and *CG* genotypes, which account for 0.549 and 0.399 standard deviations for the trait, respectively.

Association analysis of haplotypes with SFD. As indicated above, three SNPs were associated with SFD in the reference population. Therefore, we decided to determine how their haplotypes affect the trait. Any haplotype with five or less animals was excluded in the analysis. Figure 5a shows an association plot of haplotypes between *c.10718G>C* and *c.10936G>C* with SFD measurements in inches. The SFD of haplotype *GGGG* was 0.146 inches (P<0.05) greater than its *CCCC* counterpart. Animals with CC*GG* (Figure 5b) had SFD that was 0.102 inches (P<0.05) greater than animals with

Table 1. Association analysis of the bovine *CRH* gene with SFD and BMS in Wagyu x Limousine F2 crosses.

Marker	Genotype	#Animals	SFD (in inches)			BMS (in inches)		
			LSM ± S.E.	P_{GLM}	P_{QTDT}	LSM ± S.E.	P_{GLM}	P_{QTDT}
g.9657C>T	CC	43	0.485 ± 0.024[a]	0.001	0.0002	6.010±0.147[a]	0.669	0.026
	CT	107	0.396 ± 0.015[b]			5.882±0.093[a]		
	TT	82	0.365 ± 0.017[b]			5.809±0.106[a]		
c.10718G>C	CC	84	0.357 ± 0.017[a]	0.002	0.0004	5.791±0.106	0.477	0.254
	CG	111	0.403 ± 0.015[b]			5.939±0.092		
	GG	43	0.458 ± 0.024[c]			5.973±0.148		
c.10841G>A	AG	36	0.441 ± 0.026	0.067	0.0653	5.921±0.160	0.847	0.846
	GG	204	0.388 ± 0.011			5.887±0.068		
c.10936G>C	CC	33	0.333 ± 0.028[a]	0.002	0.0005	5.493±0.168[a]	0.022	0.009
	CG	118	0.383 ± 0.014[a]			5.892±0.088[b]		
	GG	88	0.438 ± 0.017[b]			6.042±0.103[b]		

* Means within a column with different superscripts are significantly different (P<0.01)

TTCC haplotype. The same trend was observed (Figure 5c) with a difference of 0.177 inches when comparing *CCGG* and *TTCC* haplotypes.

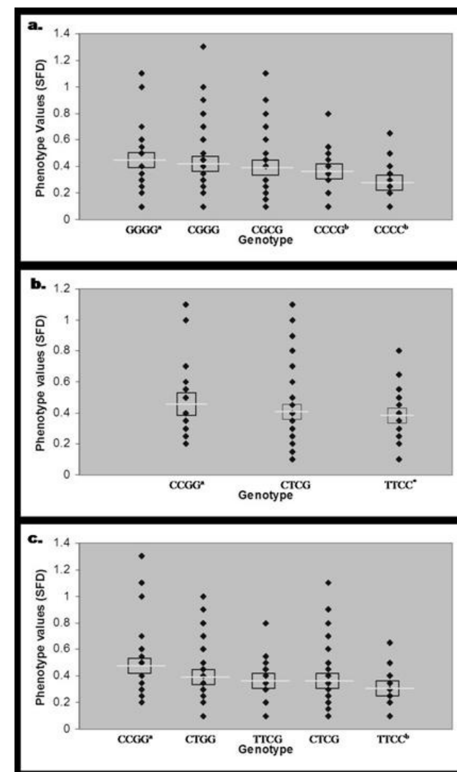

Figure 5. Association plot of haplotypes with SFD values (in inches). a) haplotypes between *c.10718G>C* and *c.10936G>C*, b) haplotypes between *g.9657C>T* and *c.10718G>C* and c) haplotypes between *g.9657C>T* and *c.10936G>C*. Different superscripts show significant differences (P values<0.05) between the two compared haplotypes.

Discussion

CRH, released from the hypothalamus to the anterior pituitary under stress condition, stimulates secretion of *ACTH*, which up-regulates the cortisol level. Cortisol has profound metabolic effects, such as stimulating

gluconeogenesis (in the liver), inhibiting glucose uptake (in muscle and adipose tissue) and stimulating fat breakdown (in adipose tissue). Transgenic mice that over-express CRH exhibits muscle wasting, decreased linear growth and obesity, which is caused by an increase in endogenous cortisol. Furthermore, SNPs in the porcine CRH gene were significantly associated with back fat thickness, carcass length, average daily gain and longissimus muscle area (Murani et al., 2006). In a Charolais-cross steer population, Buchanan and colleagues (2005) reported that three SNPs in the bovine *CRH* gene were highly associated with end-of-test rib eye area ($P < 0.034$) and hot carcass weight ($P < 0.0015$). All these data indicate that *CRH* is a strong candidate gene for concordant QTLs related to body composition and energy metabolism.

In the present study, a SNP found in the promoter region is located 138 bases from the putative transcriptional start site and forms a CpG site when allele C occurs. If this CpG site is methylated, transcriptional activity could be severely suppressed by inhibition of a sequence-specific transcription factor binding region because of the alteration by the methylated cytosine in the recognition sites, blockage by some CpG binding protein (such as MeCP-1 and MeCP2) and alteration of chromatin structures (Kudo et al., 1995). Furthermore, the allele T eliminates four potential regulatory binding sites: NRSF, E2F transcription factor, CDF-1 and transcription factor CP2. Cross species alignment indicated that the proximal promoter region flanking the polymorphic site is highly conserved in nine mammalian species, suggesting evolutionary importance of the region in the transcription regulatory sites of CRH. However, only NRSF is conserved among species. Seth et al. (2001) showed that NRSF was found in the first intronic region of CRH and it represses the gene expression through a HDAC-dependent mechanism. However, NRSF also acts as an enhancer of transcription activity. When a RE-1/NRSE region is either disrupted or deleted from the intronic region of CRH, a significant 1.2-2.5 fold up regulation in reporter activity was observed (Seth et al., 2001).

Four SNPs were also detected in the exon 2 coding region of the bovine *CRH* gene. One mutation (*c.10893A>C*) results in silent mutation, where the remaining, *c.10718G>C*, *c.10841G>A*, and *c.10936G>C,* are missense mutations that lead to amino acid alterations from arginine to proline, from serine to asparagine and from aspartic acid to histidine, respectively. According to Majewski and Ott (2003), arginine, aspartic acid and histidine are the least mutable amino acids with mutability of 0.365, 0.424 and 0.482, respectively. The missense mutation *c.10841G>A* was associated with neither marbling nor SFD in the reference population, but it impacted the secondary structure of the bovine CRH mRNA significantly (Figure 4). The *c.10718G>C* was significantly associated with SFD, while the *c.10936G>C* affected both SFD and marbling (Table 1). Therefore, in this case it appears that there is no association between mRNA secondary structure and phenotype. In addition, three SNPs (*g.9657C>T*, *c.10718G>C* and *c.10936G>C*) had also significant haplotype effects on SFD (Figure 5). Overall, our study confirmed that *CRH* is a strong candidate gene that regulates lipid metabolism in mammals. However, the center locations of QTLs on BTA14 detected in different experiments vary for both marbling and SFD (Casas et al., 2003). This implies that other possible candidate genes on the chromosome might be involved in lipogenesis, which needs to be further explored.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

GENETIC PARAMETER ESTIMATES FOR ULTRASOUND INDICATORS OF CARCASS

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ABSTRACT: Inclusion of embryo transfer data in large scale genetic evaluations has been severely limited, mainly due to concerns over the ability to properly account for maternal effects of the foster dam. As an animal ages, the effect of his or her dam on measured traits typically decreases. For traits like ultrasound indicators of carcass performance, usually measured around a year of age, the absence of maternal effects would allow the inclusion of ultrasound observations from embryo transfer animals without the need to adjust for maternal ability of the recipient dam. The objective of this study was to determine whether maternal effects significantly influence ultrasound observations measured on the population of non-embryo transfer Red Angus cattle. Ultrasound data from the Red Angus Association of America, consisting of 26,193 ultrasound observations for traits scan weight (SCWT), ultrasound rib eye area (UREA), ultrasound back fat (UBF), percent intramuscular fat (%IMF) and percent retail product (%RP), were used in the analyses. Each ultrasound trait was first analyzed using a single trait animal model with a random additive genetic effect. A second analysis, evaluated each trait using univariate animal models with random direct and maternal effects. Full and reduced models were compared using likelihood ratio tests. Heritability estimates for the direct additive effect were 0.23, 0.35, 0.30 0.42 and 0.48 and maternal effect were 0.06, 0.04, 0.03, 0.06 and 0.02 for SCWT, UREA, %IMF, UBF and %RP, respectively. Likelihood ratio tests indicated that the model containing maternal effects fit the data significantly better ($P < 0.01$) in all cases. These results indicate a significant maternal effect on yearling ultrasound observations. Therefore, including data from embryo transfer animals would not be appropriate if the maternal effects of foster dams are unaccounted for.

Key Words: Beef Cattle, Heritability, Ultrasound, Maternal Effects

Introduction

Inclusion of data from embryo transfer (**ET**) calves in large scale genetic evaluations has been largely limited due to concerns over how to properly account for the maternal effects of the foster dam. Methodologies for properly accounting for these effects have been outlined by Schaeffer and Kennedy (1989) and Van Vleck (1990). These methods could be incorporated into a genetic evaluation, although, concerns over how to properly account for selection and management of foster dams still exist in these methods.

As an animal ages, the extent to which maternal effects influences performance decreases. Given the nature of ultrasound observations which are typically recorded around a year of age, maternal effects may no longer have an important influence on performance. If this were the case, data from ET calves could be included in genetic evaluations without having to account for foster dam effects.

While a multitude of studies have evaluated the importance of maternal effects on traits through weaning (Koots et al., 1994), few studies have reported the strength of maternal influence on ultrasound observations. Previous research has shown the magnitude of these effects varies depending on the type of trait, with back fat being the most heavily influenced followed by rib eye area (Johnson et al., 1993; Robinson et al., 1993; Shephard et al., 1996). Scan weight, percent intramuscular fat and percent retail product were not evaluated in these studies.

Currently, ET data is not included in national cattle evaluations performed for the Red Angus Association of America (**RAAA**). If it can be determined that an animal's maternal ability does not influence their calf's ultrasound observations using non-ET RAAA population data, ET observations could also be included in genetic evaluation. Therefore, the objective of this study was to determine whether maternal effects significantly influence ultrasound observations measured on the population of non-ET Red Angus cattle.

Materials and Methods

A total of 26,193 animals with ultrasound observations were obtained from the RAAA. Traits included in the analysis were scan weight (**SCWT**), ultrasound rib eye area (**UREA**), percent intramuscular fat (**%IMF**), ultrasound back fat (**UBF**) and percent retail product (**%RP**).

Ultrasound contemporary groups were formed using the following information.

1. Weaning Work Group
2. Weaning Management Group
3. Weaning Computer Generated Group
4. Yearling Work Group
5. Yearling Management Group
6. Yearling Computer Generated Group
7. Ultrasound Management Code

8. Ultrasound Work Group
9. Ultrasound Date
10. Sex of the Individual

Formation of contemporary groups in this manner resulted in 2,640 unique groups with an average size of 9.9 animals per group. The minimum and maximum contemporary group size was 3 and 79 animals, respectively. In order for observations to be included in the analysis scan weights were required to be less than 816 kg and ultrasound rib eye areas less than 129 cm^2. Animals were removed from the analysis if they were outside the ranges for average daily gain (less than 0 or greater than 3.2 kg / day), scan age (less than 300 days of age or greater than 480 days of age), and if scan weight was less than 204 kg for bulls and less than 181 kg for heifers. These observations were then adjusted to a 365 d endpoint for bulls and 385 d endpoint for heifers (D. E. Wilson, 2005, unpublished data).

Editing the data in this manner resulted in a final data file consisting of 26,087 records. For the purpose of estimating variance components, a 3-generation pedigree was built for the individuals in this final data file. This final pedigree consisted of 70,240 individual animals, along with 6,687 unique sires, 39,280 unique dams and 5,301 unique maternal grandsires. The average inbreeding of animals in the pedigree was 0.02.

Variance Component Estimation

Ultrasound observations were analyzed using two separate models. First, heritability estimates were obtained for additive direct genetic effects for each trait singly using the model represented in matrix form below,

$$y = Xb + Z_d u_d + e$$

where **y** was a vector of observations, **X** and $\mathbf{Z_d}$ were known incidence matrices relating the fixed effects in **b** and the random direct animal genetic effects in $\mathbf{u_d}$ to the observations in **y**, and **e** was a vector of uncorrelated residual errors. The random effects in the model were assumed to have means of zero and variances represented by

$$\mathrm{var}\begin{bmatrix} u_d \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_d^2 & 0 \\ 0 & I_N\sigma_e^2 \end{bmatrix}.$$

In the above equation, **A** was Wright's numerator relationship matrix and $\mathbf{I_N}$ was an identity matrix with an order equal to the number of animals with observations. The values σ_d^2 and σ_e^2 represented the direct additive genetic and residual variances, respectively.

Second, these traits were analyzed using a model that contained an additional random maternal genetic effect and a direct – maternal genetic covariance. Estimates of heritability for direct and maternal genetic effects for each trait were obtained using the equation

$$y = Xb + \begin{bmatrix} Z_d & Z_m \end{bmatrix}\begin{bmatrix} u_d \\ u_m \end{bmatrix} + e$$

In the above equation $\mathbf{Z_m}$ was a known incidence matrix relating the maternal random animal effects in $\mathbf{u_m}$ to observations in **y.** Other terms were as previously defined. Random effects in this model were assumed to have means of zero, and variances represented by

$$\mathrm{var}\begin{bmatrix} u_d \\ u_m \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_d^2 & A\sigma_{d,m} & 0 \\ A\sigma_{d,m} & A\sigma_m^2 & 0 \\ 0 & 0 & I_N\sigma_e^2 \end{bmatrix},$$

where $\sigma_{d,m}$ and σ_m^2 represented the direct – maternal genetic covariance and maternal additive genetic variance, respectively.

Ultrasound contemporary group was the sole fixed effect in both analyses. Heritabilities and genetic correlations were estimated using the statistical software package ASREML (Gilmour et al., 2002).

Model Comparison

Likelihood ratio tests similar to those described by Beckman et al. (2007), were used to determine whether or not the full model (direct and maternal effects) fit the data significantly better than the reduced (direct effects only) model. This type of test can only be applied when the parameters of the full model completely encompass the parameters of the reduced model with no differences in data. The test statistic was calculated as follows

$$D = 2\left|\log L_F - \log L_R\right|,$$

where D represents twice the absolute difference between full model REML logL ($\log L_F$) and reduced model REML logL ($\log L_R$). The null hypotheses (H_0) stated full models were not significantly better than simple models. The D is distributed as a Chi-square distribution with degrees of freedom being the difference in the number of parameters fit in the model.

Results and Discussion

Ultrasound summary statistics are shown below in table 1. Percent retail product is a composite trait calculated from SCWT, UREA and BF, and the total number of observations is equal to the number of animals with known SCWT, UREA and UBF observations.

Table 1. Summary statistics for each of the analyzed traits.[1]

	N	Mean	SD[2]	Min[2]	Max[2]
SCWT	26,017	499	55.9	259.1	758.6
UREA	25,884	79.68	10.58	36.45	131.61
%IMF	25,610	3.255	0.801	0.707	8.977
UBF	25,930	0.635	0.281	0.058	1.826
%RP	25,820	65.9	1.5	59.4	72.9

[1]SCWT = Scan Weight (kg); UREA = Ultrasound Rib Eye Area (cm^2); % IMF= Percent Intramuscular Fat (%); UBF = Ultrasound Back Fat (cm); % RP = Percent Retail Product (%)

[2]Standard Deviation, Min = Minimum Value, Max = Maximum Value

Direct heritability estimates obtained from model 1 are shown below in table 2. Percent retail product had the highest heritability estimate (0.50 ± 0.02) of all the traits. Ultrasound back fat heritability was estimated to be 0.47 ± 0.02, which was similar to estimates reported by Robinson et al., (1993) and Shephard et al. (1996), however much larger than the estimate of 0.11 reported by Johnson et al., (1993) describing a population of Brangus cattle. The cattle in this study were all scanned near a year of age, but typically at that age animals have yet to reach puberty and are still growing which may greatly reduce the phenotypic variability of fat depth.

Results from model 2 are also shown below in table 2. Percent retail product still had the highest direct heritability estimate (0.48 ± 0.03), and the remaining direct estimates were similar to those in model 1. Maternal heritability estimates were 0.06 ± 0.01, 0.04 ± 0.01, 0.02 ± 0.01, 0.06 ± 0.02 and 0.02 ± 0.01 for SCWT, UREA, %IMF, UBF and %RP, respectively. The addition of the maternal component to model 2 caused an average decrease in the direct heritability estimate of 8.3%. A similar trend was noticed by Robinson et al., (1993) where they observed direct heritability estimates to be biased upwards of 2 and 5% for ultrasound rib eye area and ultrasound back fat when maternal genetic effects were ignored.

Although, the maternal heritability estimates are quite small (≤ 0.06), the inclusion of the maternal genetic effect in model 2 did more than just re-partition the additive genetic variation. All traits except UREA had an increase in residual variance and decreased additive genetic variance. A similar trend was noticed by Crews et al. (1999) for carcass traits.

Previous studies have shown non-zero maternal heritability estimates for UBF (Johnson et al., 1993; Robinson et al., 1993), and rather large genetic correlations (-0.69) between UBF and weaning weight maternal (Shephard et al., 1996). When lean traits such as UREA have been considered, other studies have failed to show proof of any maternal genetic influence which is opposite of the results seen here.

Log likelihood estimates, likelihood ratio test statistics and associated p-values are shown in table 3. The p-values reported were calculated from a Chi-Square distribution with 2 degrees of freedom. Percent retail product was the least significant trait with a p-value of 0.0045. These reported p-values indicate that the inclusion of a maternal genetic effect significantly increased the likelihood of the model for each of the traits.

Results presented above suggest that a maternal genetic effect influences ultrasound observations measured in RAAA cattle. These maternal genetic effects are influencing UBF and SCWT by as much as 6% of the total phenotypic variation. Although these effects are statistically significant, the question that remains is whether there a biological basis for including these effects in or excluding these effects from the ultrasound analysis, and whether the inclusion of maternal effects leads to meaningful re-ranking of bulls in the RAAA genetic evaluation.

Implications

Analysis of ultrasound observations has shown that the inclusion of a maternal genetic effect significantly improves the ability of a model to account for the variability in ultrasound observations. As a result, inclusion of embryo transfer data into an ultrasound genetic evaluation without properly accounting for maternal effects of foster dams is not recommended.

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Table 2. Summary of parameters[1] obtained from direct and maternal ultrasound models[2] including heritability and genetic correlations (SE) for each of the evaluated ultrasound traits[3].

	Model 1			Model 2					
	σ^2_d	σ^2_e	h^2_d	σ^2_d	σ^2_m	σ^2_e	h^2_d	h^2_m	r_{dm}
SCWT	1953.5	4454.5	0.30 (0.02)	1451	351	4527	0.23 (0.02)	0.06 (0.01)	0.02 (0.14)
UREA	0.4757	0.9127	0.34 (0.02)	0.4831	0.0503	0.8994	0.35 (0.03)	0.04 (0.01)	0.29 (0.10)
% IMF	0.1307	0.2742	0.32 (0.02)	0.1191	0.0105	0.2756	0.30 (0.03)	0.02 (0.01)	0.06 (0.15)
UBF	0.00176	0.00202	0.47 (0.02)	0.00157	0.00022	0.00204	0.42 (0.03)	0.06 (0.02)	0.17 (0.10)
% RP	0.8093	0.8189	0.50 (0.02)	0.7699	0.01381	0.8263	0.48 (0.03)	0.02 (0.01)	-0.08 (0.01)

[1] h^2_d = Direct heritability; h^2_m = Maternal heritability; r_{dm} = direct-maternal correlation; σ^2_d = Direct genetic variance; σ^2_m = Maternal genetic variance; σ^2_e = Error variance

[2] Model 1 = Model containing a direct genetic effect only; Model 2 = Model containing direct, maternal and direct / maternal covariance.

[3] SCWT = Scan Weight (kg); UREA = Ultrasound Rib Eye Area (cm^2); % IMF= Percent Intramuscular Fat (%); UBF = Ultrasound Back Fat (cm); % RP = Percent Retail Product (%)

Table 3. Log likelihood (logL) estimates for each of the models[1] and associated significance values for each of the ultrasound traits[2].

	Model 1	Model 2		
	logL	logL	Test Statistic	P value[3]
Scan Weight	-115854	-115834	40	2.06 e-9
Rib Eye Area	-17008.1	-17001.4	13.4	0.0012
% IMF	-2744.4	-2735.8	17.1	0.00019
Fat	52253.9	52266.6	25.4	3.05 e-6
% RP	-18189.2	-18183.8	10.8	0.0045

[1] Model 1 = Model containing a direct genetic effect only; Model 2 = Model containing direct, maternal and direct / maternal covariance.

[2] SCWT = Scan Weight (kg); UREA = Ultrasound Rib Eye Area (cm^2); % IMF= Percent Intramuscular Fat (%); UBF = Ultrasound Back Fat (cm); % RP = Percent Retail Product (%)

[3] These values were obtained from a Chi Square Distribution with 2 degrees of freedom.

Proceedings, Western Section, American Society of Animal Science
Vol. 58, 2007

NITRIC OXIDE AND POLYAMINE RESPONSE TO PROSTAGLANDIN $F_{2\alpha}$ IN THE EARLY AND MID STAGE OVINE CORPUS LUTEUM[1]

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ABSTRACT: Nitric oxide (NO) and polyamines (PA) are local mediators of corpus luteum (CL) function. The requisite enzymes in the NO and PA biosynthetic pathways are endothelial (eNOS) and inducible (iNOS) nitric oxide synthases and ornithine decarboxylase (ODC), respectively. This study determined mRNA encoding eNOS, iNOS, and ODC, NO concentration and ODC activity in early (d 4; d 0 = estrus; n = 54) and mid (d 10; n = 54) stage ovine CL in response to $PGF_{2\alpha}$. Ovine CL were collected via ovariectomy on d 4 or d 10 at 0, 4, 12, or 24 h following $PGF_{2\alpha}$ (10 mg, i.m. at -4 and 0 h) or saline (2 mL, i.m. at -4 and 0 h). Real time RT-PCR was used to quantify relative amounts of mRNA. Message for eNOS, iNOS and ODC were greater ($P \leq 0.01$) in d 4 CL compared to d 10 CL. Relative amounts of mRNA encoding eNOS increased ($P < 0.05$) at 0 h in d 10 but not d 4 CL in response to $PGF_{2\alpha}$. In both d 4 and d 10 CL treated with $PGF_{2\alpha}$, mRNA encoding eNOS decreased ($P < 0.05$) at 12 and 24 h. Relative amounts of mRNA encoding iNOS in response to $PGF_{2\alpha}$ were greater ($P \leq 0.01$) in d 4 CL compared to d 10 CL (0.72 vs. 0.43 ± 0.06 arbitrary units, respectively). On both d 4 and d 10, $PGF_{2\alpha}$ resulted in reduced ($P \leq 0.01$) iNOS mRNA at 0 and 4 h. No response was detected to $PGF_{2\alpha}$ on d 4 or d 10 in relative amounts of mRNA encoding ODC ($P \geq 0.05$). Day 4 CL responded to $PGF_{2\alpha}$ with increased ($P \leq 0.05$) NO from 0 to 4 h (11.1 vs. 21.8 ± 3.6 nmol/g), which then returned to 0 h concentrations. Day 4 CL had greater ($P \leq 0.01$) ODC activity than d 10 CL (44.3 vs. 4.8 ± 4.1 cpm/µg). No differences were observed among d 10 CL in ODC activity ($P > 0.61$). Day 4 CL responded to $PGF_{2\alpha}$ with increased ($P \leq 0.01$) ODC activity from 0 to 24 h (29.4 vs. 69.1 ± 11.7 cpm/µg). These results indicate both mRNA for NO and PA synthetic enzymes and corresponding product or enzyme activity fluctuate during the ovine luteal phase and in response to $PGF_{2\alpha}$.

Key Words: Nitric oxide, polyamines, corpus luteum

Introduction

Establishment and regression of a functional corpus luteum (**CL**) is required for pregnancy and estrous cyclicity in mammalian species. While $PGF_{2\alpha}$ is the endocrine signal for luteolysis, other autocrine/paracrine signals may mediate the effect of $PGF_{2\alpha}$ on the CL. Nitric oxide (**NO**) and polyamines (**PA**), which originate from L-arginine, may be part of the luteal response to $PGF_{2\alpha}$.

Nitric oxide has been implicated as a mediator of the $PGF_{2\alpha}$ luteolytic mechanism. Nitric oxide is synthesized by NO synthase (**NOS**) enzymes, which are present in the corpus luteum (Bryant et al., 2003). Intraluteal inhibition of NOS blocked the luteolytic effect of $PGF_{2\alpha}$ in d 17 bovine CL, extending cycle length to 25 d, and increased progesterone secretion from mid and late stage CL (Jaroszewski and Hansel, 2000). Conversely, administration of a NO donor decreased progesterone from bovine CL in vivo (Nelson et al., 2004) and cultured bovine luteal cells (Jaroszewski et al., 2003).

Polyamines are required for optimal cell growth and differentiation (Tabor and Tabor, 1984) and are abundant in rapidly proliferating tissues such as tumors (Bachrach, 2004). The developing CL undergoes processes similar to those observed in carcinogenesis. Depletion of PA inhibited angiogenesis (Jasnis et al., 1994), and decreased translation of an extra cellular matrix protein (Wallon et al., 1994) in tumor cells. Angiogenesis and extracellular matrix remodeling are critical to early luteal development. The preovulatory rise in PA is critical for establishing progesterone production in the mouse (Bastida et al., 2002).

The requisite enzymes in production of NO and PA are endothelial (**eNOS**) and inducible (**iNOS**) NOS and ornithine decarboxylase (**ODC**), respectively. This study investigated the role of NO and PA in autoregulation of the CL by determining mRNA encoding eNOS, iNOS, and ODC, NO concentration and ODC activity in early and mid ovine CL in response to $PGF_{2\alpha}$.

Materials and Methods

All animal procedures were approved by the New Mexico State University animal care and use committee (2004-017). Ewes were exposed to vasectomized rams and monitored for estrus behavior. Day of estrus (d 0) was recorded and ewes were assigned to one of sixteen treatments in a 2 x 2 x 4 factorial arrangement using a completely random design. On d 4 or d 10, ewes received two injections i.m. of 10 mg $PGF_{2\alpha}$ (Lutalyse, Upjohn, Kalamazoo, MI) or 2 mL saline (pH 7.4) 4 h apart. Time of the second injection was recorded as 0 h and CL were harvested at 0, 4, 12, or 24 h via ovariectomy. Upon collection, CL were snap frozen in liquid nitrogen and stored at -80°C.

Total RNA was extracted from CL for analysis of steady state levels of mRNA encoding eNOS, iNOS, and ODC using Tri Reagent RNA/DNA/protein isolation reagent (Molecular Research Center, Inc., Cincinnati, OH). Concentration and purity of RNA samples were determined by spectrophotometric analysis of absorbance at 260 and

[1] Research funded by 2004-35203-14717 NRI-CSREES-USDA to D. E. Hawkins and K. K. Kane.

280 nm. Relative quantification of mRNA for eNOS, iNOS and ODC, normalized with β-2 microglobulin, was performed by real-time RT-PCR using Bio-Rad SYBR Green Mastermix and iCycler iQ detection system (Bio-Rad Laboratories, Hercules, CA). Primers were generated with Beacon Designer (Bio-Rad Laboratories, Hercules, CA) utilizing GenBank sequences for the appropriate enzyme. Relative abundance of each mRNA was quantified using Genex Gene Expression Macro (Bio-Rad Laboratories, Hercules, CA). Intraassay CV were 1.0, 1.1, and 1.5% and inter-assay CV were 1.1, 2.9 and 2.9% for eNOS, iNOS and ODC, respectively.

Nitric oxide concentration was determined by quantification of nitrate, a stable metabolite of NO (Li et al., 2001), in filtered luteal tissue extract using colormetric Griess reagent nitrite/nitrate kit (Active Motif, Carlsbad, CA), with modifications. Luteal tissue extract was filtered over a 10 kDa membrane (Millipore, Billerica, MA). Nitrite and NO were oxidized to nitrate by reaction with NADPH and nitrate reductase. Nitrate concentration was determined using a 96 well plate reader with absorbance at 540 nm. Concentration of NO was expressed as nmol per g luteal tissue. Intra- and interassay CV were 2.6 and 6.9%, respectively.

Activity of ODC was determined via radiometric assay described previously (Coleman and Pegg, 1998), with modifications. Luteal tissue homogenate was incubated with 1-[14-C]-L-ornithine and cofactors of the ODC reaction. Labeled carbon dioxide, a stoichiometric byproduct of the ODC reaction, was collected on filter paper soaked in 10% (w/v) KOH. Amount of radiolabeled carbon dioxide produced was determined by quantification of beta emission. Ornithine decarboxylase activity was expressed as cpm per µg protein, as determined using a protein determination kit based on the Bradford method (Cayman Chemical, Ann Arbor, MI). Intra- and interassay CV were 7% and 13%, respectively.

Relative amounts of mRNA, NO concentration, and ODC enzyme activity were analyzed using the GLM procedure of SAS (SAS Institute, Cary, NC) with CL as the experimental unit. Day, hour, and treatment ($PGF_{2\alpha}$ or saline) were included in the class statement, and response variables were modeled against day, hour, treatment and their interactions. No interactions were detected among class variables for any response variable.

Results

Greater ($P < 0.01$) amounts of eNOS mRNA were detected in both $PGF_{2\alpha}$ (1.95 vs. 1.17 ± 0.11 arbitrary units, n = 26) and saline (2.17 vs. 0.97 ± 0.10 arbitrary units, n = 28) treatments in d 4 compared to d 10 CL. In d 4 CL, eNOS mRNA did not differ ($P > 0.16$) between $PGF_{2\alpha}$ and saline treatments at any time (Table 1). Amounts of eNOS mRNA decreased ($P < 0.05$) from 0 and 4 h to 12 and 24 h in d 4 CL following treatment with $PGF_{2\alpha}$ (Table 1). Messenger RNA encoding eNOS did not change ($P > 0.09$) in response to treatment with saline in d 4 CL (Table 1). In d 10 CL, eNOS mRNA increased ($P < 0.05$) at 0 h in $PGF_{2\alpha}$ compared to saline, but did not differ ($P > 0.13$) between treatments at 4, 12, or 24 h (Table 1). Messenger RNA encoding eNOS did not respond ($P > 0.28$) to treatment with saline in d 10 CL (Table 1). However, within $PGF_{2\alpha}$-treated d 10 CL, eNOS mRNA remained high ($P > 0.60$) from 0 to 4 h, then decreased ($P \leq 0.01$) at 12 and 24 h (Table 1). Similar response to $PGF_{2\alpha}$ was observed for eNOS mRNA in d 4 and d 10 CL (Table 1).

Day 4 CL treated with $PGF_{2\alpha}$ had greater amounts of ($P < 0.01$) iNOS mRNA than $PGF_{2\alpha}$-treated d 10 CL (0.72 vs. 0.43 ± 0.06 arbitrary units, n = 26), but no difference ($P > 0.87$) in iNOS mRNA was detected between d 4 and d 10 CL (1.02 and 1.00 ± 0.06 arbitrary units, respectively, n = 28) treated with saline. In d 4 CL, iNOS mRNA was less ($P < 0.01$) at 0 h and tended to be less ($P < 0.07$) at 4 h in $PGF_{2\alpha}$ as compared to saline (Table 1). At 12 and 24 h, iNOS mRNA was similar ($P > 0.12$) in $PGF_{2\alpha}$ and saline-treated d 4 CL (Table 1). Messenger RNA encoding iNOS remained constant ($P > 0.08$) in d 4 saline-treated CL (Table 1). However, in d 4 CL following the initial decrease in $PGF_{2\alpha}$ compared to saline at 0 and 4 h, iNOS mRNA increased ($P < 0.01$) at 12 and 24 h (Table 1). In response to $PGF_{2\alpha}$, iNOS mRNA in d 10 CL increased ($P < 0.05$) from 0 h to 12 and 24 h (Table 1). In d 10 saline-treated CL, mRNA encoding iNOS decreased ($P < 0.05$) from 0 to 4 and 12 h, then returned ($P < 0.05$) to values similar to 0 h at 24 h (Table 1). Nonetheless, in d 10 CL, less ($P < 0.05$) iNOS mRNA was detected in $PGF_{2\alpha}$ compared to saline treatments at all times (Table 1).

Concentration of NO did not differ ($P > 0.24$) between d 4 and d 10 (15.3 and 15.8 ± 1.2 nmol/g, respectively, n = 54) nor $PGF_{2\alpha}$ and saline-treated (16.5 and 14.5 ± 1.2 nmol/g, respectively, n = 52) CL. In response to $PGF_{2\alpha}$, d 4 CL NO concentration almost doubled ($P < 0.05$) from 0 to 4 h, then returned to concentrations similar ($P > 0.14$) to 0 h at 12 and 24 h (Table 1). In d 10 CL, NO concentration did not respond ($P > 0.31$) to treatment with saline or $PGF_{2\alpha}$ at any time (Table 1).

Day 4 CL had greater ($P < 0.01$) amounts of mRNA encoding ODC in both $PGF_{2\alpha}$ (3.65 vs. 1.18 ± 0.19 arbitrary units, n = 26) and saline (3.47 vs. 1.49 ± 0.19 arbitrary units, n = 29) treatments than d 10 CL. In d 4 CL, ODC mRNA tended to increase ($P < 0.08$) in response to $PGF_{2\alpha}$ compared to saline at 0 h (Table 2). However, no differences ($P > 0.14$) in ODC mRNA were detected between $PGF_{2\alpha}$ and saline treatments at 4, 12, or 24 h (Table 2). No response ($P > 0.21$) in ODC mRNA to treatment with $PGF_{2\alpha}$ was detected over time in d 4 CL (Table 2). Messenger RNA encoding ODC was less ($P < 0.05$) in d 4 CL at 0, 4, and 12 h compared to 24 h following treatment with saline (Table 2). In d 10 CL, ODC mRNA was similar ($P > 0.27$) from 0 to 12 hr, but decreased ($P < 0.05$) at 24 h in $PGF_{2\alpha}$ compared to saline-treated CL (Table 2). In response to treatment with $PGF_{2\alpha}$ in the d 10 CL, mRNA encoding ODC decreased ($P < 0.05$) at 12 and 24 h compared to 0 and 4 h (Table 2). Saline did not affect ($P > 0.36$) ODC mRNA at any time in d 10 CL (Table 2).

Greater ($P < 0.01$) ODC activity was detected in both $PGF_{2\alpha}$ (46.7 vs. 5.0 ± 5.8 cpm/µg, n = 26) and saline (44.8 vs. 4.7 ± 5.5 cpm/µg, n = 29) treatments in d 4 compared to d 10 CL. In response to $PGF_{2\alpha}$, d 4 CL ODC

activity tended to be less ($P < 0.08$) at 4 h, but remained similar ($P > 0.29$) to saline at 0, 12, and 24 h (Table 2). Ornithine decarboxylase activity remained similar ($P > 0.18$) at all times in saline-treated d 4 CL (Table 2). By 24 h following $PGF_{2\alpha}$ in d 4 CL, ODC activity was greater ($P \leq 0.01$) than activity at 0 or 4 h (Table 2). In d 10 CL, no ($P > 0.61$) differences in ODC activity were detected in response to treatment or over time (Table 2).

Discussion

Early luteal development entails cell growth and angiogenesis following luteinization. Luteolysis results in the functional and structural demise of the CL. Nitric oxide and PA are potential mediators of these processes, which determine the lifespan and progesterone secretory function of the CL.

Nitric oxide is a potent vasodilatory and angiogenic hormone that may be important in luteal development and maintenance. The highly vascular CL uses the most oxygen and receives the most blood supply of any tissue on a per gram basis (Niswender et al., 1976; Niswender et al., 2000). In agreement with Grazul-Bilska et al. (2006), greater mRNA encoding eNOS was detected in early as compared to mid ovine CL. Intriguingly, greater iNOS mRNA was detected in d 4 as compared to d 10 CL from $PGF_{2\alpha}$ but not saline-treated ewes. This is in contrast to Rosiansky-Sultan et al. (2006) who found greater iNOS mRNA in untreated early compared to mid stage bovine CL. In d 4 CL, mRNA for iNOS decreased at 0 h whereas mRNA for eNOS decreased 12 h following $PGF_{2\alpha}$. Since one injection of $PGF_{2\alpha}$ was administered at -4 h, changes at 0 h in CL from $PGF_{2\alpha}$ compared to saline treated ewes may be attributed to the first injection of $PGF_{2\alpha}$. Nitric oxide concentration was not different in early compared to mid stage CL. However, in d 4 CL, NO concentration increased 4 h following $PGF_{2\alpha}$.

Nitric oxide inhibits progesterone secretion from mid stage cultured bovine mixed luteal cells (Jaroszewski et al., 2003) and bovine CL in vivo (Nelson et al., 2004) and upregulates pro-apoptotic genes in dispersed bovine luteal cells (Korzekwa et al., 2006). In vivo inhibition of NOS counteracts the luteolytic effect of $PGF_{2\alpha}$ (Skarzynski et al., 2003). In $PGF_{2\alpha}$-treated d 10 CL, mRNA encoding eNOS increased at 0 h compared to saline then began to decrease over time, whereas mRNA encoding iNOS decreased at 0 h compared to saline then began to increase over time. In both early and mid stage CL, although both eNOS and iNOS mRNA responded to treatment with $PGF_{2\alpha}$, changes in amounts of mRNA encoding iNOS occurred sooner following treatment and(or) were of greater relative magnitude than changes in mRNA encoding eNOS. These data indicate that $PGF_{2\alpha}$ may play a regulatory role in NO synthesis in the early and mid stage ruminant CL.

Early luteal development involves cell growth of the newly differentiated luteal cells to form the fully functional corpus luteum. Polyamines are required for cell growth and differentiation (Tabor and Tabor, 1984) and may be involved in regulation of luteal growth, CL maintenance, and luteolysis (Kane et al., 2004). Early CL had more mRNA encoding ODC and greater ODC activity than did mid stage CL. This is in agreement with previous data from our laboratory showing elevated mRNA for ODC and greater PA concentrations in early as compared to mid stage CL (Raymond, 1996; Kane et al., 2004). Furthermore, in early CL, despite persistent levels of mRNA encoding ODC in response to $PGF_{2\alpha}$, $PGF_{2\alpha}$ tended to decrease ODC activity at 4 h, indicating post-transcriptional regulation of ODC activity in response to $PGF_{2\alpha}$. Greater mRNA and activity for ODC in early compared to mid stage CL support a role for PA in early luteal growth. Although ODC activity in d 10 CL was almost tenfold less than ODC activity in d 4 CL, decreased mRNA encoding ODC in response to $PGF_{2\alpha}$ in the d 10 CL may contribute to decreased cell viability following $PGF_{2\alpha}$ in mid stage CL.

In summary, early CL had greater amounts of mRNA encoding eNOS and ODC and had greater ODC activity than mid stage CL. Messenger RNA for iNOS was similar in early and mid stage CL treated with saline, but was greater in early compared to mid stage CL treated with $PGF_{2\alpha}$. Early and mid stage CL responses to $PGF_{2\alpha}$ were similar for eNOS and iNOS mRNA. Concentration of NO responded to $PGF_{2\alpha}$ in early but not mid stage CL. Messenger RNA for ODC responded to $PGF_{2\alpha}$ in mid but not early stage CL. Activity of ODC responded to $PGF_{2\alpha}$ in early CL, but no response was detected in mid stage CL.

Implications

This research demonstrates that mRNA encoding NO and PA synthetic enzymes and their activities differ in early and mid stage CL and respond to treatment with $PGF_{2\alpha}$. Thus, NO and PA may mediate early luteal growth and luteolytic processes in the ruminant CL.

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Table 1. Relative amounts of mRNA encoding endothelial (eNOS) and inducible (iNOS) nitric oxide synthase (arbitrary units) and nitric oxide concentration (NO; nmol/g tissue) in early (d 4) and mid stage (d 10) ovine corpora lutea following two injections i.m. of saline (2 mL per injection) or $PGF_{2\alpha}$ (10 mg per injection) administered 4 h apart (0 h = time of second injection).

			Time after second injection of treatment (h)			
Variable[1]	Day	Treatment	0	4	12	24
eNOS mRNA	4	Saline	2.06	2.53	2.02	2.08
		$PGF_{2\alpha}$	2.33^{a}	2.19^{a}	1.63^{b}	1.67^{b}
	10	Saline	1.08^{x}	1.07	0.79	0.92
		$PGF_{2\alpha}$	1.65^{ay}	1.50^{ab}	0.91^{bc}	0.62^{c}
iNOS mRNA	4	Saline	1.01^{x}	0.84	1.09	1.12
		$PGF_{2\alpha}$	0.32^{ay}	0.52^{a}	1.18^{b}	0.88^{b}
	10	Saline	1.20^{ax}	0.80^{bx}	0.83^{bx}	1.18^{ax}
		$PGF_{2\alpha}$	0.27^{ay}	0.24^{ay}	0.62^{by}	0.59^{aby}
NO	4	Saline	9.2	16.3	13.6	17.4
		$PGF_{2\alpha}$	11.1^{a}	21.8^{b}	14.6^{a}	18.3^{a}
	10	Saline	13.9	17.1	16.4	12.5
		$PGF_{2\alpha}$	15.5	14.5	17.9	18.7

[1]Most conservative standard errors: ± 0.22 arbitrary units, 0.13 arbitrary units, and 3.6 nmol/g tissue for eNOS mRNA, iNOS mRNA and NO concentration, respectively (n = 6).

[ab]Row means with different superscripts differ ($P \leq 0.05$).

[xy]Column means within same day and variable with different superscripts differ ($P \leq 0.05$).

Table 2. Relative amounts of mRNA encoding ornithine decarboxylase (ODC; arbitrary units) and ODC activity (cpm/µg protein) in early (d 4) and mid stage (d 10) ovine corpora lutea following two injections i.m. of saline (2 mL per injection) or $PGF_{2\alpha}$ (10 mg per injection) administered 4 h apart (0 h = time of second injection).

			Time after second injection of treatment (h)			
Variable[1]	Day	Treatment	0	4	12	24
ODC mRNA	4	Saline	2.98^{a}	3.24^{a}	3.23^{a}	4.43^{b}
		$PGF_{2\alpha}$	3.99	3.28	3.63	3.68
	10	Saline	1.29	1.47	1.39	1.80^{x}
		$PGF_{2\alpha}$	1.49^{ab}	1.77^{a}	0.81^{b}	0.66^{by}
ODC Activity	4	Saline	35.8	45.7	41.1	56.7
		$PGF_{2\alpha}$	29.4^{ab}	17.7^{a}	58.5^{bc}	69.1^{c}
	10	Saline	2.8	7.1	3.1	5.7
		$PGF_{2\alpha}$	6.1	10.5	1.7	1.6

[1]Most conservative standard errors: ± 0.40 arbitrary units and 11.7 cpm/µg protein for ODC mRNA and activity, respectively (n = 6).

[ab]Row means with different superscripts differ ($P \leq 0.05$).

[xy]Column means within same day and variable with different superscripts differ ($P \leq 0.05$).

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EXPRESSION AND DISTRIBUTION OF UREA TRANSPORTER-B IN LAMBS FED INCREASING DIETARY PROTEIN

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ABSTRACT: Level of dietary CP may affect the expression and distribution of urea transporter-B (UT-B) in tissues important to N recycling in ruminants. Fifteen Dorset wether lambs (initial BW = 45.8 ± 1.3 kg) were blocked by initial BW and assigned to one of three treatments within a randomized complete block design. Lambs were fed a basal diet of mature crested wheatgrass hay (4.2% CP, 59% NDF) for ad libitum consumption plus one of three soybean meal-based supplements to achieve concentrations of 6, 9, or 12% dietary CP. Lambs were randomly euthanized within block on d 28 and samples (5 g) taken from the gastrointestinal tract, liver, kidney, and parotid salivary gland were snap frozen and later processed for Western blot analyses for UT-B. Immunoblotting using a rabbit polyclonal antibody to UT-B confirmed the presence of distinct 32 kDa (consistent with a non-glycosylated UT-B protein) and 47 kDa (probable N-glycosylated form of UT-B) protein bands in all nine tissues. A broad 32 kDa band and a slight 47 kDa band were detected in samples from the liver, reticulum, dorsal rumen, and ventral rumen. The cecum, large colon, spiral colon, and parotid salivary gland displayed a slight 32 kDa band and a visible band at 47 kDa. The kidney displayed slight bands at both 32 kDa and 47 kDa. No treatments differences in the abundance (arbitrary densitometry units) of the 32 kDa ($P \geq 0.15$) or 47 kDa ($P \geq 0.51$) UT-B bands or in the 32 kDa/47 kDa ratio ($P \geq 0.38$) were detected within tissues. However, the 32 kDa/47 kDa ratio differed ($P = 0.05$) across tissues, being greatest for the ventral rumen (92.5), liver (79.7), and reticulum (27.7), intermediate for the dorsal rumen (8.0), and lowest for the kidney (1.8), large colon (0.8), spiral colon (0.8), cecum (0.6), and parotid salivary gland (0.2). Although dietary CP level had no effect on expression of either form of UT-B, differences in the 32 kDa/47 kDa ratio among tissues may suggest a possible role of N-glycosylation in the regulation of UT-B function.

Key Words: lambs, nitrogen recycling, urea transporters

Introduction

Ruminant livestock consuming low-quality (< 6% CP) forages often rely upon their ability to recycle blood urea back to the rumen to sustain microbial metabolism. Blood urea may be recycled via diffusion across the ruminal wall from the bloodstream, or it may enter the rumen via saliva (Kennedy and Milligan, 1980). Harmeyer and Martens (1980) noted that consumption of a low-protein diet increases the permeability of the gastrointestinal tract to urea, challenging the conventional wisdom that recycling occurs solely via simple diffusion (Kennedy and Milligan, 1980). The discovery of urea transporters (**UT**) in mammalian systems (You et al., 1993) and their role in conservation of N in animals fed low protein diets (Isozaki et al., 1994) provides a potential mechanism by which the N recycling process could be regulated. The predominant UT present in gastrointestinal tract tissues is UT-B, which has been identified in sheep (Marini et al., 2004) and cattle (Marini and Van Amburgh, 2003; Stewart et al., 2005). Despite early research, little is known about the expression, tissue distribution, and physiological role of UT-B in the N recycling process. Moreover, the effects of dietary protein on UT-B expression requires further investigation, given the importance of N recycling in ruminant livestock consuming seasonally low quality forages in the Western U.S. We hypothesize that UT-B is widely distributed in the gastrointestinal tract and associated tissues of ruminants, and that UT-B expression will be up-regulated when those animals consume low protein diets. Therefore, our objectives were to 1) evaluate the expression and distribution of UT-B within the gastrointestinal tract, liver, kidney, and parotid salivary gland of lambs consuming low quality forage, and 2) to assess the role of dietary protein supply on UT-B abundance in these tissues.

Materials and Methods

Animals and diets

Fifteen Dorset wether lambs (45.8 ± 1.3 kg initial BW) were blocked by initial BW and randomly assigned to one of three treatments within a randomized complete block design. Wethers were housed in individual metabolism crates (1.4 × 0.6 m) in a temperature controlled room (20ºC) under constant lighting. Wethers were fed a basal diet of mature crested wheatgrass hay (4.2% CP, 59% NDF) for ad libitum consumption in two portions daily at 0630 and 1800. Forage refusals were collected and weighed daily, and amount of forage adjusted to induce a minimum of 10% refusal rate. Wethers were supplemented at 0600 and 1800 daily with one of three protein supplements to achieve dietary CP concentrations (% DM) of 6, 9, or 12% CP (Table 1). Wethers had free access to clean water and a trace mineralized salt block (Iofix T-M, Morton Salt; Chicago, IL; guaranteed analysis [% of DM] 97.1% NaCl, and ≤ 0.35% each of Zn, Mn, Fe, Cu, I, and Co). All procedures were approved by the University of Wyoming Animal Care and Use Committee.

[1]This project was supported in part by the University of Wyoming Faculty Grant-In-Aid Program.

Table 1. Composition of supplements

	LOW[1]	MED	HIGH
Ingredient, % of DM			
Beet pulp	78.3	53.4	28.2
Molasses	10.0	10.0	10.0
Soybean meal	5.5	27.4	49.2
Urea	0.6	3.1	5.6
Calcium carbonate	2.3	3.2	4.2
Vitamin ADE Premix[2]	2.8	2.8	2.8
Chemical			
DM, %	90.1	89.6	88.6
CP, % of DM	10.4	26.1	45.3

[1]Supplements fed to achieve 6% (LOW), 9% (MED) or 12% total dietary CP (% of DM).

[2]Contained 3,628,739 IU vitamin A, 3,628,739 IU vitamin D_3 and 18,144 IU vitamin E per kg.

Tissue Collection

After receiving their supplemented treatments for 28 days, wethers were euthanized randomly within block by injection of Beuthanasia-D Special (Schering-Plough Animal Health, Union, NJ) according to label directions. The lambs were immediately eviscerated, and tissue samples (5 g) collected from the dorsal and ventral rumen, reticulum, cecum, large colon, small (spiral) colon, liver, kidney, and parotid salivary gland. Samples were rinsed with PBS, and immediately snap frozen in liquid N. Samples were stored at -80ºC until protein isolation and Western blot analyses were performed.

Polyclonal antibody production

Polyclonal antibodies were prepared against a peptide (NeoMPS Inc., San Diego, CA) containing the sequence for the carboxy-terminal 19 amino acids known for human UT-B1 (EENRIFYLQAKKRMVESPL) plus an additional cysteine residue at the amino terminus to facilitate conjugation to the antigen carrier keyhole limpet hemocyanin (KLH). Antibodies directed against the human UT-B1 peptide have been successfully used to detect UT-B in cattle (Marini and Van Amburgh, 2003) and sheep (Marini et al., 2004). Two female rabbits received multiple subcutaneous injections of the KLH-conjugated peptide for the production of polyclonal antibodies. Rabbits were bled from the central ear artery and pre-immune serum was compared to post immunization serum using ELISA to ensure that the immunized rabbits were sustaining high antibody titers to UT-B. The anti-UT-B polyclonal antibody was purified using the T-gel method (Pierce, Rockford, IL), and preabsorption with immunizing peptide was used to demonstrate polyclonal antibody specificity.

Western blot analyses

Samples (500 mg tissue) were homogenized in 3 mL RIPA buffer (phosphate buffered saline with 1%NP 40, 0.1% SDS, and 0.5% sodium deoxycholate). Protein concentration was determined using a Bradford Assay Kit (Pierce, Rockford, IL) and 25 µg of protein was loaded per lane on 12% polyacrylaminde SDS gels. Gels were electrophoresed and protein was transferred to 0.2 µm polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA). Membranes were probed with the T-gel purified polyclonal anti-UT-B antibody diluted 1:2,500 in TBST containing 5% non-fat dry milk for 2.5 h at 20ºC. Membranes were subsequently incubated in horseradish peroxidase-conjugated goat anti-rabbit IgG diluted 1:10,000 (Jackson ImmunoResearch, West Grove, PA) in 5% milk/TBST solution for 1 h at 20ºC. Immunoreactive protein bands were visualized by chemiluminescent substrate (Pierce, Rockford, IL). All Western blots within a tissue were processed together, with exposure time dependent upon intensity of signal within tissue type. To normalize samples within tissues, membranes were re-probed using polyclonal antibody for β-actin (Cell Signaling, Danvers, MA) diluted 1:1000 as previously described for UT-B. Autoradiographs were digitized (UN-SCAN-IT_{TM}, Orem, UT) to obtain total pixel counts for the 32 kDa and 47 kDa UT-B bands as well as the 42 kDa β-actin band. Blots were normalized by dividing the total pixels for each band (32 kDa or 47 kDa) by the total pixels obtained for the corresponding β-actin of the same lane/sample. Results were expressed as arbitrary densitometry units per 32kDa and 47kDa UT-B band.

Statistical Analyses

All densitometry data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) using the model for a randomized complete block design. Because the same tissue sample from all lambs was included in a single set of Western blots assayed simultaneously, the model used to detect treatment differences in the 32 and 47 kDa protein bands consisted of weight block and treatment only, and was conducted within a given tissue type. The model used to detect differences in the 32 kDa/47 kDa ratio also included the effects of tissue type and the treatment × tissue type interaction. No treatment × tissue type interactions ($P \geq 0.86$) were detected, and thus only main effects of treatment and tissue type will be discussed. When necessary, separation of treatment means was accomplished using least squares means and Fisher's protected LSD ($P \leq 0.05$).

Results and Discussion

Immunoblotting confirmed the presence of two distinct UT-B protein bands (32 kDa and 47 kDa) in all nine tissues analyzed. Despite their presence in all tissues analyzed, the visual intensity of these UT-B bands differed among tissues. A broad 32 kDa band and a slight 47 kDa band were detected in the dorsal rumen, ventral rumen (Figure 1), reticulum and liver. The expression of UT-B within the liver is significant, as the bulk of recycled urea is derived from ureagenesis within the liver. Conversely, the cecum, large colon, and small (spiral) colon displayed a slight 32 kDa band, and a more intense band at 47 kDa. Both protein bands were apparent in the kidney at nearly the same visual intensity. Interestingly, the parotid salivary gland (Figure 2) displayed an intense protein band at 47 kDa, and a much lighter band at 32 kDa. This is the first known study to have identified UT-B within the parotid salivary gland of any species. Because the parotid salivary gland plays a

major role in the recycling of blood urea back to the rumen via saliva, particularly in ruminants consuming forage-based diets (Kennedy and Milligan, 1980), the identification of UT-B within the salivary glands may prove to be beneficial in improving our understanding of N recycling via the salivary route.

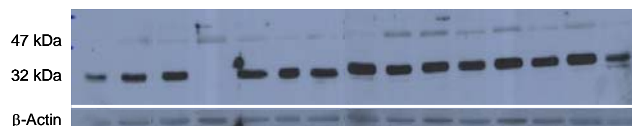

Figure 1. Western blot of samples from the ventral rumen

Figure 2. Western blot of samples from the parotid salivary gland.

Marini and Van Amburgh (2003) reported a broad UT-B protein band in rumen papillae of dairy heifers, and later (Marini et al., 2004) in the duodenum, ileum, and cecum of lambs that is similar in size to our 47 kDa UT-B protein. Likewise, Stewart et al (2005) verified the presence of a 43-54 kDa UT-B protein band in the bovine rumen. However, we believe that the two distinct protein bands detected by our anti-UT-B antibody represent *N*-glycosylated (47 kDa) and non-glycosylated (32 kDa) forms of the UT-B protein. Lucien et al. (2002) predicted that the structure of human UT-B1 contained a single unique functional *N*-glycosylation site, which is consistent with the presence of two distinct UT-B bands as observed in the current study and that of others. Timmer et al. (2001) reported the presence of a broad UT-B protein band between 45 and 65 kDa in human erythrocytes, and between 37 and 51 kDa in rat erythrocytes. However, these broad bands were converted to a distinct tight band at 32 kDa following deglycosylation of those tissues. Similarly, Olives et al. (1995) confirmed the presence of a diffuse 46-60 kDa UT-B band present in human erythrocytes that, when deglycosylated, was reduced to 36 kDa.

Contrary to our initial hypothesis that UT-B abundance would be greatest for animals on the low protein diet, no treatment differences in the abundance (arbitrary densitometry units) of the 32 kDa ($P \geq 0.15$) or 47 kDa ($P \geq 0.51$) UT-B protein bands (Table 2) were detected. Similarly, Marini et al. (2004) reported no differences in UT-B abundance in any of the gastrointestinal tissues analyzed in response to increasing dietary N. In contrast, Marini and Van Amburgh (2003) observed greater expression of UT-B (based upon visual evaluation) in the ruminal mucosa of dairy heifers fed a high N diet (2.97 – 3.4% N) as compared to a low N diet (1.45 – 1.89 % N).

While increasing CP did not influence ($P \geq 0.38$) the 32 kDa/47 kDa ratio within a given tissue, the 32 kDa/47 kDa ratio differed ($P = 0.05$) across tissues, being greatest for the ventral rumen (92.5), liver (79.7), and reticulum (27.7), intermediate for the dorsal rumen (8.0), and lowest for the kidney (1.77), large colon (0.83), spiral colon (0.78), cecum (0.57), and parotid salivary gland (0.20). Differences in the 32 kDa/47 kDa ratio among tissues, reflecting relative differences in the degree of *N*-glycosylation of UT-B, may suggest a possible role of *N*-glycoslylation in the regulation of UT-B function in the ruminant. The importance of *N*-glycosylation to the function of UT-B remains largely unexplained. In other systems, *N*-glycosylation often determines membrane expression level and addressing of polypeptides to the membrane (Varki et al., 2002). However, site-directed mutagenesis to delete the *N*-glycosylation site of human UT-B expressed in *Xenopus* oocytes demonstrated that the lack of *N*-glycosylation did not affect urea uptake when compared to the wild-type UT-B (Lucien et al., 2002). The regulatory pathways affecting UT-B function in *Xenopus* oocytes may differ from that of ruminants, and thus further research into the physiological role of *N*-glycosylation on UT-B function is needed.

Implications

Both *N*-glycosylated and non-glycosylated forms of urea transporter-B (UT-B) are expressed in the gastrointestinal tract, kidney, liver, and parotid salivary gland of lambs fed a forage-based diet. Although dietary protein level had no effect on abundance of either form of UT-B, the relative abundance of the *N*-glycosylated form of UT-B differed across tissues. Further research into the physiological significance of *N*-glycosylation in the regulation of UT-B function is needed.

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Table 2. Effect of dietary crude protein concentration on the abundance (arbitrary densitometry units) of urea transporter-B protein in the gastrointestinal tract and related tissues in lambs fed a forage-based diet.

	Treatment[1]				
Item	Low	Med	High	SEM	$P <$[2]
Ventral Rumen					
32 kDa	7.063	5.095	7.681	1.180	0.31
47 kDa	0.580	0.526	0.492	0.200	0.95
Ratio[3]	22.400	14.660	240.429	125.710	0.38
Liver					
32 kDa	15.041	21.726	25.156	4.833	0.35
47 kDa	0.670	0.523	1.001	0.333	0.60
Ratio	43.328	93.017	102.897	47.038	0.64
Reticulum					
32 kDa	1.599	1.793	1.979	0.155	0.26
47 kDa	0.160	0.140	0.159	0.062	0.97
Ratio	26.665	15.730	40.768	13.388	0.44
Parotid Salivary Gland					
32 kDa	0.271	0.304	0.290	0.044	0.86
47 kDa	1.346	1.641	1.667	0.211	0.51
Ratio	0.211	0.196	0.184	0.034	0.86
Kidney					
32 kDa	0.896	0.517	0.644	0.254	0.58
47 kDa	0.423	0.408	0.382	0.088	0.95
Ratio	2.102	1.410	1.799	0.470	0.60
Dorsal Rumen					
32 kDa	3.809	2.799	3.678	0.367	0.15
47 kDa	0.467	0.431	0.485	0.064	0.84
Ratio	9.296	6.843	7.857	1.331	0.45
Cecum					
32 kDa	0.088	0.072	0.209	0.050	0.15
47 kDa	0.231	0.350	0.515	0.196	0.60
Ratio	0.445	0.715	0.536	0.237	0.72
Small (Spiral) Colon					
32 kDa	0.240	0.248	0.244	0.063	0.99
47 kDa	0.439	0.279	0.366	0.117	0.64
Ratio	0.662	0.886	0.804	0.195	0.72
Large Colon					
32 kDa	0.190	0.332	0.250	0.090	0.55
47 kDa	0.345	0.316	0.568	0.170	0.53
Ratio	0.623	1.133	0.729	0.261	0.38

[1]Treatments consisted of 6% (Low), 9% (Med), and 12% (High) dietary CP (% of DM).
[2]*P*-value of differences between treatments.
[3]Ratio = 32 kDa/47 kDa.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECT OF DURATION OF CIDR TREATMENT ON CONCEPTION AND PREGNANCY RATES IN BEEF HEIFERS USING A TIMED-AI PROTOCOL

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ABSTRACT: The objective of this experiment was to determine the effect of reducing the length of CIDR exposure in a CIDR-based timed-AI synchronization protocol (CIDR-PGF2α-GNRH and AI) on conception and pregnancy rates in beef heifers. British cross-bred heifers (n = 82) were stratified by body weight (BW) and body condition score (BCS) and were randomly subjected to one of the following two treatments: 1) heifers (n = 41) received CIDR (d -7) for 7 d, $PGF_{2\alpha}$ (25 mg) at CIDR removal (d 0), GnRH (75 μg) 56 h after CIDR removal and immediate AI d 3; (7-d CPG); or 2) heifers (n = 41) received CIDR (d -5) for 5 d, $PGF_{2\alpha}$ (25 mg) at CIDR removal (d 0), GnRH (75 μg) 56 h after CIDR removal and immediate AI d 3; (5-d CPG). Signs of behavioral estrus were monitored four times daily two days before CIDR insertion and three days following CIDR removal. Blood samples were collected from all heifers on the day of CIDR insertion (d 7 in 7-d CPG and d 5 in 5-d CPG) and at breeding (d 3). Heifers were exposed to bulls fourteen days after AI. Pregnancy status was determined via transrectal ultrasonography at d 35 and 82 after AI. Data were analyzed by logistic regression. Percentage of heifers detected in estrus was not different between groups (71% and 66% for 7-d CPG and 5-d CPG, respectively). Based on serum progesterone (P_4) results, the synchronization rates were similar between groups. Treatment had an effect ($P < 0.03$) on conception to AI (39% and 65.8 %) for 7-d CPG and 5-d CPG, respectively. Age tended to effect conception to AI ($P = 0.07$), in that as age increased, conception decreased. BW and BCS did not effect conception to AI. Overall pregnancy rate was not different between treatment groups. Body weight had a marginal effect on pregnancy rate ($P = 0.07$), whereas age and BCS did not have an effect on pregnancy rate. Results from this study indicate that reducing the length of CIDR treatment (5 d vs. 7 d) in a CIDR-based timed-AI synchronization protocol may improve conception to AI in beef heifers.

Key Words: Beef Heifers, CIDR, Timed-AI

Introduction

To alleviate the difficulties associated with estrus detection and to increase the AI submission rate, researchers have developed timed-AI protocols. Cosynch (Geary et al., 1998) and Ovsynch (Pursley et al., 1998) are effective timed-AI breeding protocols for use in cows but typically result in unacceptable conception per AI in heifers (Pursley et al., 1997; Martinez et al., 2002). It is not completely understood why these protocols are ineffective in heifers. The low conception percentage resulting from these timed-AI protocols in heifers may be due to the difference in the pattern of follicular growth in heifers vs. cows and the time of initiation of these protocols relative to the estrous cycle (Day et al., 2004; Nebel and Jobst, 1998; Rivera et al., 2004). Heifers have a faster rate of follicular growth than lactating cows (Sartori et al., 2001; Moreira et al., 2000). Sartori et al., (2001) found nearly half of all dairy heifers have three follicular waves, thereby increasing the likelihood of heifers not responding to the first GnRH injection in timed-AI protocols with ovulation or luteinization of the dominant follicle. Heifers that fail to respond to the initial GnRH injection without ovulation also fail to initiate a new follicular wave. The reduced ovulation rate following the first injection of GnRH may also increase the incidence of heifers with a shortened luteal phase characterized by premature estrus (Schmitt et al., 1996). The premature onset of estrus before timed-AI results in reduced conception.

The discovery of the follicular dynamics in heifers has led to modification of timed-AI protocols to improve conception to AI in heifers. In an attempt to further control estrus and ovulation and improve fertility, it has been proposed that ovulation can be synchronized by inducing synchronous emergence of a new follicular wave in the presence of high blood P_4 concentrations. The use of CIDR offers an opportunity to realize the benefits of P_4 in a timed-AI protocol. The Ovsynch-CIDR protocol has been developed in an effort to take advantage of the benefits of a progestin-based regimen and timed-AI. When a CIDR was incorporated into the Cosynch protocol, the pregnancy rate in beef heifers was improved compared to Cosynch alone (Martinez et al., 2002).

In an effort to further improve conception in beef heifers, scientists studied methods to modify CIDR-based protocols. Due to the dynamic nature of follicular waves in heifers, long P_4 exposure may inhibit follicular turnover and delay or inhibit heifers from reaching estrus at a desired synchronized time (Day et al., 2004). These authors concluded that that induction of a new follicular wave with GnRH in the presence of short P_4 exposure, prevents the development of a persistent follicle, thus increasing conception to AI. Therefore, the objective of this study was to determine the effect of reducing the length of CIDR treatment in a CIDR-based timed-AI synchronization protocol (CIDR-PGF2α-GnRH and AI) on conception and

pregnancy rates in beef heifers. It is hypothesized that shortening the length of CIDR treatment from 7 to 5 days would improve reproductive performance in beef heifers.

Materials and Methods

The University of Idaho Institutional Animal Care and Use Committee approved all experimental procedures used in this study. Eighty-two British cross-bred heifers (Angus x Hereford x Simmental) were used in this trial. Before experiment initiation, heifers were weighed and stratified by body weight (**BW**) and then age in one of two treatments: 1) CIDR on d -7, $PGF_{2\alpha}$ (25 mg; Lutalyse) and CIDR removal on d 0, GnRH (75 µg, Cystorelin, Cystorelin; Merial Ltd, Duluth, GA) (im) injection and timed-AI simultaneously on d 3, (**7-d CPG**) (n=41); or 2) CIDR on d -5, $PGF_{2\alpha}$ (25 mg, im; Lutalyse) and CIDR removal on d 0, GnRH (75 µg, im; Cystorelin,) injection and timed-AI simultaneously on d 3 (**5-d CPG**) (n=41). Heifers were observed for signs of estrus 48 h (d -9) before experiment initiation four times daily for a period of one h at 7:00 and 11:00 a.m. and 3:00 and 7:00 p.m. At initiation of the experiment body condition scores (**BCS**) were assessed by two qualified individuals and averages taken from both individual's assessments. Body conditions scores were based on a scale of one to nine, with one being extremely emaciated and nine being overly obese. Using aseptic procedures, on d -7, 7-d CPG heifers received a CIDR and on d -5, 5-d CPG heifers received a CIDR. Both groups were checked for CIDR retention. To avoid potential loss of CIDR devices, tails of the devices were cut, leaving approximately three to four inches of string for removal. On d 0, CIDR inserts were removed from both groups. All heifers were given 25 mg $PGF_{2\alpha}$. All injections included in the treatment protocols were administered in the neck region with 18 gauge needles in accordance with standard beef quality assurance (BQA) guidelines. All heifers were tail chalked to aid in detection of estrus. Fifty-six h following $PGF_{2\alpha}$ injection (d 3), all heifers received 75 µg GnRH and were artificially inseminated by a single inseminator. Semen was used from three Angus sires stratified across treatment groups. Estrous activities were recorded. Two bulls were used for natural service 14 d following timed-AI for a bull to heifer ratio of 1:41. One bull was removed at 22 d and the second bull was removed 51 d after natural service.

To determine P_4 concentrations, blood samples were collected via jugular venipuncture into Vacutainers® from both groups on the day of CIDR insertion (d -7 or d -5) and at insemination (d 3). Blood samples were immediately placed on ice after withdrawal and maintained at 6º C to allow clotting for a period of 24 h. After 24 h of refrigeration, blood samples were centrifuged (International Equipment Company) at 2500 $\times g$, serum was harvested, and kept at -20º C until assayed for P_4. Progesterone concentrations were then determined by radioimmunoassay.

Plasma P_4 concentrations on day of CIDR insertion were used to classify heifers as having high (≥ 1 ng/ml) or low (<1 ng/ml) P_4 concentration. If the first blood sample contained plasma P_4 levels ≥ 1 ng/ml, heifers were classified as cyclic before treatment initiation. The second blood samples were collected to determine if heifers responded to $PGF_{2\alpha}$ with luteolysis and thus synchronized. If samples contained plasma P_4 concentrations <1 ng/ml, it was considered that luteolysis had occurred and heifers were synchronized.

Visual heat detection was conducted 48 h before treatment four times daily for one h each observation. Visual heat detection was also conducted immediately following CIDR removal four times daily for one h each observation until timed-AI.

Pregnancy status was determined via transrectal ultrasonography (Sonovet Co., Mure, Mitaka-Shi Tokyo, Japan; Aloka 500, Aloka, Tokyo, Japan) on d 35 and 82 after AI. Conception percentage was defined as number of animals pregnant per treatment group divided by the total number of animals inseminated per treatment group. Pregnancy rates were defined as number of heifers pregnant after AI and natural service divided by the total number of animals in each treatment group.

Statistical Analysis. Conception and pregnancy data were analyzed by logistical regression using SAS (SAS Inst. Inc. Gary, NC). The full statistical model included the effect of treatment, treatment by BCS, BW and age.

Results and Discussion

Average age for heifers was 397 ± 22.3 and average BW was 329.7 ± 31.5 (Table 1). Mean BCS were 5.6 ± 0.4 for both 7-d CPG and 5-d CPG (Table 1). Treatment had a significant effect on conception to AI ($P < 0.05$). Conception percentages were 39.02% and 65.85% for 7-d CPG and 5-d CPG, respectively (Table 2). Heifers treated with a P_4 releasing device during the second part of the estrous cycle may maintain the second or third wave dominant follicle for an extended period of time after regression of the corpus luteum (Schmitt et al., 1996). This may explain the reduced fertility when progestogens are used for an extended time period (Schmitt et al., 1996; Odde, 1990). Therefore, it is plausible that induction of a new follicular wave with GnRH in the presence of short P_4 exposure, prevents the development of a persistent follicle thus increasing conception to AI (Day et al., 2004).

Treatment did not affect pregnancy rates (95.1%) for either the 7-d CPG or 5-d CPG treatment groups (Table 2).

Table 1. Mean[1] age, body weight (BW) and body condition scores (BCS) of heifers before treatment with 7-d CPG or 5-d CPG protocols.

Treatment[2]	Age (days)	BW	BCS
7-d CPG (n = 41)	396 ± 18.8	327.9 ± 32.4	5.6 ± 0.4
5-d CPG (n = 41)	399 ± 25.4	331.6 ± 30.9	5.6 ± 0.4
Overall (n = 82)	397 ± 22.3	329.7 ± 31.5	5.6 ± 0.4

[1]Means ± SD

[2] 7-d CPG: 7 d CIDR exposure (d -7) + 25 mg $PGF_{2\alpha}$ (d 0) + 75 µg GnRH (56 h after CIDR removal) + TAI; 5-d CPG: 5 d CIDR exposure (d -5) + 25 mg $PGF_{2\alpha}$ (d 0) + 75 µg GnRH (56 h after CIDR removal) + TAI.

Table 2. Incidence of conception and pregnancy rates in beef heifers treated with 7-d CPG or 5-d CPG.

Treatment[1]	Conception per AI	Pregnancy Rate
7-d CPG (n = 41)	39.02%[a]	95.12%
5-d CPG (n = 41)	65.85%[b]	95.12%

[1]7-d CPG: 7 d CIDR exposure (d -7) + 25 mg $PGF_{2\alpha}$ (d 0) + 75 µg GnRH (56 h after CIDR removal) + TAI; 5-d CPG: 5 d CIDR exposure (d -5) + 25 mg $PGF_{2\alpha}$ (d 0) + 75 µg GnRH (56 h after CIDR removal) + TAI.
[a,b]Percentage with different superscript, within the same column differ *($P < 0.05$).*

Mean serum P_4 concentrations were different between groups at CIDR insertion and were 2.3 ng/ml ± 0.23 and 1.41 ng/ml ± 0.23 for 7-d CPG and 5-d CPG, respectively (Table 3). In 7-d CPG, eight heifers had serum P_4 concentrations of less than 1 ng/ml P_4 and 33 heifers had P_4 concentrations above 1 ng/ml. In contrast, in 5-d CPG, 16 heifers had serum P_4 concentrations less than 1 ng/ml and 25 heifers had P_4 concentrations above 1 ng/ml (Table 3). These results indicate that 7-d CPG heifers likely had more cyclic heifers than 5-d CPG. Mean serum P_4 concentrations on the day of breeding showed no difference between groups. Mean serum P_4 concentrations for 7-d CPG were 0.51 ng/ml ± 0.04 and for 5-d CPG were 0.53 ± 0.04 ng/ml. Regardless of treatment, $PGF_{2\alpha}$ caused luteolysis in 95% of heifers, indicating that both groups were successfully synchronized. At breeding, two animals in the 7-d CPG treatment group had P_4 levels above 1 ng/ml, indicating complete luteolysis did not occur in those individuals. However, both of these heifers conceived to AI. In 5-d CPG, two animals also had P_4 levels above 1 ng/ml and only one of these animals conceived to AI.

Table 3. Mean[1] serum progesterone (P_4) levels of beef heifers before CIDR insertion and at day of breeding.

Treatment	P_4 ng/ml Day of CIDR insertion	P_4 ng/ml Day of Breeding
7-d CPG (n = 41)	2.3 ng/ml ± 0.23[a]	0.51 ng/ml ± 0.04
5-d CPG (n = 41)	1.41 ng/ml ± 0.23[b]	0.53 ng/ml ± 0.04
Overall (n = 82)	1.72 ng/ml ± 1.72	0.52 ng/ml ± 0.04

[1]LSMEANS ± SE

[a,b] Means with different superscript within the same column differ *($P < 0.05$).*

Before initiation of the experiment, 34% of heifers for 7-d CPG and 24% of heifers for 5-d CPG were observed in estrus. After CIDR removal and before AI, 71% of heifers for 7-d CPG and 66% of heifers for 5-d CPG were observed in estrus (Table 4).

Table 4. Percentage of heifers observed in estrus before and after CIDR treatment with 7-d CPG or 5-d CPG.

Treatment[1]	% in estrus prior to treatment	% in estrus after treatment
7-d CPG (n = 41)	34%	71%
5-d CPG (n = 41)	24%	66%
Overall (n = 82)	29%	69%

[1]7-d CPG: 7 d CIDR exposure (d -7) + 25 mg $PGF_{2\alpha}$ (d 0) + 75 µg GnRH (56 h after CIDR removal) + TAI; 5-d CPG: 5 d CIDR exposure (d -5) + 25 mg $PGF_{2\alpha}$ (d 0) + 75 µg GnRH (56 h after CIDR removal) + TAI.

Body condition score and BW had no effect on conception percentages. Pruitt and Momont (1990) demonstrated that cows in poor body condition (<5 on a 9-point scale) are less likely to become pregnant during the breeding season and are unlikely to be inseminated early in the breeding season. In the present study, the mean BCS for all cows were 5.6 . The majority of the heifers in the present study had a BCS greater than 5, with only a few heifers scoring less than 5.

Although there is not a clear explanation, age tended to have a marginal effect ($P = 0.07$) on conception. This effect was apparent in that as age increased, conception decreased (Table 5).

Table 5. Odds ratio estimates on the effect of treatment,[1] body condition score (BCS), body weight (BW), and age on incidence of conception in beef heifers.

Effect	Point Estimate[2]	P value	95% Wald Confidence Limits
Treatment	0.35	0.03	0.14 – 0.92
BCS	4.04	0.22	0.43 – 37.56
BW	1.00	0.74	0.99 – 1.01
Age	0.98	0.07	0.95 – 1.00

[1]7-d CPG: 7 d CIDR exposure (d -7) + 25 mg $PGF_{2\alpha}$ (d 0) + 75 µg GnRH (56 h after CIDR removal) + TAI; 5-d CPG: 5 d CIDR exposure (d -5) + 25 mg $PGF_{2\alpha}$ (d 0) + 75 µg GnRH (56 h after CIDR removal) + TAI.

[2]Odds ratio estimates: a value higher than 1 indicates increased risk for conception whereas a value lower than 1 indicates a reduced risk of conception.

Age and BCS did not have an effect on pregnancy rate (P = 0.24, 0.59), respectively. Body weight tended to have an effect on pregnancy rate (P = 0.06) in that as BW increased, pregnancy rate decreased (Table 6). This was an unexpected result as just the opposite would be expected in heifers. No clear explanation is known for this result.

Table 6. Odds ratio estimates on the effect of treatment,[1] body condition score (BCS), body weight (BW), and age on pregnancy rates in beef heifers.

Effect	Point Estimate[2]	P value	95% Wald Confidence Limits
Treatment	1.65	0.67	0.16 – 17.00
BCS	3.62	0.59	0.03 – 405.25
BW	0.98	0.07	0.96 – 1.00
Age	0.97	0.24	0.92 – 1.02

[1]7-d CPG: 7 d CIDR exposure (d -7) + 25 mg $PGF_{2\alpha}$ (d 0) + 75 µg GnRH (56 h after CIDR removal) + TAI; 5-d CPG: 5 d CIDR exposure (d -5) + 25 mg $PGF_{2\alpha}$ (d 0) + 75 µg GnRH (56 h after CIDR removal) + TAI.
[2]Odds ratio estimates: a value higher than 1 indicates increased risk for pregnancy whereas a value lower than 1 indicates a reduced risk for pregnancy.

Pregnancy loss was determined if a heifer was diagnosed pregnant at 35 days post-AI and was either open or had a very early pregnancy at 82 days post-AI. In 7-d CPG, no pregnancy losses were observed. In 5-d CPG, two heifers experienced pregnancy loss. One heifer was diagnosed open at 82 days after AI, whereas the other heifer was pregnant with a 30-day embryo.

Implications

Shortened P_4 exposure for five d in beef heifers increased conception. The 5-d CPG protocol was also desirable as it eliminated one injection of GnRH and reduced the need for additional animal handling. All characteristics of the protocol are desirable from a beef management view point. This protocol may have a significant impact on increased conception in beef heifers.

Acknowledgements

The authors wish to thank Pfizer Animal Health (New York, NY) and Merial (Duluth, GA) for their support of this research through the donation of Lutalyse® and Cystorelin®, respectively. Additionally, the authors wish to thank the Idaho Agricultural Experiment Station for their support of this research.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

SELECTION INTENSITIES, GENERATION INTERVALS AND POPULATION STRUCTURE OF RED ANGUS CATTLE

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ABSTRACT: Livestock industries have a nucleus-multiplier-commercial structure, with nucleus herds dictating the rate and direction of genetic change. The nucleus typically represents a small fraction of the total population but these animals dominate the pedigrees, particularly on the sire pathways. The objectives of this study were to quantify pathways of selection (sires to produce sires, SS; sires to produce dams, SD; dams to produce sires, DS; dams to produce dams, DD) in terms of selection proportions, intensities and generation intervals, and use this information to characterize parental origins within and across herds. Pedigree and breeder records from 1955 to 2006 were obtained from the Red Angus Association of America. Birth records were traced forwards to identify bull (or heifer) calves that contributed to SS/SD (or DS/DD) paths. Proportions of animals that became grandparents in each birth yr were computed to determine selection intensities. The age of grandparents when sons (or daughters) became sires (or dams) was calculated. In each herd, grandparents were coded as having been bred in that herd or an outside herd based on available breeder identification. The percentage of herds that produced animals in each pathway was determined. Average selection intensities based on selected proportions were 2.07, 1.71, 0.96, and 1.83 standard deviations; generation intervals were 4.5, 5.0, 4.4, and 4.9 yr for SS, DS, SD, and DD respectively, from 1955 to 1999. Selection intensities increased over the first 30 yr then declined over the next decade. This resulted from a preference for younger animals reflected in reductions in generation intervals for all paths during the same time. Theoretical genetic gain assuming a correlation between predicted and actual merit of 0.5 had a maximum value of some 0.2 genetic standard deviations per yr from 1975 onwards, up from 0.1 standard deviations More than 50% of animals in all paths were bred in outside herds. Some two thirds herds produced offspring that became dams of dams or dams of sires, while less than half produced paternal animals. Results support the division of pedigree herds into nucleus and multiplier categories.

Key Words: Generation interval, Red Angus cattle, selection intensity.

Introduction

Beef cattle populations exhibit a pyramidal structure, with nucleus, multiplier and commercial sub-populations. The nucleus comprises a small group of herds that produce animals (sires in particular) for multiplier herds. Some nucleus breeders also sell animals directly to commercial producers. Multipliers, as their name implies, multiply genes from the nucleus to generate parents ultimately purchased by commercial producers. Many characteristics of a whole breed reflect that of its nucleus, which is the driver of genetic change (Baker and Davey, 1960; Harris, 1998). Generation intervals and selection intensities affect the rate of genetic change in a population. The maximum rate of genetic change is realized when selection intensities are high and generation intervals are low. The maximum possible selection intensities can be calculated if the proportion of animals selected for breeding is known, assuming truncation selection.

Some animals used for breeding generate sale animals but never produce any replacements. Others might contribute male offspring that become sires to produce sires (**SS**), and/or sires to produce dams (**SD**), or female offspring that become dams to produce sires (**DS**) and/or dams to produce dams (**DD**). Population structure can be described by quantifying aspects of these four pathways of selection. The objectives of this study were to identify animals contributing to each selection path and quantify their generation intervals and selection intensities. Furthermore, the contribution of each herd in producing replacements in the four pathways was calculated. This information identifies nucleus herds and characterizes some aspects of the structure of the registered Red Angus cattle population.

Materials and Methods

Description of Data

Pedigree information was obtained from the Red Angus Association of America. Data included animal registration number, birth yr (**BY**), sex, and breeder. Three extracts were constructed for different analyses.

Dataset 1 included all animals with BY between 1955 and 2006 (n=2,053,371). Dataset 2 was a subset of dataset 1 that omitted animals born after 1999 and further required both parents known(n=1,318,917). Births since 1999 were omitted as offspring may not have had opportunity to appear in the pedigree as grand-sires or grand-dams. Dataset 3 was a subset of dataset 2, including only those herds (identified by breeder) represented in the pedigree for a minimum of 10 yr (n=355,776 with 4,301 herds). These herds had the opportunity to produce replacements for selection in one or more of the four pathways.

Generation Interval

The ages of parents when their progeny were born were calculated for the four pathways of selection using dataset 2, to quantify generation intervals. Animal identification numbers were traced forward from their birth to identify bull or heifer calves that became grand-sires (i.e., SS, SD), or grand-dams (i.e., DS, DD). The ages when they became grand-sires or grand-dams were calculated and averaged according to BY. Overall generation intervals for each pathway were calculated by averaging generation intervals over BY.

Proportion Selected and Selection Intensities

Total bull and heifer calves born were counted according to BY, and the proportions that became grand-sires or grand-dams were computed. Selection intensities were obtained from the proportion selected assuming truncation selection. Selection intensities were computed as $\bar{i} = \frac{z}{p}$, where $\bar{i}$ is intensity of selection, z is the height of the ordinate at the truncation point, t, (i.e. $z = \frac{1}{2\pi} e^{-1/2t^2}$), with t being found so that 1 minus the integral of the density function equals the proportion p of animals selected ($p = 1 - \int_t^{+\infty} \frac{1}{2\pi} e^{-1/2x^2}$).

Theoretical Rate of Change

Genetic gain was calculated for each BY using $\Delta \hat{B}V/t = \frac{r(\bar{i}_{SS} + \bar{i}_{SD} + \bar{i}_{DS} + \bar{i}_{DD})}{L_{SS} + L_{SD} + L_{DS} + L_{DD}}$ where $\Delta BV/t$ is the genetic gain or change in breeding values per yr, $\bar{i}$ is selection intensity and L is generation interval, with subscripts for each pathway. Accuracy, r, was assumed to be 0.5, equivalent to mass selection with heritability 0.25.

Population Structure

Dataset 3 was used to find, for each herd, the percent of animals by pathway that were not born in that herd. The number of herds in dataset 3 that produced animals in each pathway were counted. Herds with the greatest contribution of parents to a particular pathway were accumulated until those herds had produced 25, 50, 75 or 100% of animals in that pathway. The number of such herds was represented as a proportion of all herds.

Results and Discussion

Generation Intervals, Selection Proportions and Intensities

Average ages of parents were in general increasing for the four pathways of selection until reaching maximum values in 1975, then decreased to minimum values in 1985, and increased again from 1985 (Table 1). Sire pathways had lower generation intervals than dam pathways. Similar results have been reported in Italian and Japanese beef cattle breeds (Bozzi et al., 2006; Nomura et al., 2001).

Table 1. Generation intervals in four pathways of selection[1].

Birth year	SS	DS	SD	DD
1955	3.8	5.0	4.4	4.5
1965	4.6	5.4	4.4	4.8
1975	5.1	5.5	4.7	5.2
1985	4.1	4.8	4.2	4.8
1995	4.3	5.1	4.2	5.2
1999	4.8	5.4	4.3	5.2
Average	4.5	5.0	4.4	4.9

[1]SS= sires to produce sires; DS= dams to produce sires; SD= sires to produce dams; DD= dams to produce dams.

Selection proportions declined and therefore selection intensities increased in all pathways of selection in the first 30 yr from 1955 (Figure 1). Selection intensities declined from 1985 and increased again after approximately 1990 (Figure 2). The decline and subsequent increase in selection intensities corresponds to a similar decline and increase in generation intervals at the same time (Table 1).

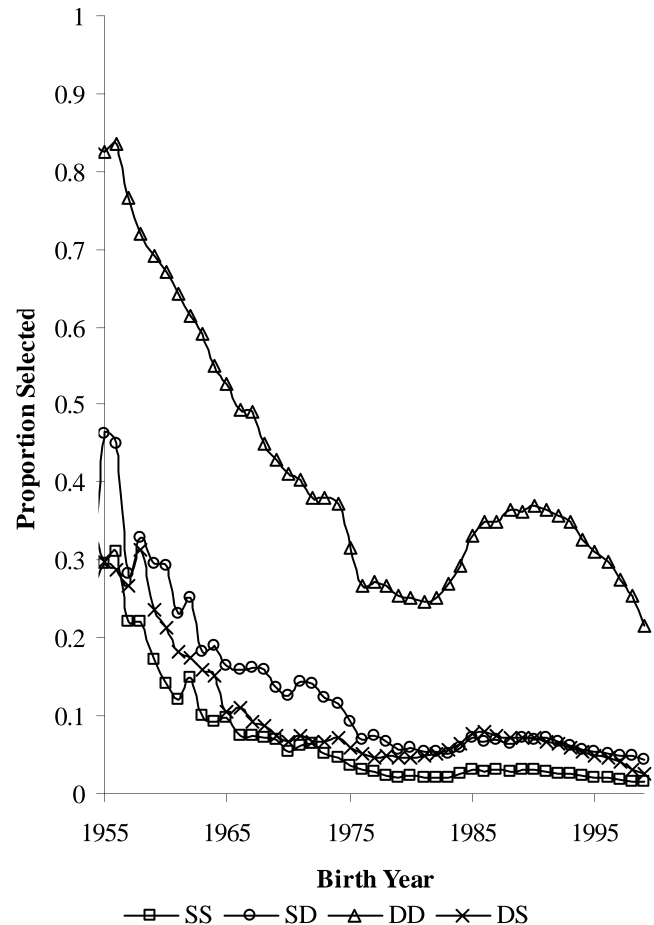

Figure 1. Proportion of animals selected to become sires of sires (SS), sire of dams (SD), dams of dams (DD), and dams of sires (DS) from birth yr 1955 to 1999.

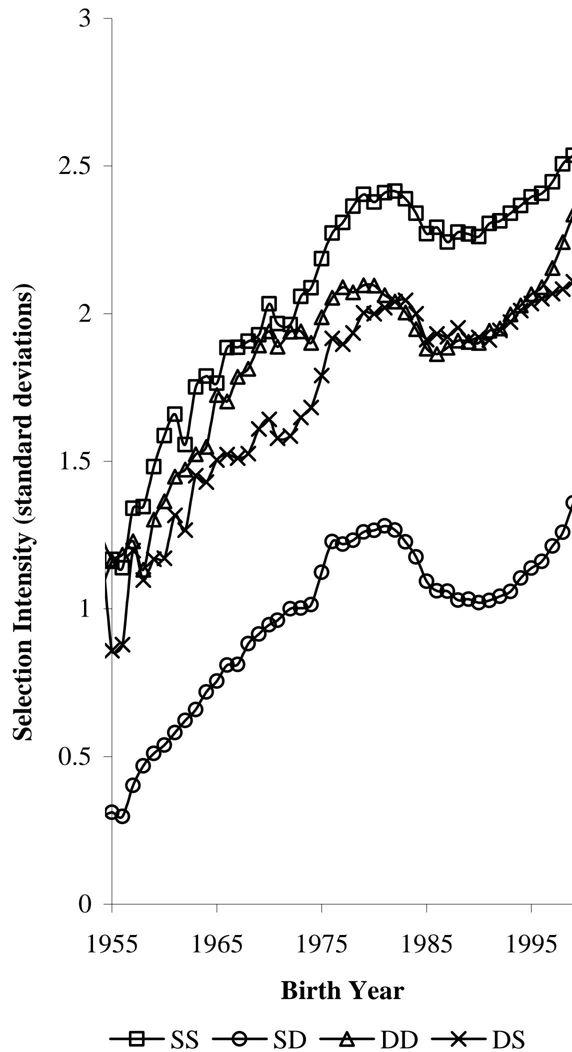

Figure 2. Selection intensities in standard deviation units computed from selection proportions for sires of sires (SS), sire of dams (SD), dams of dams (DD), and dams of sires (DS) from birth yr 1955 to 1999.

A greater proportion of young animals used as parents for a decade from 1980. Shorter generation intervals were observed, and were reflected by increased selection proportions and decreased selection intensities over the same period. The relationship between generation interval and selection intensity reflects the fact that greater use of young animals as parents required an increased replacement rate.

The average standardized selection intensities were 2.07, 1.71, 0.96, and 1.83 for SS, DS, DD, and SD, respectively. Selection proportions were highest for DD, followed by SD, DS, and SS, respectively (Figure 2). The low selection intensities of DD are similar to what has been reported, where higher selection intensities occur in males, because of higher reproductive rates (Smith and Banos, 1991). It was expected that SS would have the lowest proportion selected and highest selection intensity, because the fewest of these animals are needed to maintain the population

Theoretical Rate of Genetic Change

The potential rate of genetic change assuming truncation selection and an accuracy of 0.5 increased over time. In the foundation yr, gain was 0.1 standard deviations per yr. The maximum rate of gain of 0.21 standard deviations per yr, was observed in 1982. This is similar to reported rates of gain of approximately 0.2 in dairy cattle (Lopez-Villalobos et al., 2000). In the current study a slight decrease was observed, followed by nearly constant annual gains from 1985 to 1995, after which an increasing trend was again noted (Figure 3).

Trends in the rate of genetic gain corresponded to collective changes in generation intervals and selection intensity for the breed. The period around 1980 characterized by lower generation intervals and lower selection intensity had slightly lower genetic gain. Selecting younger animals can result in lower gain, because these animals have fewer progeny and therefore lower accuracy. Results suggest that if truncation selection was practiced, the tradeoff between generation intervals and selection intensities were not beneficial in terms of genetic progress. However, actual selection would have been on multiple traits with varying accuracy by trait and selection pathway. Furthermore, selection decisions are unlikely to be uniform for every breeder.

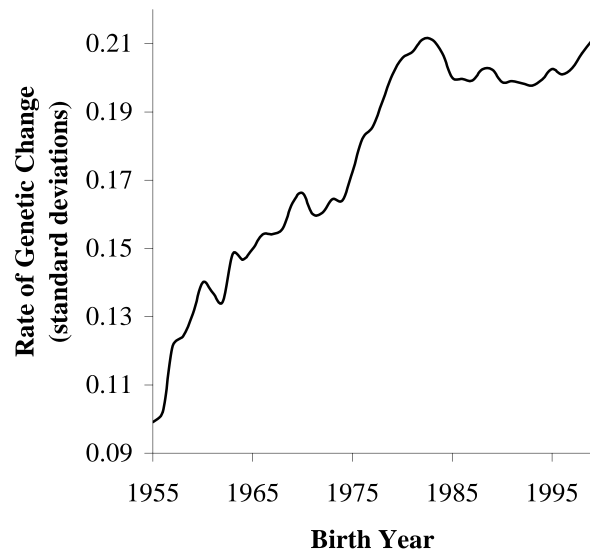

Figure 3. Rate of theoretical genetic change derived from observed generation intervals and selection proportions from birth yr 1955 to 1999.

Population Structure

Most animals representing the four pathways of selection were bred in herds other than where they were used as parents. On average 94, 83, 85, and 58% of SS, DS, SD, and DD animals respectively were bred in outside herds. The percentages of herds that produced one or more animals in the four pathways of selection were 30, 43, 61, and 85% for SS, DS, SD, and DD, respectively. Only 1,271 herds (30% of the total) produced SS, and only 153 of those (3.6% of all herds in the pedigree) produced 50% of SS animals that

appeared in the pedigree. Similar results were observed in each of the four pathways of selection, with few breeders contributing the majority of animals that appear in the pedigree (Table 2).

More DD than other animals came from within the herd, because most breeders keep their own replacement females, so breeders eventually produce a DD. Most breeders do not produce bulls to breed bulls (SS) in their own herds, as evident from the analysis. These results correspond to previous findings in Canadian Herefords, where over two thirds of sires and one third of dams were bred in outside herds (Koots and Crow, 1989). Less than half of herds produced animals in the paternal pathways (SS and SD) because fewer males are required in the population, so the production of males can be specialized in a few herds. Results suggest that a subsector of the registered population must produce most of the SS, SD, and DS animals.

The hierarchical structure of the breed becomes more evident by examining the number of individual herds producing animals in the four pathways, the majority came from only a few herds. These are the nucleus breeders that provided most of the improvement for the breed. The multiplier herds pass those improved genes on to the rest of the producers. This structure has been proposed as representative for other breeds (Baker and Davey, 1960; Koots and Crow, 1989).

Table 2. Number of herds that produce 25, 50, 75 or 100% of animals in the four pathways of selection, and percentage out of all herds in the pedigree these represent.

	Number of herds producing an animal in each pathway[1] (percent out of total herds)			
Percent of animals	SS	DS	SD	DD
25%	37	45	54	48
	(1%)	(1%)	(1%)	(1%)
50%	153	175	230	193
	(4%)	(4%)	(5%)	(4%)
75%	447	494	647	560
	(10%)	(11%)	(15%)	(13%)
100%	1271	1865	2617	3662
	(30%)	(43%)	(61%)	(85%)

[1]SS= sires to produce sires; DS= dams to produce sires; SD= sires to produce dams; DD= dams to produce dams.

Implications

Selection proportions decreased and generation intervals increased in the Red Angus breed. Collectively these two factors appear to be contributing to a slight increase in genetic gain. A few herds in the registered population were breeding most of the animals in the four pathways of selection (sires to produce sires, sires to produce dams, dams to produce sires, and dams to produce dams). Genetic change in the entire registered population reflected progress made by this subset of the industry. Knowledge of the hierarchical structure of the industry can be used to advantage by targeting genetic recommendations and new technologies at nucleus herds, thus making more cost-effective genetic gain in the whole breed, to the benefit of the industry. It would be beneficial to compare these results to data on actual genetic change of individual traits in the nucleus herds, and thus gain a more realistic understanding of the genetic progress made in the breed.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

THE ABILITY OF A YEAST-DERIVED CELL WALL PREPARATION TO MINIMIZE TOXIC EFFECTS OF HIGH-ALKALOID TALL FESCUE STRAW IN BEEF CATTLE

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ABSTRACT: Two experiments were conducted to evaluate the influence of a yeast-derived cell wall preparation (YCW) on forage intake and digestibility, ruminal fermentation characteristics, serum prolactin and prolactin stores, and milk production in beef cattle consuming high-alkaloid tall fescue straw. In Exp. 1, 16 ruminally cannulated steers (200 ± 6 kg BW) were blocked by BW and within block assigned to one of four treatments (TRT) containing YCW at 0, 20, 40, or 60 $g \cdot hd^{-1} \cdot d^{-1}$. Tall fescue straw (579 ppb ergovaline) was provided at 120% the previous 5-d average intake with soybean meal (SBM) used as a CP supplement. In the 29-d digestion study, total DMI and DM digestibility were not different ($P > 0.05$). A linear decrease in ruminal liquid dilution rate ($P = 0.03$) was noted as YCW increased. Weekly serum prolactin was not affected by TRT ($P > 0.05$); however, prolactin stores linearly increased as YCW increased ($P = 0.05$). In Exp. 2, 60 cows (517 ± 5 kg BW; approximately 200 d gestation) were stratified by BCS and randomly assigned to the same four YCW treatments as Exp. 1 (447 ppb ergovaline high-alkaloid straw) with the addition of a low-alkaloid straw (149 ppb ergovaline; no YCW supplementation) as a positive control (CON). Cows were provided ad libitum access to straw and were supplemented with SBM daily. One cow was removed from the 40 $g \cdot hd^{-1} \cdot d^{-1}$ TRT due to clinical signs of fescue toxicosis. The CON cows gained more weight ($P = 0.02$) pre-calving compared to 0 $g \cdot hd^{-1} \cdot d^{-1}$ cows. A linear increase ($P = 0.04$) in milk production was observed as YCW increased at 60 d post-partum. Serum prolactin post-calving and change from initial to post-calving increased linearly ($P = 0.02$ and $P = 0.05$, respectively) with increasing YCW supplementation. Also, post-calving serum prolactin was higher ($P = 0.002$) in CON compared to 0 $g \cdot hd^{-1} \cdot d^{-1}$ cows. The YCW seems to alleviate some symptoms of the fescue toxicosis and, therefore, has the potential to be used successfully with other management practices when feeding or grazing high-alkaloid tall fescue.

Keywords: Ergot alkaloids, ergovaline, prolactin

Introduction

Production of grass seed in the U.S. is centered in the Pacific Northwest where over 200,000 tons of tall fescue straw (*Festuca arundinacea;* **TFS**) is produced annually (NASS, 2005). Historically, the disposal method for TFS was burning; however, the environmental implications and danger associated with the process necessitated a reduction in open-field burning. Currently, the most common use of TFS is as a forage source by ruminant livestock (Hovermale and Craig, 2001), but use of some varieties is limited due to the endophyte *Neotyphodium coenophialum* (Morgan-Jones and Gams, 1982) and associated ergot alkaloids. Many metabolic activities and physiological responses including decreased feed intake, elevated body temperature, decreased reproductive efficiency, decreased peripheral circulation, fescue foot, fat necrosis, and agalactia are associated with the consumption of endophyte-infected tall fescue (Paterson, et al., 1995). Economic losses, in the U.S. beef industry, have been estimated at $609 million annually (Hoveland et al., 1993). Considering inflation and losses to other livestock, the cost may currently approach $1 billion per year (Panaccione et al., 2001).

Preliminary data suggests a yeast-derived cell wall preparation (YCW) may minimize, or alleviate, the negative effects of endophyte toxins on animal performance (Akay et al., 2003a, b). Therefore, the objectives of our study were to determine the influence of YCW on: forage intake and digestibility, ruminal fermentation characteristics, serum prolactin and prolactin stores, and milk production in beef cattle consuming high-alkaloid TFS.

Materials and Methods

*Experiment 1 (**E1**): Steers Digestion/Physiology Study*

Sixteen ruminally cannulated, Angus x Hereford steers (200 ± 6 kg BW) were used in a randomized complete block design. Steers were blocked by weight and, within block, randomly assigned to treatments and housed in individual pens (2 m x 4 m) within an enclosed barn with continuous lighting. Steers had unrestricted access to fresh water. Before straw feeding (0700) a trace mineralized salt mix and soybean meal (**SBM**) were supplemented (0.068 % BW; CP basis) intraruminally via cannula to meet 100% of the estimated degradable intake protein requirement assuming a microbial efficiency of 11% (NRC, 1996; model 1). A yeast-derived cell wall preparation (MTB-100™; Alltech Inc., Nicholasville, KY; **YCW**) was provided to yield the following treatments (**TRT**): 0, 20, 40 and 60 $g \cdot hd^{-1} \cdot d^{-1}$ YCW. The appropriate quantity of YCW was added to each steers SBM/trace mineralized salt supplement daily. All steers consumed chopped (4-8 cm), high-alkaloid (579 ppb ergovaline; DM basis) TFS provided at 120% of the previous 5-d average intake at

0730, with orts from the previous day determined before feeding. Nutrient content of straw and SBM is provided in Table 1. The experimental protocol was approved by the Animal Care and Use Committee at Oregon State University (ACUP # 3721).

The experimental period was 29 d, with 19 d of diet adaptation and 10 d of sampling. Intake and orts were monitored throughout the experiment; however, official measurements were taken on d 20 through 25 and d 21 through 26 for intake and orts, respectively. Straw and orts samples for alkaloid analysis were air-dried, ground in a Wiley mill (1-mm screen), and stored (-20ºC) for later analysis. Also, additional samples of TFS, SBM, and orts were collected, dried in a forced-air oven (55° C; 48 h), reweighed for calculation of DM, ground in a Wiley mill (1-mm screen), and composited by source for straw and SBM and by steer for orts.

At 0700 on d 21, immediately following supplementation, each steer was intraruminally pulse-dosed with 4 g of Co-EDTA in a 150-mL aqueous solution (Uden et al., 1980) for determination of ruminal liquid fill and dilution rate. Ruminal fluid (approximately 100 mL) was collected by suction strainer at 0 (prior to SBM supplementation), 3, 6, 9, 12, 18, and 24 h after Co-EDTA adminstration. Samples were immediately analyzed for pH and sub-sampled by placing 5 mL of ruminal fluid in 1 mL of 25% (wt/vol) meta-phosphoric acid and stored (-20°C) for later analysis of NH_3-N and VFA. Also, 20 mL of ruminal fluid were stored (-20°C) for later analysis of Co concentration. Volatile fatty acids were analyzed as described by Harmon et al. (1985) and NH_3-N was analyzed by a modification (sodium salicylate was substituted for phenol) of the procedure described by Broderick and Kang (1980) using a UV/visible light spectrophotometer. Cobalt concentration in ruminal fluid was analyzed by atomic absorption using an air/acetylene flame.

Spot urine samples were collected at 0700 on d 22 to 27 using polyethylene bags (25 cm x 35 cm) split diagonally and secured with string over the withers and hips of the animal. Bags were left on until sample was collected, which never exceeded 1.5 h. Urine samples were composited by steer and stored (-20ºC) for later analysis of creatinine and ergot alkaloids as described by Hovermale and Craig (2001) and Lodge-Ivey et al. (2006).

On d 22 through 27, fecal grab samples were collected 2 times/day at 12-h intervals with a 2-h increment added between days to shift sampling times. This allowed sampling on every even hour of the 24-h day. Fecal subsamples (200 g) were composited by steer, stored (-20ºC), lyophilized, and ground in a Wiley mill (1-mm screen).

On d 28, TRT effects on ruminal DM and indigestible ADF (**IADF**) fill were determined by manually removing reticuloruminal contents 4 h after feeding. Total ruminal contents were weighed, thoroughly mixed by hand, and sub-sampled (300 g) in triplicate. The remaining ruminal contents were replaced into the steer. Ruminal samples were weighed, dried in a force-air oven (55°C; 96 h), reweighed for DM, ground to pass a 1-mm screen in a Wiley mill, and composited by steer.

Ground samples were analyzed for DM and OM (AOAC, 1990), N (Leco CN-2000), 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. Also, samples were analyzed for IADF as described by Bohnert et al. (2002). Air-dried TFS and orts and lyophilized fecal samples were analyzed for ergovaline and lysergic acid using HPLC as described previously for urine.

Four hours after feeding on d 1, 8, 15, and 22, 10 mL of blood were collected by coccygeal venipuncture using serum tubes. Blood samples were allowed to clot overnight at 4ºC, centrifuged (1,500 x *g*; 20 min; 4°C) and serum harvested and stored (-20°C) for prolactin analysis as described by Hockett et al. (2000).

Steers were subjected to a thyrotropin-releasing hormone (**TRH**) challenge to measure pituitary prolactin stores on d 29. The afternoon prior to the challenge, steers were catheterized via the jugular vein. The day of the challenge, each steer was dosed with 1 µg TRH/kg BW via the catheter. Blood samples were collected at -30, -15, 0 (before TRH administration), and 5, 10, 15, 20, 30, 45, 60, 90, 120, and 150 min. Blood samples were handled as previously described for prolactin analysis. Area under the curve was determined for prolactin using the trapezoidal summation method.

Statistical Analysis. Data were analyzed as a randomized complete block using the GLM procedure of SAS (SAS Inst., Inc., Cary NC). Steer, TRT, and block were included in the model. Contrast statements were linear effect and quadratic effect of increasing YCW. Ruminal pH, NH_3-N, and VFA, and TRH challenge data were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included steer, TRT, block, hour, and TRT × hour. Also, weekly temperature and serum prolactin data were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included steer, TRT, block, day, and TRT × day. The same contrasts described above were used to partition TRT effects for ruminal pH, NH_3-N, VFA, temperature, and serum prolactin.

*Experiment 2 (**E2**): Cow Performance and Production*

Sixty Angus × Hereford cows (517 ± 5 kg BW; approximately 200 d gestation) were stratified by BCS (5.0 ± 0.1; Herd and Sprott, 1986) and assigned randomly to one of 20 pens and one of five TRT (three cows/pen; four pens/TRT) in a randomized complete block design. Animals had ad libitum access to high-alkaloid TFS or a low-alkaloid TFS (Table 1) used in formulating the following TRT: low-alkaloid TFS (**CON**) and high-alkaloid TFS with 0 (**0**), 20 (**20**), 40 (**40**), or 60 $g \cdot hd^{-1} \cdot d^{-1}$ YCW (**60**). Also, SBM was provided daily to all TRT to meet 100% of the estimated degradable intake protein requirement assuming a microbial efficiency of 11% (NRC, 1996; model 1). Straw samples for alkaloid analysis were obtained weekly and stored (-20ºC) and analyzed as described in E1. Also, additional samples of low-alkaloid and high-alkaloid TFS and SBM were collected weekly, dried in a forced-air oven (55° C; 48 h), reweighed for calculation of DM, ground in a Wiley mill (1-mm screen),

and composited by source and period for analysis of NDF, ADF, N, and OM as described in E1.

An evaluation of all cows was conducted daily at 0630 and assigned a locomotion score from 1 to 5 (adapted from Sprecher et al., 1997). A locomotion score of 3 or higher was considered indicative of fescue foot and necessitated removal.

Cow BW and BCS was measured at study initiation, d 28, and every 14 d thereafter until calving. All weights were obtained following an overnight shrink (16 h). Also, cow BW and BCS were obtained within 24 h following parturition.

Blood samples (~10 mL) were collected by coccygeal venipuncture using serum tubes on d 1 and within 24 h after parturition. Blood samples were prepared for prolactin analysis as previously described (E1 and E2; inter-assay CV = 5.0 and intra-assay CV = 8.0).

Following parturition, cows and calves were placed in a common 7.3 ha pasture and were provided approximately 11.2 $kg{\cdot}cow^{-1}{\cdot}d^{-1}$ (DM basis) of meadow hay (6.3 % CP; DM basis). One week after the last cow calved, pairs were moved to the Northern Great Basin Experimental Range (NGBER) 72 km west-southwest of Burns, OR. Animals grazed mixed sagebrush, bunch grass as described by Ganskopp (2001) and were managed according to NGBER management practices. Approximately 60 ± 2 d post-partum, milk production was estimated by weigh-suckle-weigh (Williams et al., 1979) with an 8 h separation.

Statistical Analyses. Data were analyzed as a randomized complete block using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Pen, TRT, and block were included in the model. Contrast statements were linear effect and quadratic effect of increasing YCW supplementation and CON vs. 0.

Results and Discussion

Experiment 1: Steer Digestion/Physiology Study

No animals in this experiment exhibited physical symptoms of tall fescue toxicosis. Neither TFS (18.6 g/kg BW), total (19.8 g/kg BW) DMI, nor DMD (46.3 %) was affected by increasing YCW supplementation ($P > 0.05$). These results are inconsistent with Akay et al. (2003b) who noted increased DMI in steers fed endophyte-infected tall fescue seed with YCW compared to those without (8.46 vs. 7.81 kg/d). However, steers were at an ambient temperature of 30° C compared to the low average ambient temperature of -2.3^{o} C in the current study. Hemken et al. (1981) suggests DMI of cattle consuming endophyte-free or endophyte-infected tall fescue does not differ in an environment of 23°C or less.

Means for ruminal fermentation characteristics were averaged across time because no TRT x hour interactions occurred ($P > 0.05$). Increasing supplementation of YCW did not affect ($P > 0.05$) ruminal NH_3-N, pH, or total VFA. Molar proportions of acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate or acetate:proprionate ratio were not affected by increasing YCW ($P > 0.05$).

No differences ($P > 0.05$) were detected in liquid volume, IADF intake, fill, outflow, or passage with increasing YCW supplementation; though, dilution rate linearly decreased ($P = 0.03$) with increasing YCW (7.8, 6.1, 7.0, and 6.2 %/h for TRT 0, 20, 40, and 60 $g{\cdot}hd^{-1}{\cdot}d^{-1}$ YCW, respectively).

Paterson et al. (1995) reported decreased serum prolactin as a consistent result of fescue toxicosis. Analysis of weekly serum prolactin concentration detected no TRT x day interaction ($P > 0.05$) or effect of increasing level of YCW ($P > 0.05$; 1.9 ng/mL). During the TRH challenge, data from one steer on TRT 0 was excluded due to loss of catheter patency. Prolactin area under the curve **(AUC)** for the TRH challenge increased linearly ($P = 0.05$) with increasing YCW (Figure 1). These results suggest YCW may mediate prolactin depression normally associated with fescue toxicosis.

No differences ($P > 0.05$) were detected in intake or excretion, fecal or urine, of ergovaline, lysergic acid or the combination.

Experiment 2: Cow Performance and Production Study

During the course of the experiment, one cow from TRT 40 was removed on d 55 due to a lameness score of 4 and a second cow was removed from TRT 40 due to misdiagnosis of pregnancy. These animals were completely removed from the dataset.

Contrary to Akay et al. (2003a) who reported an increase in BW of cows supplemented with YCW at 20 $g{\cdot}hd^{-1}{\cdot}d^{-1}$ compared to those not supplemented during a 5 month period (May to Oct.) grazing tall fescue; we noted no differences ($P > 0.05$) in pre- or post-calving change in BW or BCS as YCW increased. These differing results may be a function of heat stress in the Akay study compared to the winter and spring temperatures associated with the current experiment. Nevertheless, pre-calving BW change increased ($P = 0.02$) in CON (44.0 kg) compared to 0 (21.7 kg). In agreement, Paterson et al. (1995) reported a loss in ADG of cows grazing endophyte-infected fescue compared to an increase in ADG of cows grazing non-endophyte infected fescue.

Peters et al. (1992) reported daily milk production was 25% lower in animals consuming endophyte infected compared to endophyte-free tall fescue. In our study, milk production increased linearly ($P = 0.04$) as YCW increased (Table 2). Also, these differences were similar to the observed differences in serum prolactin concentration. Suppression of the periparturient surge of prolactin has been reported in cattle administered ergot alkaloids and is associated with decreased metabolic activity of the mammary cells (Tucker, 1985). In this study, post-calving, and change from initial to post-calving, serum prolactin concentrations increased linearly ($P = 0.02$; $P = 0.05$, respectively) with increasing YCW and decreased ($P = 0.002$; $P = 0.003$, respectively) with 0 compared to CON (Table 2). This coincides with the TRH-challenge AUC data in E1.

Increasing YCW resulted in greater prolactin stores, alleviated prolactin depression, and increased milk production of beef cattle consuming high-alkaloid TFS. Consequently, YCW appears to ameliorate some of the negative consequences observed with intake of high-alkaloid tall fescue.

Implications

The yeast-derived cell wall preparation alleviated some signs of fescue toxicosis. This data provides grass seed straw consumers with information that will help in designing safe and effective management strategies for the use of high-alkaloid tall fescue straw. In addition, this research is directly applicable to ruminant livestock producers in the eastern United States that rely on endophyte-infected tall fescue as a forage base.

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Table 1. Feedstuff nutrient content (DM basis)

	Experiment 1 (steer study)		Experiment 2 (cow study)		
Item	High-alkaloid tall fescue straw	Soybean Meal	High-alkaloid tall fescue straw	Low-alkaloid tall fescue straw	Soybean meal
CP, %	5.8	54.0	5.6	6.5	52.7
OM, %	94	93	92	92	93
NDF, %	69	13	72	71	13
ADF, %	44	5	43	43	5
Ergovaline, ppb	579	NA[a]	449	147	NA[a]
Lysergic acid, ppb	68	NA[a]	11	>10	NA[a]

[a] NA= Not analyzed.

Table 2. Effect of a yeast-derived cell wall preparation (YCW) on milk production and serum prolactin in cows consuming high- or low-alkaloid tall fescue straw.

	Treatment[a]						*P*-value[c]		
Item	CON	0	20	40	60	SEM[b]	L	Q	CON vs. 0
Milk production, kg[d]	12.5	9.8	11.2	13.6	14.2	1.5	0.04	0.79	0.22
Serum prolactin, ng/mL									
Initial[e]	26.7	40.6	37.8	54.9	38.8	13.0	0.84	0.62	0.46
Postcalving[f]	145.7	62.4	111.9	100.5	126.9	15.7	0.02	0.29	0.002
Postcalving-Initial	119.0	21.8	74.1	57.3	88.1	18.9	0.05	0.58	0.003

[a] CON= no YCW + low-alkaloid tall fescue straw (147 ppb ergovaline); 0, 20, 40, and 60= 0, 20, 40, or 60 g·hd^{-1}·d^{-1} YCW + high-alkaloid tall fescue straw (449 ppb ergovaline).
[b] n= 4.
[c] Probability of linear (L) and quadratic (Q) effects of increasing YCW; CON vs. 0 = CON Treatment (TRT) vs. 0 TRT.
[d] 60 ± 1 d after parturition.
[e] From study initiation.
[f] Within 24 h after parturition.

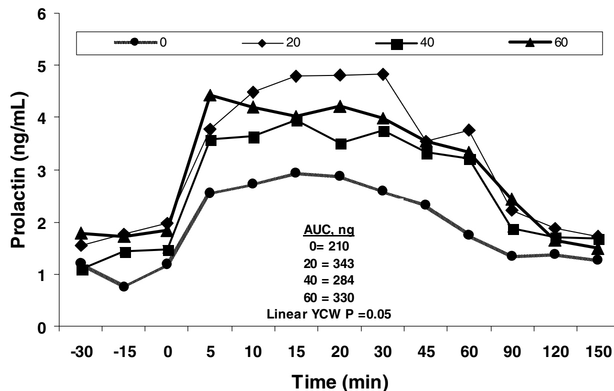

Figure 1. Effect of a yeast-derived cell wall preparation (YCW) supplemented at 0, 20, 40 or 60 g·hd^{-1}·d^{-1} on area under the curve (AUC) for steers experiencing a Thyrotropin-releasing hormone challenge while consuming high-alkaloid tall fescue straw (579 ppb ergovaline). Linear effect (P = 0.05) of YCW was observed for AUC.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECTS OF MILD STRESS ON EWE MATERNAL BEHAVIOR

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ABSTRACT: Maternal behavior is important for the survival of offspring. It was hypothesized that mild recurrent stress diminishes maternal behavior and this effect is reversed by administration of the antidepressant sertraline. Commercial western white-faced ewes (n=12) were exposed to short duration stress three times per wk beginning on d four postpartum (PP). Stress included social isolation and presence of a barking dog. Control ewes (n=6) were not exposed to direct stress. Response to stress was quantified by changes in serum concentrations of cortisol. Beginning on d eight PP, ewe behavior was observed following lamb removal and following reunion of ewe and lamb. During lamb absence, number of high-pitched bleats and time spent pacing were used as measures of agitation. Upon reunion, a maternal score was computed based on number of times the ewe nuzzled her lamb, number of low pitched grunts, and amount of time lambs were allowed to nurse. Beginning on d 21 PP, six stressed ewes were treated daily with 200 mg sertraline and behavior testing continued for four wks. Data analysis indicates that stress acutely increased ($P < 0.05$) concentrations of cortisol, but did not chronically affect ($P > 0.05$) cortisol secretion. Prior to d 21 PP, stress decreased ($P < 0.05$) maternal behavior and agitation during separation. After d 21, maternal behavior did not differ ($P > 0.05$) across groups. Stressed untreated ewes continued to show decreased ($P < 0.05$) agitation compared to control ewes, while sertraline-treated ewes were not different ($P > 0.05$) from either control or stressed-untreated ewes. By wk four PP, stress no longer elicited an increase in cortisol from ewes not receiving sertraline; however, concentrations of cortisol were acutely elevated in sertraline-treated ewes. These data indicate that mild stress early in the PP period decreases maternal behavior and agitation due to lamb removal. Sertraline treatment partially reverses this effect, but is associated with increased cortisol secretion in response to stress. This is similar to the increased agitation seen in humans during early antidepressant treatment.
Key Words: ewe, postpartum, maternal

Introduction

For sheep, the combination of bearing precocious offspring, high levels of sociability, and predator pressures necessitates rapid and secure bonding between ewe and lamb (Terrazas et al. 1999). This bond is formed within hours of lambing (Poindron 1980) and continues for several months (Poindron et al. 1993). Specific and reliable measures of ewe maternal behavior have been thoroughly characterized and replicated (Dwyer et al. 1999; Dwyer & Lawrence 2000; Everett-Hincks et al. 2005; Poindron et al. 1994).

Specific behavioral measures of distress in sheep are well researched. The most often used procedure for evaluating a fear or stress response in sheep is isolation from conspecifics (Broadbear et al. 2004b; Broadbear et al. 2004a; Cockram 2004; Romeyer & Bouissou 1992; Turner et al. 2002; Vandenheede et al. 1998). Other types of stress include being in the presence of humans and surprise situations (Vandenheede et al. 1998), audiovisual stress of a barking dog (Turner et al. 2002). Indications of stress include increased motor activity, increased immobilization, decreased feeding, defecation, increased vocalizations, and attempts to escape.

Interestingly, the stress response to social isolation, restraint and transportation is reduced in ewes during late pregnancy and the postpartum period (Poindron et al. 1994; Poindron et al. 1997; Roussel et al. 2006; Viérin & Bouissou 2001). According to the research conducted by Tilbrook and colleagues (Tilbrook et al. 2006), the presence of offspring can reduce the hypothalamic-pituitary-adrenal system response to stress of restraint and social isolation. During the postpartum period, the most potent inducer of stress is separation from lambs, and this response continues up to two months postpartum (Cockram et al. 1993; Poindron et al. 1994; Poindron et al. 1997).

Objectives

While the long range goal is to understand the neurobiology of the impact of stress on maternal behavior, the goal of the present study was to determine the effects of repetitive mild stress on maternal behavior, cortisol levels, and behavior during separation from lambs; and to determine the effects of administration of sertraline, an selective serotonin reuptake inhibitor (SSRI) antidepressant, on these same measures.

Materials and Methods

Design

In this study, the delivery of unpredictable and repetitive mild stressors was used in an effort to disrupt but not eliminate maternal behaviors in postpartum ewes. Animals (n=6/group) in the Stress and Stress + TX groups were exposed to the repeated stress-inducing conditions. Animals in the Stress + TX group received daily injections of sertraline, beginning before four weeks postpartum. Control group animals (n=6) received neither stress nor drug treatment.

Animals and Housing

Eighteen postpartum Western white-faced ewes from the University of Wyoming commercial sheep flock were used. Ewes ranged in age from 2 years (n = 1) to 9 years (n = 1). All but one of the ewes was multiparous, and only sheep giving birth to singletons (n = 7) or twins (n = 11) were used.

During the first week postpartum, all ewes and lambs were housed singly in pens adjacent to other ewes and lambs. At five to 10 days postpartum, ewes and lambs were moved into larger pens containing up to five ewe/lamb combinations, where they remained for another two to three weeks. At that time, the ewes and lambs were housed in large groups of up to 20 ewes with their lambs, with access to the outside.

Stress Induction

Animals in the Stress and Stress + TX groups were exposed to short duration stress three times per wk beginning on d four postpartum (PP) and continuing for the duration of the study. Types of stressors were varied and included: isolation from conspecifics and lambs, presence of sheep dog, and presence of unfamiliar human within the pen. The stress-inducing period lasted for at least 5 minutes and ewes were exposed to such experiences 14 times over a 60 day period. Control ewes (n=6) were not exposed to direct stress.

Blood Sampling and Hormone Analysis

Beginning on day four PP, weekly or bi-weekly blood samples were collected from all ewes. For the Stress and Stress + TX ewes, blood samples were collected by jugular venipuncture (approximately 10 ml) immediately before and after stress exposure. Samples from control ewes were taken on the same schedule. Blood samples were allowed to clot overnight at 4° C and serum was then separated by centrifugation at 1500 *g* for 20 min. Serum was decanted and stored at -20° C until analysis. Samples were analyzed in duplicate utilizing a commercial RIA kit.

Early postpartum behavioral testing

Tests of ewe behavior began on d eight PP. The first three tests were conducted prior to initiation of sertraline treatment. For these tests, the ewe remained in her pen and the lamb(s) was moved to a separate portion of the barn. Ewe behavior was recorded for one minute during lamb separation. The lamb was returned to the ewe's pen and maternal behavior was recorded for an additional 5 min. The total time of separation was between 5 and 10 min.

Antidepressant administration

Antidepressant treatment with sertraline began on d 21 PP, at an initial dosage of 50 mg and increasing over a three day period to 200 mg or approximately 2 mg/kg (doses for humans and other animals are within the range of 1-3 mg/kg). The sertraline (Zoloft) tablets were dissolved in sterile buffer for s.c. injection. Ewes in the Stress Group received s.c. injections of the buffer only beginning on approximately d 40 PP.

Late postpartum behavioral testing

Three additional, weekly behavioral tests were conducted beginning week three postpartum. For these, both the ewes and lambs were moved to a nearby barn. The ewe was placed in a testing pen that was approximately 3 meters square, and the lambs were contained in a separate pen, allowing for auditory and partial visual contact between ewe and lamb. Behavior of the ewe was recorded for 5 min, at which the time the lamb(s) were placed with the ewe in the testing pen, and behavior was recorded for an additional 5 min with the lamb present.

Data analysis

Ewe behaviors were analyzed using the JWatcher event recording program. During lamb absence, the number of high-pitched bleats and time spent pacing were used as a measure of agitation. Maternal behavior, following reunion, was computed based on number of times the ewe nuzzled her lamb, number of low pitched grunts she emitted, and amount of time lambs were allowed to nurse.

Results and Discussion

Cortisol levels

Data analysis indicates that stress acutely increased ($P < 0.05$) concentrations of cortisol during weeks one and two postpartum, but did not affect baseline ($P > 0.05$) cortisol secretion. By postpartum week three, the mild stress no longer resulted in cortisol elevation for the Stress group animals. However, those animals receiving sertraline continued to respond to stress with an elevation ($P < 0.05$) in cortisol levels during postpartum weeks three and four. This elevation failed to reach significance for postpartum week five ($P < .1$) or six ($P > .1$).

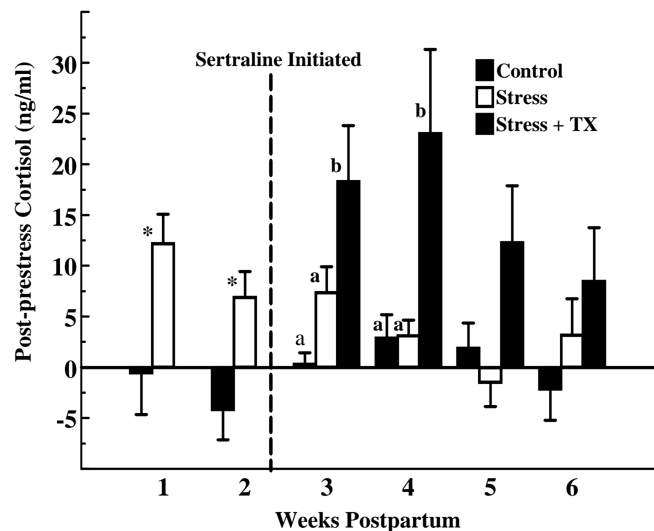

Figure 1. Difference in cortisol levels (Post-stress concentration – Pre-stress concentration, ng/ml) for Control, Stress, and Stress + TX groups.
[a] vs. [b] Numbers within a column with different superscripts are different ($P < 0.05$).
* $P < .05$

Ewe behavior: early postpartum

Three tests of ewe response to 60 seconds of separation from their lambs were conducted between d 6 and d 21 PP. Ewe behavior during the separation b was typified by agitation and frequent high pitched bleats for both Stress and Control animals. However, animals in the Stress group emitted fewer high pitched bleats (P < 0.05) than did the Control animals. Ewes responded to the lamb's return with typical maternal behaviors including nuzzling and allowing the lamb to suckle. Maternal behavior scores, calculated as the amount of time spent nursing, the number of low-pitched grunts, and the number of times the ewe nuzzled or licked the lamb, were calculated for the subsequent 5 min period. The Stress animals had a reduction (P < .01) in maternal scores compared to the Control animals.

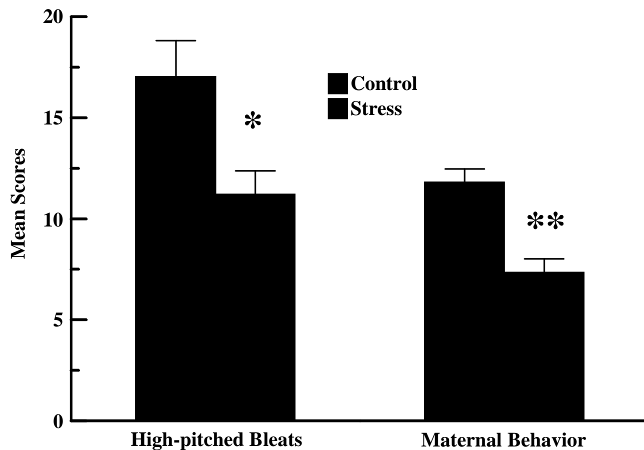

Figure 2. Mean Agitation and Maternal Behavior scores for behavior tests conducted during postpartum weeks 2 and 3.
* $p < .05$
** $p < .01$

Ewe behavior: late postpartum

One week after sertraline treatment was started, an additional set of three behavioral tests were given, at weekly intervals. These tests were conducted in a large pen, allowing additional behaviors to be recorded. Ewe behavior during the five min separation period included high pitched bleats, rearing on the gate, and pacing/walking around the pen and these behaviors were used to calculate an agitation score for each animal. Agitation scores were averaged for the three tests. A significant difference, P<.05, was found across the three groups, such that the Stress group had scores exhibited lower agitation than either the Control or the Stress + TX group. No difference (P >.1) was found between the Control and the Stress + TX groups or between the Stress + TX and Stress groups. Maternal behavior scores, based on time suckling, nuzzling the lamb and low-pitched bleats, did not differ across groups.

These data indicate that mild stress decreases maternal behavior, and decreases the level of agitation exhibited during separation from the lamb during the early postpartum period. Sertraline treatment partially reverses this effect.

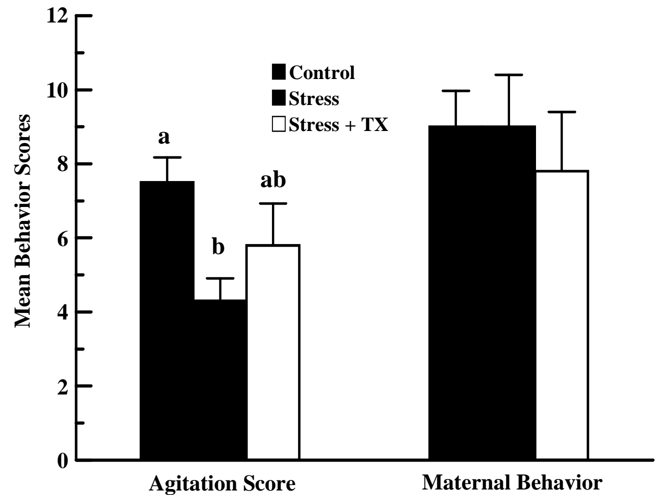

Figure 3. Agitation and maternal behavior scores for three tests, conducted during sertraline treatment, weeks three to five PP.
[a vs. b] Numbers within a column with different superscripts are different (*P* < 0.05).

The effectiveness of these mild stressors in elevating cortisol levels diminished within the first three weeks PP. The low level of reactivity to stress is consistent with earlier findings that the HPA axis response to stress is decreased in postparturient ewes (Tilbrook et al. 2006). However, this diminishment was not seen in those animals receiving sertraline injections. In fact, these animals showed an enhanced HPA response to the mild stress during the first two weeks of administration. While other studies have reported that acute administration of selective serotonin reuptake inhibitors (SSRIs) such as sertraline, stimulates ACTH and cortisol secretion in ewes (Broadbear et al. 2004b; Broadbear et al. 2004a), this is the first report of a such stimulation in response to chronic administration or sertraline.

It is interesting that the increase in cortisol levels of the sheep in the Stress + TX group during the first two weeks of treatment were only noted following acute exposure to the stress, and this elevation was not seen in the pre-stress blood levels. Further, it is especially important to note that these same animals did not show an increase in agitation when separated from their offspring nor a decrease in maternal behavior relative to the Stress, untreated group animals. Therefore, we have not found a generalized anxiety response to the sertraline, but rather an interaction between the stress and sertraline administration.

Implications

Sertraline, as well as other SSRI antidepressants, have been used successfully in treating both anxiety and depressive disorders in humans. However, the therapeutic response to treatment is generally delayed, with little reduction in symptom seen prior to two to four weeks of treatment. In contrast, side effects of these medications are seen much earlier. One such side effect in humans is

agitation, which is especially likely during the early stages of treatment (Belzung et al. 2001; Healy & Whitaker 2003; Nadeem et al. 2004) . In fact, concerns have been raised regarding the potential for increased suicidality in patients treated with these antidepressants.

The use of repeated mild stress to postparturient ewes may provide insight into the neurobiology of the interactions between stress and reproductive status. In addition, this model may prove useful in understanding the human conditions of depression, anxiety and postpartum mood disorders.

Acknowledgements

This research was funded in part by a University of Wyoming Grant-in-Aid provided to the authors. This publication was made possible by NIH Grant # P20 RR016474 from the INBRE Program of the National Center for Research Resources. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIH. We would like to thank Edward van Kirk and Sarah Hoerle for their assistance with this project.

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Proceeding, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECTS OF PROGESTERONE ON RAM REPRODUCTIVE BEHAVIOR

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ABSTRACT: Progesterone is necessary in males for spermiogenesis and testosterone biosynthesis. The current study tested the hypothesis that progesterone is also a crucial modulator of sexual behavior in rams. Intact Columbia rams (n = 6) and Columbia rams gonadectomized (GNX; n = 5) at 6 – 7 mo of age were exposed to ewes in estrus at 10 – 11 mo of age. Expressed reproductive behaviors were recorded and categorized as investigatory (investigatory sniffs, flehmen, foreleg kicks, nudge, vocalization) or consummatory (mount attempts, mounts, ejaculations) behaviors. Following determination of baseline behaviors, intact rams were treated with the specific progesterone receptor antagonist, mifepristone (RU486; 25 mg), twice daily, and re-tested. Gonadectomized rams were implanted subcutaneously with 4 doses of Synovex-h (200 mg testosterone and 20 mg estradiol per dose). Concentrations of serum testosterone did not differ among intact and GNX males treated with Synovex-h. Expression of reproductive behavior was determined one month following insertion of Synovex-h implants. Pursuant to behavior testing, GNX rams were treated with 5 mg of progesterone twice daily and behavior was monitored. Two intact rams with were removed from the study due to a lack of sexual behavior. Mifepristone treatment did not affect ($P = 0.3$) the expression of investigatory behavior in intact rams, but tended ($P = 0.09$) to decrease the expression of consummatory behavior. Testosterone and estradiol (Synovex-h) alone tended ($P = 0.07$) to increase the number of investigatory behaviors in GNX males, but consummatory behavior was not observed. Investigatory behaviors tended ($P = 0.09$) to be further increased following treatment of GNX males with progesterone. Mounts and mount attempts were observed in two of the five GNX males following progesterone treatment, but consummatory behavior was not increased ($P = 0.25$) overall. Progesterone appears to facilitate the expression of ram reproductive behavior and may be especially important for the expression of consummatory behavior.

Keywords: Progesterone, Ram, Reproductive Behavior

Introduction

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

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

The physiological significance of progesterone, outside of its role as a precursor for androgen production, is not well understood. Traditionally progesterone was thought to have little or no function in the control of male sexual behavior. Early studies, utilizing supraphysiological doses of progesterone, were shown to inhibit male sexual behavior (reviewed in Wagner, 2006). However, treatment of rodents with physiological concentrations of progesterone facilitated mounting and copulatory behavior (Andersen and Tufik, 2006). Therefore the objective of this experiment was to determine the role of progesterone in the expression of ram reproductive behavior.

Materials and Methods

Sexually naive Columbia rams (n = 6) and rams gonadectomized (GNX; n = 5) at 6 – 7 mo of age were tested for sexual behavior at 10 – 11 mo of age. Rams were housed together, fed alfalfa hay, and provided water ad libitum. Estrus was induced in ovariectomized ewes by intravaginal progesterone treatment (CIDR) for 10 d followed by 50 μg daily injections (i.m.) of estradiol-17β. Ewes exhibited estrus by 48 h following CIDR removal and estradiol treatment. Ewes remained in estrus for 96 h when treated daily with 50 μg estradiol (i.m.). Sexual behavior was evaluated in rams tested individually for three minutes in a 2.0 x 2.0 m pen with three estrous ewes. The testing environment was a partition of their home pen with rams tested in sight of pen mates. Sexual behaviors exhibited by the rams were recorded and classified as either investigatory (investigatory sniff,

flehmen, nudge, foreleg kick, and vocalizations) or consummatory (mount attempts, mounts, and ejaculations) behavior.

Baseline behaviors were established in all rams by the average of expressed sexual behavior in rams on three consecutive days. Following baseline testing, GNX rams were implanted with four Synovex-h implants (200 mg testosterone proprionate and 20 mg estradiol benzoate each; Syntex Laboratories, Inc. Palo Alto, CA). Approximately 30 d following insertion of Synovex-h implants, GNX rams were exposed to ewes in estrus and observed for the expression of sexual behavior on three consecutive days. Effect of progesterone on the expression of sexual behavior was determined in Synovex-h treated rams following the administration of exogenous progesterone (5 mg s.c. in 95% ethanol) twice per day. Progesterone treatment was initiated 2 d prior to testing and continued throughout the 3 d testing period.

Following the establishment of baseline behavior, intact rams were treated with 25 mg mifepristone (RU486; 20% ethanol 80% TWEEN 80) twice per day and exposed to estrus ewes. Mifepristone treatment was initiated 2 d prior to behavior testing.

Blood samples for analysis of serum concentrations of testosterone were collected from GNX rams prior to castration and from both GNX and intact rams three times during the testing period. Serum was separated by centrifugation and stored at -20 ^{o}C until testosterone analysis.

Concentrations of testosterone were determined in a single RIA using a solid phase DPC (Diagnostic Products Corporation, Los Angeles, CA) kit with 6.1% intra-assay variation. Standards were diluted in charcoal-stripped wether serum and were parallel to kit standards.

Statistical Methods. Effects of mifepristone (RU486) and Synovex-h (testosterone and estradiol) on sexual behavior were determined by a paired T Test (SAS 9.1, Cary, NC). Additive effects of progesterone in GNX rams were determined by paired T Test utilizing behavior expressed pre-Synovex-h or following Synovex-h as the baseline. Differences in concentration of serum progesterone were determined by GLM methods of SAS (Ver. 9.1, Cary, NC).

Results

Two intact rams were removed from the study due to a lack of sexual behavior. One of these rams had serum concentrations of testosterone less than 1 ng/mL. The other ram had levels of testosterone comparable to the other sexually-active rams in this study.

All GNX rams had detectable quantities (4.4 ± 0.9 ng/mL) of testosterone prior to castration. Following treatment with Synovex-h, serum concentrations of testosterone were not different ($P = 0.62$) from intact rams (7.1 ± 2.9 ng/mL). Serum concentrations of testosterone in GNX rams did not differ ($P = 0.43$) over time, and was not increased ($P > 0.21$) during progesterone treatment.

Treatment of intact rams with mifepristone did not affect ($P = 0.70$) investigatory behavior, but tended ($P = 0.09$) to decrease consummatory behaviors (Figure 1).

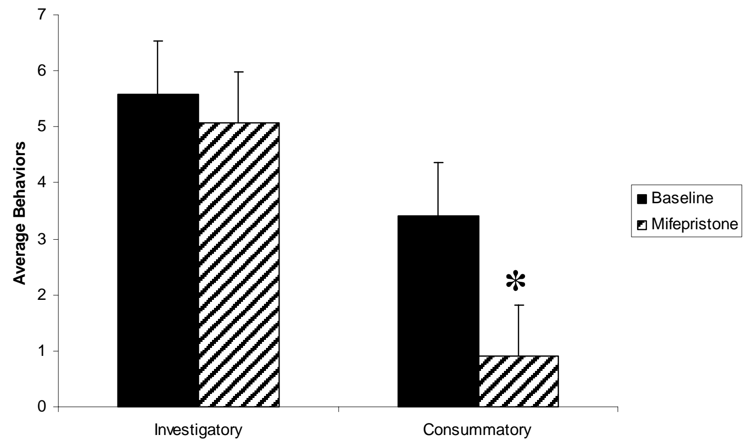

Figure 1. Investigatory and consummatory behavior expressed by intact rams prior to (Baseline) and following mifipristone treatment. * $P = 0.09$ paired T Test change from Baseline.

Synovex-h implants in combination with exogenous progesterone increased ($P = 0.02$) the expression of investigatory behavior compared to pretreatment baseline (Figure 2). Synovex-h implants alone tended ($P = 0.06$) to increase investigatory behavior. Progesterone in combination with Synovex-h treatment tended ($P = 0.09$) to further increase the expression of investigatory behavior when compared to Synovex-h alone (Figure 2).

Mounting behavior was not observed in any of the GNX rams prior to progesterone treatment. Following progesterone treatment mounting behavior was observed in two of the five GNX rams, but consummatory behavior did not increase overall ($P = 0.25$).

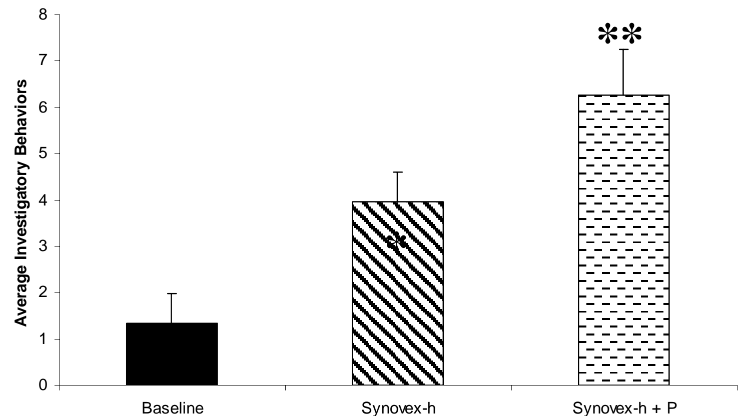

Figure 2. Investigatory behavior expressed in gonadectomized rams prior to treatment (Baseline), following treatment with Synovex-h implants, and Synovex-h implants in combination with progesterone. **$P = 0.02$ *$P = 0.07$, Paired T Test change from Baseline.

Discussion

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

Two intact rams in the current study were non-performers with an absolute absence of any observed sexual behaviors. One of these rams had low serum concentrations of progesterone and may have been sexually immature. The other ram had serum concentrations of testosterone comparable to his sexually-active cohorts.

The testes and the adrenal cortex are the sites of synthesis for progesterone in the male. Circulating progesterone in the male is most likely of adrenal origin (Wagner, 2006). In this study, effects of progesterone seem to be independent of its role as a precursor hormone. Treatment with progesterone did not increase serum concentrations of testosterone in GNX rams. Similar to rodents (Wagner, 2006), progesterone appears to be more important for the expression of mounting behavior than for sexual interest in the ram. Intact rams treated with the progesterone receptor antagonist, mifepristone, had decreased numbers of mounts while investigatory behavior was unaffected. Mounting behavior was only observed in GNX rams after treatment with progesterone, and was not observed when rams were treated with testosterone and estradiol (Synovex-h) alone. Clearly GNX rams displayed increased sexual interest, as indicated by the increased expression of investigatory behaviors; however, this increase may reflect learning (possibly independent of a hormonal affect) in these sexually naïve rams. Although results depict only a trend, numbers were limited and more robust results would be expected with a larger experimental population.

In conclusion progesterone specifically appears to facilitate the expression of ram sexual behavior and may be especially important for the expression of mounting behavior.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

ESTIMATION OF HETEROTIC EFFECTS ON HEIFER PREGNANCY IN BEEF CATTLE

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ABSTRACT: Reproduction is the most important factor to profitability of the cow herd when compared with other economically important traits, such as growth and carcass characteristics. By increasing the proportion of heifers calving at two yr of age, producers can increase the revenue and selection intensity of their herds. Therefore, the primary objective of this project was to estimate breed effects and heterosis values for heifer pregnancy. Reproductive records collected on 651 purebred and crossbred commercial beef females, comprised of Hereford, Simmental, and Tarentaise, from Montana State University's Northern Agricultural Research Center in Havre, Montana were analyzed as a binary trait. Females were born from 1976 to 1995. Data included calving records on two- and three-yr-old cows. Heifer Pregnancy was defined as a success if her first calving record was at two yr of age, but a failure if her first calving record was at three yr of age. Heifer pregnancy was able to be defined this way because young cows in this herd were not culled unless they were open for two consecutive years. Comparison of direct genetic effects showed that Simmental females were the most likely and Hereford females were the least likely to calve at two yr of age with Tarentaise being intermediate to the other two breeds. Comparison of maternal genetic effects showed that females out of Tarentaise dams were the most likely and females out of Hereford dams were the least likely to calve at two yr of age with no estimation on females out of Simmental dams. Comparison of paternal genetic effects showed that females out of Tarentaise sires were most likely and females out of Simmental sires were least likely with females out of Hereford sires being intermediate. Heterosis was estimated to be -2.79%, -0.52%, and 9.56% for direct, maternal and paternal heterosis, respectively, which is a favorable response that indicates that crossbreeding will increase the proportion of females calving at two yr of age.
age.

Key Words: Heterosis, Heifer Pregnancy, Reproduction

Introduction

Reproduction is one of the most economically important traits in a commercial cow/calf operation. Willham (1973) stated that, at the commercial level, reproduction was 10 times as important as growth and 20 times greater than end-product traits. Reproduction is essential when the overall goal for a commercial producer is to raise a viable calf annually.

In the past, reproductive traits have been reported as being lowly heritable, ranging from zero to 0.20 (Matthiews et al., 1995; Doyle et al., 2000), causing producers to seek management alternatives to increase reproduction.

By increasing the proportion of heifers that calve at 2 yr of age, producers can minimize the high investment cost of replacement heifer development, and increase total revenue, while increasing selection intensity of the herd. Given the increasing popularity of crossbred cattle for improved performance of other economically important traits, such as growth traits and carcass characteristics, crossbreeding may also be beneficial in the terms of heifer pregnancy rate. Heterosis has generally been reported to be higher in those traits that are lowly heritable, making heifer pregnancy an ideal candidate to improve through the use of crossbreeding. Therefore, implementation of any crossbreeding system should yield increased reproductive efficiency. Cassady et. al. (2002) stated that the efficiency of various crossbreeding systems is determined by differences among breed effects relative to magnitudes of heterosis and recombination effects. Therefore, estimation of these genetic effects provides essential information to guide efficient use of genetic resources in crossbreeding programs.

The experimental objective of this study was to estimate breed and heterotic effects of heifer pregnancy in crossbred beef cattle.

Materials and Methods

All procedures for data collection were approved by Montana State University. Data for this study were obtained from the Montana State University Northern Agricultural Research Center in Havre, Montana and at the Thackeray Ranch (summer range) in the Bear Paw Mountains. These data were a subset of a long-tern crossbreeding experiment that was conducted at the research center and Thackeray Ranch and has been described by Anderson et al., 1996; Davis et al., 1996; Anderson et al., 2001; Boss et al., 2001; Davis et al., 2001a; Davis et al., 2001b; and Bailey et al., 2001. The data included 651 reproductive records on 2 and 3-yr-old females born from 1976 to 1995. The records were from

purebred Hereford and Tarentaise and crossbred Hereford, Simmental, and Tarentaise females as shown in Table 1. Due to small groups of animals, breed groups with Simmental or Simmental cross dams were omitted for this study.

Heifer Pregnancy was defined as the ability of the heifer to conceive and carry the fetus to parturition. Heifer pregnancy was determined to be a success if her first calving record was observed at two yr of age, but a failure if her first calving record was observed at three yr of age. Records observed at two yr of age were coded as "1", and records observed at three years of age were coded as "2." Heifer pregnancy was able to be defined this way because young cows in this herd were not culled unless they were open for two consecutive years.

Data were analyzed using the IML procedures of SAS (SAS Inst., Inc., Cary, NC) using the following equation:

$$\mathbf{s} = \mathbf{C}^{-}\mathbf{r}$$

where **s** is the solution vector of genetic and heterotic effects, **C** is the matrix of genetic expectations (Table 2) matched to each breed type, and **r** is the vector of phenotypes where "1" designates females that first calved as 2-yr-olds and "2" designates females that calved as 3-yr-olds.

Results and Discussion

Estimates of breed effects and heterosis are shown in Tables 3 and 4, respectively. Comparisons of direct genetic effects showed that Simmental females were the most likely, and Hereford females were the least likely, to calve at 2 yr of age with Tarentaise being intermediate to the other two breeds. Comparison of maternal genetic effects showed that females out of Tarentaise dams were more likely, and females out of Hereford dams were less likely, to calve at 2 yr of age. Estimations of maternal genetic effects were unable to be calculated for Simmental due to the fact that there were no purebred Simmental or Simmental cross dams in this project. There were several breed groups representing Simmental that had to be omitted because they were considered to have too few records. Comparison of paternal genetic effects showed that females out of Tarentaise sires were most likely, and females out of Simmental sires were least likely, to calve at 2 yr of age, with females out of Hereford sires being intermediate.

Individual heterosis (Table 4) was estimated to be -2.79%. This is a favorable response due to open heifers being assigned a phenotype of "2" and bred heifers assigned a phenotype of "1". This estimate indicates that crossbreeding will increase heifer pregnancy rates in commercial cow/calf operations. It can be assumed that crossbred females have a 2.79% greater chance of calving at 2 yr of age than do purebred females.

Maternal heterosis was estimated to be -0.52%. This is a favorable response that indicates that daughters of crossbred dams would have increased pregnancy rates compared to their contemporaries out of purebred dams.

Paternal heterosis was estimated to be 9.56%. This is an unfavorable response, indicating that females out of crossbred sires are less likely to calve at 2 yr of age than those heifers out of purebred sires. The biological reason for this is not understood and warrants further investigation.

Implications

The results of this experiment were favorable for the estimation of heterosis on heifer pregnancy rate. The implementation of crossbred females into a cow/calf operation would prove valuable to the increase of heifer pregnancy rates. However, producers must be aware that crossbreeding is only a tool that may be utilized to increase the reproductive efficiency of a cow herd, along with good selection and management practices

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Table 1. Number and average heifer pregnancy of females per breed group.

Breed Group[1]	Females	Average Heifer Pregnancy
H x H	220	81.4%
H x HT	17	82.4%
H x T	39	84.6%
H x TH	17	88.2%
S x H	44	88.6%
SH x H	60	71.7%
T x H	70	90.0%
T x HT	16	93.8%
T x T	104	89.4%
T x TH	22	81.8%
TH x H	15	66.7%
TH x T	14	92.9%
TH x TH	13	84.6%

[1] H - Hereford; S - Simmental; T - Tarentaise

Table 2. Genetic expectation for each breed group[1]

	μ	g^I_H	g^I_T	g^I_S	g^M_H	g^M_T	g^M_S	g^P_H	g^P_T	g^P_S	g^N_H	g^N_T	g^N_S	h^I	h^M	h^P
H x H	1.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
H X HT	1.00	0.75	0.25	0.00	0.50	0.50	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.50	1.00	0.00
H x T	1.00	0.50	0.50	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
H X TH	1.00	0.75	0.25	0.00	0.50	0.50	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.50	1.00	0.00
S x H	1.00	0.50	0.00	0.50	1.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00
SH x H	1.00	0.75	0.00	0.25	1.00	0.00	0.00	0.50	0.00	0.50	1.00	0.00	0.00	0.50	0.00	1.00
T X H	1.00	0.50	0.50	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
T X HT	1.00	0.25	0.75	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.50	1.00	0.00
T x T	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
T X TH	1.00	0.25	0.75	0.00	0.50	0.50	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.50	1.00	0.00
TH x H	1.00	0.75	0.25	0.00	1.00	0.00	0.00	0.50	0.50	0.00	1.00	0.00	0.00	0.50	0.00	1.00
TH x T	1.00	0.25	0.75	0.00	0.00	1.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.50	0.00	1.00
TH x TH	1.00	0.50	0.50	0.00	0.50	0.50	0.00	0.50	0.50	0.00	1.00	0.00	0.00	0.50	1.00	1.00

[1] g^I_i, g^M_i, g^P_i, and g^N_i are direct, maternal, paternal, and maternal grandam breed effects. Subscript I represents the breed associated with the effect (H – Hereford, T – Tarentaise, and S – Simmental). h^I, h^M, and h^P are direct, maternal, and paternal heterosis effects.

Table 3. Estimates of breed effects[1]

Comparison[2]	g^I	g^M	g^P	g^N
H - T	2.66%	1.67%	3.66%	1.19%
H - S	10.08%	-	-1.84%	-
S - T	7.41%	-	-5.50%	-

[1] g^I_i, g^M_i, g^P_i, and g^N_i are direct, maternal, paternal, and maternal grandam breed effects

[2] H - Hereford; T - Tarentaise; S - Simmental

Table 4. Estimates of heterotic effects[1]

Heterosis	Estimate
h^I	-2.80%
h^M	-0.52%
h^P	9.56%

[1] h^I, h^M, and h^P are direct, maternal, and paternal heterosis effects

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

GENETIC ANALYSIS OF REBREEDING TO PRODUCE A CALF AT THREE YEARS OF AGE IN THE MONTANA LINE 4 HEREFORD HERD

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ABSTRACT: Rebreeding a first calf heifer to produce her second calf at 3 yr of age can be challenging for beef producers. Heifers generally require more recovery time following their first calf which may delay the onset of estrus to a point beyond the normal breeding season. In an effort to improve heifer rebreeding, data on beef cows at 2 and 3 yr of age were analyzed to determine if rebreeding of first calf heifers is under any degree of genetic control. Records on 399 females born from 1976 – 2001 were analyzed to determine genetic parameters for probability of rebreeding. Animals included in the analysis were the Line 4 Herefords which are maintained at Montana State University's Northern Agricultural Research Center in Havre, Montana. Young cows in this herd were only culled if they were open, so any cow calving at 2 yr of age, but not at 3 yr of age was considered to fail at rebreeding. Overall, 69.9% of females were successful in breeding back to produce a calf at 3 yr of age. To estimate the genetic parameters associated with this trait, data were analyzed using MTDFREML with year of birth as a categorical fixed effect and percentage of individual inbreeding fit as a linear and quadratic covariate. On the observed scale, heritability was estimated to be 0.14 (0.12). Converting the observed trait to the underlying scale produced a heritability estimate for rebreeding of 0.24. Probability of rebreeding to produce a second calf at three yr of age has a genetic component and selection against females who fail to rebreed should result in a positive genetic response.

Keywords: Genetic parameters, reproduction, beef cattle

Introduction

It has been long accepted that reproductive traits are the most important set of traits in a cow/calf operation (Dickerson, 1970). Estimates of the relative importance of reproductive traits compared to growth and end-product traits have ranged from 3.24 to 20 (Willham, 1973; Melton, 1995). Therefore, increasing the reproductive level of the cow herd can have a dramatic effect on the profit of the operation.

Within reproductive traits, the ability of a cow to produce a second calf at three years of age may be the most important factor in determining if a cow will remain in the herd. A large investment is generally involved in heifer development in order for a female to produce a calf at two years of age, but production of one calf does not cover this investment. Therefore, it is important for the cow to rebreed annually in order to remain in the herd and cover her expenses by producing as many calves as necessary to recoup the investment (Doyle, 2000). However, the resumption of estrous in first calf heifers is generally longer than in older cows, so this rebreeding can be a management challenge. Many producers will breed heifers so that they calve earlier than the rest of the herd, allowing them additional recovery time before breeding season, but this does not insure that those females will rebreed. Therefore, genetic predictions for the ability to rebreed could provide producers with an additional selection tool when selecting replacement females for the herd.

Therefore, the objective of this study was to determine if there is a genetic component to rebreeding following the first calf which could later be used to develop genetic prediction tools for producers.

Materials and Methods

All procedures for data collection were approved by Montana State University. Data was collected on 399 females in the Line 4 Hereford herd at Montana State University's Northern Agricultural Research Center in Havre, Montana born from 1976 to 2001. Animals in the Line 4 herd are descendents of animals from the Miles City Line 1 population. The foundation animals for the Line 4 herd were transferred from Miles City to Havre in 1962 and 1963. With the exception of some early matings back to Line 1 animals (prior to 1972), this herd has been maintained as a closed herd since its inception in Havre.

All cows were bred natural service with a breeding season of 45 d (June 1 – July 15). Two- and 3-yr-old cows were only culled from this herd if they were open for two consecutive years. Therefore, any cow that produced a calf at two yr of age, but then disappeared from the database was assumed to not rebreed to produce a calf a 3 yr of age. Cows that did produce a calf at 3 yr of age were denoted with a phenotype of "1" and cows that failed to produce a second calf were denoted with a phenotype of "2".

Number of females and proportion of females producing a second calf per year of birth are shown in Table 1. On average, 69.9% of females were successful in rebreeding and produced a second calf at 3 yr of age.

All cows were maintained together, so year was the only categorical fixed effect included in the model. Level of inbreeding was fit as a linear covariate to

account for the fact that this was a closed herd with varying amounts of inbreeding in the animals. Although inbreeding increased each year, on average, there was enough variation within each year of birth that year of birth did not account for all of the variation due to inbreeding. Average inbreeding as well as the amount of variation in inbreeding for each year is shown in Table 2.

Random effects included in the model were only direct genetic effects and residual so that the model was:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z_a a} + \mathbf{e}$$

where:

- **y** is a vector of observed rebreeding;
- **β** is a vector of fixed effects including year of birth and a linear covariate of inbreeding;
- **a** is a vector of direct genetic effects;
- **e** is a vector of random error effects;
- **X** is a known incidence matrix associating fixed effects with records in **y**; and
- $\mathbf{Z_a}$ is a known incidence matrix associating random genetic effects with records in **y** with zero columns associated with the 7,112 animals in the pedigree, including those that do not have records.

Furthermore,

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

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

Variance components, on the observed scale, were estimated using a linear animal model in the MTDFREML program (Boldman, et al., 1995) with the adjustment made by Dodenhoff et al. (1998) to calculate the standard errors for certain models. Although analysis

Table 1. Number of females and percentage producing a calf at three years of age per year.

Year of Birth	Number	Percentage
1976	23	69.6%
1977	20	90.0%
1978	12	75.0%
1979	23	73.9%
1980	33	75.8%
1981	16	75.0%
1982	26	57.7%
1983	20	50.0%
1984	13	92.3%
1985	21	47.6%
1986	7	28.6%
1987	12	58.3%
1988	0	-
1989	2	0.0%
1990	0	-
1991	4	75.0%
1992	3	100.0%
1993	15	73.3%
1994	21	85.7%
1995	21	66.7%
1996	18	77.8%
1997	16	62.5%
1998	23	60.9%
1999	19	73.7%
2000	12	58.3%
2001	19	94.7%
Total	399	69.9%

Table 2. Average inbreeding (F) and standard deviation of inbreeding per year for females in the study.

Year of Birth	F	Std. Dev.
1976	27.2%	2.2%
1977	28.4%	2.9%
1978	28.6%	2.5%
1979	29.0%	2.2%
1980	29.3%	2.9%
1981	29.3%	2.0%
1982	29.9%	2.9%
1983	29.6%	2.5%
1984	30.7%	3.5%
1985	31.3%	2.1%
1986	31.3%	1.9%
1987	31.8%	2.1%
1988	-	-
1989	32.0%	1.8%
1990	-	-
1991	31.7%	4.5%
1992	35.1%	0.1%
1993	33.7%	2.6%
1994	32.4%	2.3%
1995	34.1%	1.7%
1996	34.6%	2.2%
1997	35.0%	3.2%
1998	34.5%	1.5%
1999	34.6%	2.0%
2000	34.7%	1.9%
2001	36.3%	1.6%

using a threshold model would be more appropriate, previous studies have found that there is little difference in ranking of sires when categorical data is analyzed using linear methods (Meijering and Gianola, 1985).

Heritability was then transformed to the underlying scale using the following transformation (Roberston and Lerner, 1949; Dempster and Lerner, 1950; Hooijer *et al.*, 2001):

$$h^2_{\text{underlying}} = \frac{h^2_{\text{observed}}(p)(1-p)}{z^2}$$

where p is the proportion of animals experiencing calving difficulty and z is equal to p times the associated intensity of p (Van Vleck, 1993).

Results and Discussion

Estimates of variance components are shown in Table 3. The heritability of breed back, on the observed scale, was estimated to be 0.14 (0.12). Transformed to the underlying scale, the estimate of heritability was 0.24. Previous studies have shown that heritabilities transformed to the underlying scale are larger than their observed scale counterparts (i.e., Buddenberg et al., 1989; Evans et al., 1999). This estimate is larger than that found by Doyle et al. (2000) who estimated 0.19 (0.02) in Angus cattle using Method $\Re$. However, it is in the range of other studies which vary greatly with estimates from 0.00 to 0.18 on the observed scale (Buddenberg et al., 1989) and estimates of 0.17 to 0.49 (Buddenber et al., 1989; Snelling et al., 1996) on the underlying scale when analyzed as a threshold trait.

Table 3. Genetic parameters for breed back.

	Scale	
Parameter[a]	Observed	Underlying
σ_a^2	0.0278	
σ_p^2	0.2008	
h^2	0.14 (0.12)	0.24

[a] σ_a^2 – Additive genetic variance; σ_p^2 – Phenotypic variance; h^2 – Direct heritability

These estimates are also similar to those found for heifer pregnancy, which have been shown to range from low estimates on the observed scale (Milagres et al., 1979; Koots et al., 1994) to moderate estimates on the underlying scale of 0.21 (Doyle et al., 1996) and 0.20 (Snelling et al., 1996). Heifer pregnancy EPD are currently being published by certain U.S. beef breed associations and utilized by producers to increase fertility in their herds. The similarity of heifer pregnancy genetic parameters to the estimates found in this study are encouraging that breed back EPD may have the same usefulness for the beef industry.

Implications

Reproduction is an important part of any seedstock enterprise. Utilizing the genetic parameters estimated in this study, genetic predictions, in the form of expected progeny differences, can be developed that will allow producers to select replacements that have a higher probability of producing their second calf at three years of age. This will ultimately aid producers in their continued effort to maintain or increase the level of reproduction in their beef herds.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

RETROSPECTIVE ANALYSIS OF SELECTION APPLIED TO A RATIO

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ABSTRACT: Use of ratios to adjust one correlated trait for another is fairly commonplace. However, there are statistical arguments that restrict the appropriate use of ratios to certain circumstances. The ratio of a calf's weaning weight to that of its dam has been used as an indicator of cow efficiency and an evaluation criterion. Objectives of this study were to retrospectively assess selection applied when bulls were selected based on the ratio of their weaning weight to the coincident weight of their dam and predict correlated responses in these weights to that selection. The variance of the ratio tended to increase (P = 0.11) as weaning weight increased, but was independent of cow weight. The observed selection differential for weaning weight was independent of the selection differential for the ratio (P > 0.10). However, the selection differential for cow weight was inversely related to the selection differential for the ratio (P = 0.01). The emphasis on cow weight relative to that given weaning weight ranged from 3% to 109% (SD = 31%) and the variance of the index in retrospect fluctuated more than 5-fold. It follows that the correlated responses of weaning weight and cow weight to selection for their ratio would be variable. These results contraindicate use of the ratio of calf weaning weight to cow weight either as an evaluation criterion for beef cows or as a selection criterion upon which to choose bulls or heifers. By inference, the use of ratios of traits to evaluate animals or as selection criteria is generally likely to be inappropriate.

Key words: Breeding aims, Efficiency, Phenotypic selection, Selection index

Introduction

Use of ratios to adjust one correlated trait for another is fairly commonplace. Examples include weaning weight:cow weight to indicate cow efficiency (Kress et al., 1995; MacNeil, 2005), longissimus muscle area:carcass weight to indicate muscularity (Thomas et al., 2002), weight:height to indicate body condition (Brown et al., 2000; Turconi et al., 2006), and feed:gain to indicate efficiency of growth (Tedeschi et al., 2006). Concerns about insidious effects of ratios were first noted by Pearson (1897) and have been elaborated on numerous times subsequently. Use of a ratio for purposes of comparing subjects assumes three conditions: 1) the relationship between numerator and denominator is linear; 2) the intercept of the regression of numerator on denominator is the origin; and 3) the variance of the ratio increases with increasing values of the numerator and denominator (Weil, 1962). These conditions are rarely tested. When ratios are used to evaluate subjects for the purpose of selection among them, the ratio is often also implicitly assumed to be normally distributed. Yet, a ratio of two normally distributed variables is not normally distributed (Fieller, 1932; Hinkley, 1969). Despite these theoretical issues with the use of ratios, they continue to be commonly used to evaluate animals and as selection criteria. The objective of this study was to conduct a retrospective analysis of selection applied to calf weaning weight and cow weight when sires were selected based on the ratio of these traits.

Materials and Methods

Data for this study were compiled from MacNeil (2005). Calves were produced in 1989 to 2000 from sires born in 1987 to 1998 and selected on the ratio of their age-of-dam adjusted 200-d weight (**WW**) to the coincident mature equivalent weight of their dam (**CW**). Virtually all selection pressure was applied to males and most females were exposed to breeding as yearlings. With the following exceptions, four yearling bulls were selected and used each year. Nine yearling bulls were used in the 1989 and 1990 breeding seasons and five yearling bulls were used in 1992 and 1996 to 1999 breeding seasons. In the present study, attention is focused on the sire selection differentials and predicted responses in the component traits. Individual selection differentials were computed within year based on the adjusted phenotypes. Means and average selection differentials for sires, by birth year, are shown in Table 1.

Table 1. Adjusted weaning weight (WW, kg) and mature equivalent cow weight (CW, kg) means and sire selection differentials for WW, CW and their ratio.

Birth Year	Mean		♂ Selection Differential		
	WW	CW	WW	CW	Ratio
1987	246	486	19.7	-2.3	6.3
1988	226	472	23.3	-1.7	8.0
1989	240	499	47.7	25.3	8.9
1990	239	498	30.2	-19.9	8.4
1991	244	515	42.9	24.5	5.5
1992	250	526	47.2	3.2	8.6
1993	260	544	37.9	-25.6	10.7
1994	236	518	37.2	-40.0	11.6
1995	242	546	39.0	8.9	7.5
1996	229	511	31.7	-10.5	7.9
1997	227	508	22.6	-57.7	9.9
1998	240	517	39.0	-7.3	7.8

Obviously, variation in the selection differentials results both from the selection applied and from sampling and the latter source of variation cannot be quantified without replicating the selection line.

Index in retrospect calculations were used to compute phenotypic selection index weights (**w**) that are implied by the observed selection differentials for weaning weight and cow weight (Table 1). The phenotypic variance-covariance matrix for WW and CW (kg) was:

$$\mathbf{P} = \begin{matrix} 564.0 & 256.1 \\ 256.1 & 2906.8 \end{matrix} \qquad \text{MacNeil, 2005.}$$

Thus, $\mathbf{w} = \mathbf{P}^{-1}\Delta\mathbf{p}$, where $\Delta\mathbf{p}$ denotes the vector of phenotypic selection differentials and the index in retrospect resulting from selection on the ratio is $I = w_1WW + w_2CW$. Correlated responses to selection on the index ($\Delta\mathbf{G}$), given selection intensity equal to 1.0, are calculated as: $\Delta\mathbf{G} = \mathbf{wG}/\boldsymbol{\sigma}_\mathbf{I}$, where $\boldsymbol{\sigma}_\mathbf{I}$ = SD of the index and **G** is the genetic variance-covariance matrix for WW and CW.

$$\mathbf{G} = \begin{matrix} 270.7 & 703.7 \\ 703.7 & 2209.2 \end{matrix} \qquad \text{MacNeil, 2005.}$$

Given the phenotypic means and variance-covariance matrix, samples of 100 weaning weight – cow weight pairs were generated from the bivariate normal distribution. This Monte Carlo simulation was replicated 1000 times. Within each replicate, the average index I = **0.06575***WW -**0.0088***CW and the ratio R = WW/CW were calculated. Samples were ranked based on both I and R. The 2, 5, 10, 20, 40, 60, and 80 percent of samples with greatest values for R were selected. Ranks of I were then summarized to determine the frequency with which selection based on I and R was consistent, i.e., the same samples were selected using both criteria. In separate simulation experiments, a) the mean weaning weight was reduced 10% with the net effect of making the y-intercept of the regression of WW on CW closer to 0.0, and b) the correlation between weaning weight and cow weight increased to 0.5 increasing the slope of the regression.

Results and Discussion

As previously reported by MacNeil (2005), the relationship between age-of dam adjusted 200-d weight and mature equivalent cow weight was nonlinear ($P < 0.05$) and the y-intercept of the regression of weaning weight on cow weight was positive, complicating interpretation of the ratio. The variance of the ratio tended to increase ($P = 0.11$) as weaning weight increased, but was essentially independent of cow weight. In and of themselves, these violations of the conditions under which ratios provide for an unbiased comparison of subjects may be sufficient to negate the usefulness of the ratio.

Illustrated in Figure 1 are relationships of selection differentials for observed weaning weight and cow weight with the selection differential for their ratio. Given the present implementation of selection for the ratio of weaning weight to cow weight, the observed secondary selection differential for weaning weight was essentially independent of the primary selection differential. However, the secondary selection differential for cow weight was inversely related to the primary selection differential ($P = 0.01$). Thus, under the assumption that the Bulmer (1971) effect is negligible, the relative emphasis on weaning weight and cow weight is expected to change with increasing selection intensity.

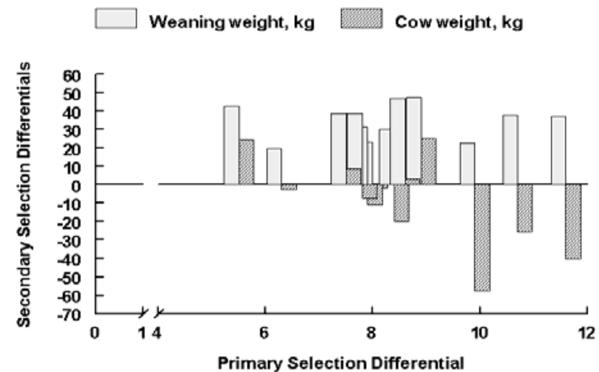

Figure 1. Secondary selection differentials for weaning weight and cow weight plotted against the primary selection differential for their ratio.

Presented in Table 2 are the yearly selection index weights applicable to weaning weight and cow weight when bulls were selected for use based on the ratio of these traits, the variance of the index, and predicted correlated responses in these traits. Use of constant genetic and phenotypic variance-covariance matrices results in changes in index weights and predicted correlated responses being due only to year to year changes in the secondary selection differentials. The emphasis on cow weight relative to that given weaning weight ranged from 3% to 109% (SD = 31%) and the variance of the index in retrospect fluctuated more than 5-fold over the course of the experiment. It follows that correlated responses would vary across the annual cycles of selection.

Table 2. Index weights for weaning weight and cow weight when selection was based on their ratio, variance of the index in retrospect (σ^2), and predicted correlated responses.

Year	Index weight		σ^2	Correlated response	
	WW	CW		WW	CW
1987	0.037	-0.0040	0.74	8.3	19.8
1988	0.043	-0.0044	1.01	8.6	20.6
1989	0.084	0.0013	4.05	11.8	30.8
1990	0.059	-0.0121	2.02	5.3	10.5
1991	0.075	0.0018	3.27	12.0	31.5
1992	0.087	-0.0065	4.07	9.3	23.1
1993	0.074	-0.0154	3.21	5.2	10.2
1994	0.075	-0.0204	3.62	3.2	4.1
1995	0.071	-0.0032	2.73	10.2	25.9
1996	0.060	-0.0089	2.01	7.1	16.1
1997	0.051	-0.0244	2.56	-2.1	-11.2
1998	0.073	-0.0090	2.92	7.9	18.6

From the simulation and illustrated in Figure 2 is the degree to which selection based on the ratio of calf weaning weight to cow weight would result in selection decisions that are inconsistent with the average index in retrospect. Thus, error rates plotted for the nominal conditions assume the index in retrospect weights are consistent with true economic values for weaning weight and cow weight. This assumption is arguable and to the extent that it is not true even greater discrepancies in sets of animals selected using the ratio *vis a vis* a selection index would be expected. Under the nominal conditions, discrepancies between sets of selected animals are most severe with low proportions selected (i.e. high selection intensity). Thus, under most practical conditions selection of bulls would be more greatly affected than selection of heifers.

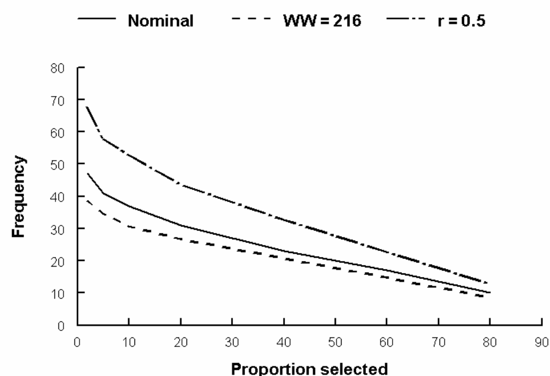

Figure 2. Frequency with which animals selected using a ratio of calf weaning weight to cow weight and a corresponding index in retrospect are inconsistent as a function of the proportion of animals selected. The nominal simulations were conducted using parameters estimated from the data and perturbed simulations conducted with the parameter altered relative to the nominal condition as indicated.

When the mean weaning weight was reduced *ceteris paribus* relative to the nominal condition making the y-intercept from the regression of weaning weight on cow weight closer to zero, the inconsistency between sets of animals selected by the index in retrospect and the ratio was reduced at all proportions selected (Figure 2). This reduction was greatest at low proportions selected. However, when the covariance between numerator and denominator of the ratio was increased *ceteris paribus,* the rate at which selection decisions were inconsistent using the two criteria also increased. Thus, problems associated with expressing efficiency as a ratio may be greater for feed to gain ratios than for use of weaning weight to cow weight as a proxy for cow efficiency.

A variety of previous studies have also addressed the use of ratios in various contexts. Dinkel et al. (1965) found the ratio of longissimus muscle area to carcass weight an unsatisfactory procedure to adjust for differences in carcass weight and suggest its use has the potential to mask treatment effects. Lang et al. (2005) demonstrate the potential for the use of ratios in rescaling phenotypic data to introduce spurious correlations with body size and lead to inaccurately identified quantitative trait loci. Packard and Boardman (1999) go so far as to recommend 1) discontinuing use of ratios in an attempt to scale data and, 2) that conclusions from studies using them not be taken too seriously as they are often unfounded or incorrect.

The present results raise three considerations that may contraindicate use of ratios of traits in selection. First, for many pairs of traits, the implicit assumptions of a ratio are violated. Second, emphasis placed on each trait will be inconsistent when selection is based on their ratio. Finally, as a result of the inconsistent selection applied, a reduction in genetic improvement relative to that attainable with index selection is to be expected. While these conclusions are drawn from a single selection line, they are consistent with a substantial body of literature regarding the statistical properties of ratios.

Implications

Use of the ratio of calf weaning weight to cow weight as a selection criterion has theoretical defects and places inconsistent emphasis on the component traits resulting in variable responses to selection. Despite these issues the practice is pervasive. These problems are most severe with high selection intensity which occurs, for instance, in the choosing bulls for use in AI. Other commonly used ratios likely pose similar problems. Thus, it is suggested that ratios of two traits not be used in describing individual animals or as a criterion to choose among candidates for selection.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

ITERATIVE SOLUTION OF LINEAR EQUATIONS FROM NATIONAL BEEF CATTLE EVALUATION

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ABSTRACT: Genetic evaluation comprises setting up and solving equations including fixed (herd, year, age-of-dam) and random (direct, maternal) effects. Animal models that include effects for every animal in the pedigree are typically used. Resulting equations are large (millions of animals times several traits) and sparse (averaging 10 or so non-zero elements per equation). Evaluations undertaken by the Center for Genetic Evaluation of Livestock at CSU utilize a sequential method of solving known as Gauss-Seidel (G) iteration, as implemented in the Animal Breeders Tool Kit. No relaxation is used, nor is account taken of equation structure. In multi-trait circumstances, Block (B) Gauss-Seidel may be used, whereby a few equations (e.g. all effects on one animal) are solved simultaneously. This technique was superior in sire-maternal grandsire models but has less advantage in animal models when blocks may be sparse, as in models assuming zero direct-maternal genetic covariance. An alternative approach providing iterative refinement is conjugate gradient (C) and is typically superior when equations can be preconditioned using the diagonal (D) or suitable B. There is little difference in arithmetic operations required, although C methods can process equations without regard to the order of the elements, whereas G requires coefficients sorted by row and column. The objective of this study was to quantify these methods for convergence rate. Datasets represented 79,690 (24,735 blocks of size 3), 193,660 (45,901×4), 421,074 (137,043×3) or 9,114,549 (2,141,596×4) equations from growth or carcass evaluations. Methods were compared after each round by computing correlations (within factors) of current round values to those obtained after 10,000 G iterations. Correlations exceeding 0.999 for all factors using G, BG, DC, BC required 634, 470, 74 and 55 rounds in dataset 1, 3,988, 2,391, 252 and 164 in dataset 2, 5,110, 3,916, 203 and 150 in dataset 3. Method DC converged to slightly different solutions for maternal genetic weaning weight in dataset 4, but correlations of at least 0.998 were achieved by G, BG, DC and BC in 3,344, 1,044, 500 and 463 iterations. Typically C required about one-tenth iterations of G. The advantage of accounting for blocks was greater in G than C.
Key Words: Mixed model equations, iterative solution, pre-conditioned conjugate gradient

Introduction

Routine genetic evaluation of livestock involves partitioning observed performance records for multiple traits into a number of explanatory effects. Growth analyses might include fixed effects for herd-year, age of dam and date of birth, and random effects for direct genetic, maternal genetic and maternal permanent environmental effects. These effects are obtained simultaneously by setting up and solving Henderson's mixed model equations that account for the variance-covariance structure of the random effects. The equations are computationally straightforward to construct by including genetic effects for all animals regardless of whether or not every animal has every effect represented in the performance records. Accordingly, there may be millions or tens of millions of equations but most equations are sparse with 10 or so non-zero elements per equation. The Center for Genetic Evaluation of Livestock (**CGEL**) at Colorado State University uses an indirect approach to solve these equations known as Gauss-Seidel (**G**) iteration as implemented in the Animal Breeders Tool Kit (ABTK, Golden et al., 1992). This method involves obtaining successively improved solutions from some starting values and may require as many as 10,000 iterations to obtain convergence. Alternative approaches for solving equations include a block (**B**) implementation of G, or the conjugate gradient (**C**) method using diagonal (**D**) or B preconditioners. Computing power has grown more rapidly than the size of the national pedigree files but pooling breeds for multi-breed national evaluation and including additional correlated traits will lead to orders of magnitude increases in numbers of equations. The objective of this study was to compare these alternate approaches in terms of convergence properties.

Materials and Methods

Datasets. Four sets of mixed model equations representative of many of the genetic evaluations undertaken by CGEL were used for comparison. Datasets 1, 2 and 4 represented growth evaluations (birth and weaning weights) whereas dataset 3 involved carcass and ultrasound traits (Table 1). Datasets represented analyses undertaken in 2006. The fitted effects in the models varied by dataset but included fixed, direct genetic, maternal genetic and maternal permanent environmental effects (Table 1). Fixed effects were not constrained. Fixed effects included various contemporary groups, cross-classified effects (e.g., age of dam) and a few covariates (e.g., age at measurement). The variance-covariance matrices between factors on a single animal ($\boldsymbol{G_0}$) varied for each dataset, as did residual variance-covariance matrices ($\boldsymbol{R_0}$). All datasets included several

generations of animals and incorporated the inverse of the numerator relationship matrix relevant to their respective pedigree file. More details of specific analyses are in Speidel et al., (2003). Datasets 1, 2 and 4 involved sparse $\boldsymbol{G_0}$ as covariances between direct and maternal genetic effects and all those involving maternal permanent environmental effects were zero. The $\boldsymbol{R_0}$ were dense. Dataset 3 involved dense $\boldsymbol{G_0}$ but sparse $\boldsymbol{R_0}$ as no animals were measured for both carcass and live animal ultrasound measures.

Iterative Methods. Suppose $\boldsymbol{A}$ defines a square left-hand side or coefficient matrix of order n, $\boldsymbol{b}$ is the right-hand side, and $\boldsymbol{x}$ is a solution such that $\boldsymbol{Ax}=\boldsymbol{b}$. Iterative methods such as G or C involve successively refining an approximate solution until a suitably accurate solution is found. A perfect solution is not required, as due to rounding errors and other issues, the equations being solved are at best a nearby representation of the theoretical equations that we would like to solve. Method G involves solving an unknown element i in iteration $k+1$, as if all the solutions for other effects are correct (e.g., Golub and Van Loan, 1983). In scalar notation, using a superscript to denote round of iteration,

$$x_i^{k+1} = (b_i - \sum_{j=1}^{j=i-1} a_{i,j} x_j^{k+1} - \sum_{j=i+1}^{j=n} a_{i,j} x_j^k)/a_{i,i} \quad [1].$$

Method C is based on updating $\boldsymbol{x}$, with the update being derived by solving the leftover or residual from the current solution. That is, $\boldsymbol{x}^{k+1}=\boldsymbol{x}^k + \alpha\, \boldsymbol{r}^k$ where $\boldsymbol{r}^k = \boldsymbol{b} - \boldsymbol{Ax}^k$ is the residual from the current solution and α is a scaling factor. Furthermore, C involves modifying equations $\boldsymbol{Ax}=\boldsymbol{b}$ to solve an equivalent symmetric set of equations $(\boldsymbol{P}^{-1}\boldsymbol{A}\ \boldsymbol{P}^{-1})\ \boldsymbol{s} = \boldsymbol{P}^{-1}\boldsymbol{b}$ that converge more rapidly for appropriate chosen symmetric $\boldsymbol{P}$. The solution $\boldsymbol{x}$ is obtained as $\boldsymbol{P}\,\boldsymbol{s}$. The matrix $\boldsymbol{P}^2$ is known as the conditioning matrix, and among other options can be diagonal elements of $\boldsymbol{A}$ or diagonal blocks of $\boldsymbol{A}$. Factor α controls the step size and changes each iteration. Implementation details are available elsewhere (e.g., Golub and Van Loan, 1983; Berger et al., 1989; Stranden and Lidauer, 1999; Mrode, 2005).

Diagonal blocks in $\boldsymbol{A}$ can be used to advantage in method G by replacing scalar elements in [1] by corresponding blocks of submatrices of the left-hand side or subvectors of the solution and right-hand side.

Methods were implemented using Lahey Fortran F95 with only diagonal and upper non-zero elements of a coefficient matrix stored in single precision in a single vector sorted by column within row. A second vector of equal length recorded the column identifiers for each non-zero element. A third vector of length equal to the order of the equations plus one stored pointers to the diagonal element where each row of non-zero elements or column identifiers began.

Method Comparison. Equations from each dataset were subjected to 10,000 G iterations from $\boldsymbol{x}^0 = \boldsymbol{0}$, to represent converged solutions $\boldsymbol{x}^c$. Each of G, BG, DC, BC methods were then undertaken from a starting solution $\boldsymbol{x}^0 = \boldsymbol{0}$ with the correlations between $\boldsymbol{x}^k$ and $\boldsymbol{x}^c$ computed for each factor in the model, each iteration.

Table 1. Characteristics of the coefficient matrices of the mixed model equations

Dataset	Name[1]	Order	# HS NZE[2]	# Fixed Effects	# Animals	Block Size	Random Effects[3]
1	Rouse	79,690	641,956	173	24,735	3	(bwd-wwd-wwm)-wmp
2	NZC	193,660	2,094,591	3,248	45,901	4	(bwd-wwd-wwm-pwd)-wwp
3	RA cc	421,074	4,127,160	9,945	137,043	3	(reac-cw-reau)
4	RA g	9,114,549	64,167,964	122,147	2,141,506	4	(bwd-bwm-wwd-wwm)-wwp

[1]Rouse = CSU Rouse Ranch ,NZC = New Zealand Charolais, RA=Red Angus Association of America, cc=carcass, g=growth
[2]half-stored non-zero elements
[3]bw=Birth Weight, ww=Weaning Weight, pw=Post-Weaning Gain, rea=Rib-Eye Area, cw=Carcass Weight, d=Direct, m=Maternal, p=Maternal Permanent Environment, c=Carcass, u=Ultrasound

Table 2. Rounds of iteration required for Gauss-Seidel (G) or conjugate gradient (C) methods, accounting for block (B) structure, to achieve correlations with converged values from 10,000 G iterations of at least 0.9, 0.99. 0.999 or 0.9999 for every random factor from four different multiple trait mixed model coefficient matrices

Dataset	0.9				0.99				0.999[1]				0.9999			
	G	BG	D[2]C	BC	G	BG	DC	BC	G	BG	DC	BC	G	BG	DC	BC
1	74	66	28	21	370	237	45	39	634	470	74	55	864	725	97	88
2	74	348	66	72	1,700	1,411	171	98	3,988	2,391	252	164	5,818	3,270	295	194
3	318	37	40	32	2,224	2,094	138	113	5,110	3,916	203	150	7,406	5,437	269	180
4	281	94	209	173	2,073	384	377	273	3,344	1,044	500	463	na[3]	na	na	na

[1]Dataset 4 did not reach convergence to a correlation of 0.999 for maternal genetic weaning weight so for that dataset rounds to correlation of 0.998 are presented
[2]Diagonal preconditioning matrix in conjugate gradient
[3]Convergence beyond correlation of 0.999 was not examined in dataset 4

Results and Discussion

Correlations between solutions for different methods are not informative in terms of comparing methods because unconstrained fixed effects can converge to different solutions according to the methods used. Accordingly, correlations were computed separately for each factor in the model and only correlations between solutions for random factors are presented. Convergence rates of the five random factors in dataset 2 are shown in terms of their correlations at each iteration with values obtained after 10,000 G iterations (Method G in Figure 1, and DC in Figure 2). It is apparent that different factors converge at different rates. Generally, mutually uncorrelated maternal permanent environmental effects converge first, followed by direct genetic effects. Maternal genetic effects tend to be the last factor to converge.

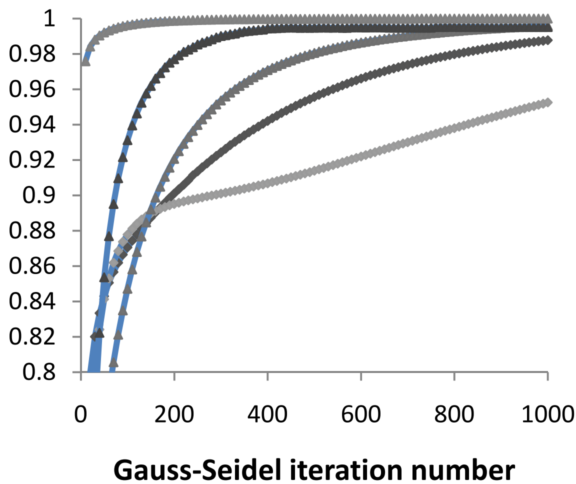

Figure 1. Convergence of weaning maternal permanent environment, weaning maternal genetic, weaning direct, birth direct and birth maternal genetic effects (listed in order of convergence at iteration 300) using Gauss-Seidel iteration on mixed model equations from the RAAA growth trait analysis.

The number of iterations required to obtain convergence to correlations of 0.9, 0.99, 0.999 or 0.9999 for all methods for the last factor to converge are shown in Table 2. The G method routinely used by CGEL was always slowest to converge, except to a correlation of 0.9 in two datasets. All four datasets included genetic effects sorted by factor within animal in order to facilitate block iteration comprising a block for each animal in the analysis. However, CGEL routinely forms mixed model equations sorted by animal within factor. The number of G iterations required to obtain convergence is sensitive to the sequence of equations in the coefficient matrix with animal within factor converging slightly faster than factor within animal (data not shown). The C methods are not sequential and therefore not sensitive to equation order.

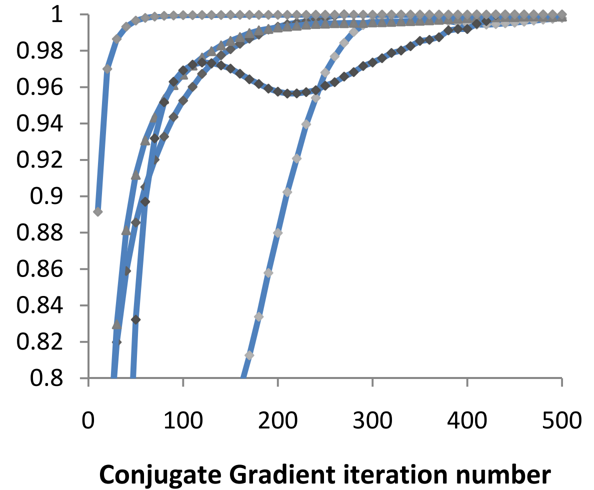

Figure 2. Convergence of weaning maternal permanent environment, birth direct, weaning direct, weaning maternal, and birth maternal genetic effects (listed in order of convergence at iteration 200) using conjugate gradient with a diagonal preconditioning matrix on mixed model equations from the RAAA growth trait.

Exploiting blocks in BG reduced the number of iterations compared to G. Compared to BG, method G seems to converge more slowly the closer it gets to convergence. Accordingly, the advantage of BG increased with the number of iterations. The superiority of BG also varied by dataset, but overall the advantage was that about half the number of iterations were required. The computing effort for BG is practically identical to the effort required in G, other than the fact that the coefficient matrix must be suitably ordered to facilitate formation of the blocks.

Conjugate gradient methods had an enormous impact on convergence rates with similar precision being achieved in around one-tenth the number of iterations required by G methods (Table 2, Figures 1 and 2). Accounting for blocks rather than using a simple diagonal preconditioning matrix in C resulted in only a modest reduction in number of iterations for BC compared to DC.

The computing effort for C methods are little different from those required for G, other than the need to compute the residuals or lack of fit at each iteration. However, C methods do not require the non-zero elements of the coefficient matrix to be stored in a particular order, unlike G which requires sequential access to coefficients by row and column. Sorting coefficients can be problematic when techniques are used that iterate on data (Schaeffer and Kennedy, 1986) rather than explicitly forming the coefficient matrix prior to iteration.

Rounding errors can impact the comparison of alternative methods as the order of arithmetic operations influences the size of these errors and different methods process coefficients in different sequences. Accordingly,

C methods may ultimately converge to slightly different values than G methods. The comparisons reported in this paper used 10,000 iterations of G as the gold standard for comparison, as G is currently used for all CGEL evaluations. Rounding errors likely prevented DC from achieving correlations exceeding 0.998 for maternal genetic effects in dataset 4.

Implications

Increases in computing speed and reductions in the cost of computer memory have occurred at a greater rate than growth in the pedigree files of most Breed Associations. This has meant that the effort and time required to set up and solve linear equations to obtain EPDs have declined over the last decade. However, recent interest in pooling data from many US Breed Associations into a single multi-breed analysis will increase the size of the equations by an order of magnitude. Furthermore, accuracies of EPDs can be increased by including additional indicator traits in multiple trait evaluations and in these circumstances a change from Gauss-Seidel to conjugate gradient with a diagonal preconditioning matrix seems well worthwhile.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

UROTENSIN 2 AND ITS RECEPTOR AS CANDIDATE GENES FOR BEEF MARBLING SCORE AND SUBCUTANEOUS FAT DEPTH

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ABSTRACT: Urotensin 2 (*UTS2*) and its receptor (*UTS2R*) are associated with insulin resistance in humans, and many studies have indicated that muscle lipid accumulation is a major contributor to insulin resistance. In beef cattle, marbling is a subjective measurement of intramuscular lipid accumulation. Therefore, the objective of this study was to validate the candidacy of both *UTS2* and *UTS2R* for fat deposition in beef cattle. Both cDNA and genomic DNA sequences of these two genes in cattle were retrieved from public databases and used to design 11 pairs of primers. Direct sequencing of the amplicons identified 5 SNPs in *UTS2* and one INDEL and 13 coding SNPs in *UTS2R*, respectively. However, only one SNP in the promoter of *UTS2* and the INDEL in the promoter of *UTS2R* were chosen for genotyping on ~ 250 Wagyu x Limousin F2 population. Statistical analysis revealed that the former gene was suggestively associated with subcutaneous fat depth (SFD) ($P<0.10$), but not with beef marbling score (BMS), while the latter gene was significantly associated with BMS ($P<0.01$), but not with SFD. Our results provide evidence that the same orthologous gene may have conserved functions in biological or biochemical pathways, and thus explain the same or similar variations of the concordant QTLs among different species. Therefore, cross-species candidate gene transfer is worth pursing to facilitate understanding of genetic complexity of quantitative traits in mammals.

Key word: *UTS2*, *UTS2R*, mutations, beef marbling score, subcutaneous fat depth.

Introduction

Urotensin II (*UTS2*) encodes a 11 amino acid mature peptide that binds to the orphan G protein-coupled receptor, GPR-14 (renamed urotensin 2 receptor, UTS2R) (Ames et al., 1999). Recent studies have indicated that both *UTS2* and *UTS2R* have significant impacts on insulin resistance (Langham et al., 2004; Suzuki et al., 2004 and Ong et al., 2006), which represents a core pathological character of patients with type 2 diabetes mellitus and obesity. Interestingly enough, many studies have also revealed that intramyocellular lipid (IMCL) accumulation, the fat droplets accumulated in human skeletal muscle, is a major contributor to insulin resistance. On the other hand, IMCL is also highly correlated with extramyocelluar (EMCL) lipid content in humans ($r=0.68$) (Sinha et al., 2002). By definition, marbling score would mostly represent the EMCL content. Therefore, we hypothesized that both *UTS2* and *UTS2R* are strong potential candidate genes for muscle fat deposition in beef cattle.

Materials and Methods

Animals. It has been well known that the Wagyu breed of cattle has been traditionally selected for high intramuscular fat or marbling, whereas the Limousin breed has been selected for heavy muscle, which leads to low marbling score. Therefore, the difference in intramuscular fat deposition between these two breeds makes them very unique for identifying genes for this economically important trait. A Wagyu x Limousin reference population was developed and used in the present study, including 6 F_1 bulls, 113 F_1 dams and ~250 F_2 progeny (Jiang et al., 2005). In addition to beef marbling scores (BMS), subcutaneous fat depth (SFD) was also measured on these animals at the 12-13th rib interface perpendicular to the outside surface at a point three-fourths the length of the longissimus muscle from its chine bone end.

Gene annotation and primer design. Both cDNA and genomic DNA sequences of the bovine *UTS2* and *UTS2R* were retrieved from the GenBank databases using a comparative approach, as previously described (Jiang et al, 2006a). We determined the genomic organization of bovine *UTS2* and *UTS2R* by aligning a bovine cDNA sequence (CO872879) with a bovine genomic DNA contig (AAFC03010889) for the former gene, and aligning a bovine cDNA sequence (BT021614) with a bovine genomic DNA contig (AAFC03013715) for the latter gene. A total of 11 pairs of primers (Table 1) were designed and used to screen genetic polymorphisms in the promoter, coding and partial 3'untranslated regions of both bovine *UTS2* and *UTS2R* genes.

Polymorphism detection and genotyping assay development. Approximately 50 ng of genomic DNA each from six F_1 bulls were amplified in a final volume of 10 µL that contained 12.5 ng of each primer, 150 µM dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl and 0.25U of Platinum Taq polymerase (Invitrogen, Carlsbad, CA). The PCR conditions were carried out as follows: 94°C for 2 min, 32 cycles of 94°C for 30 sec, 63°C for 30 sec and 72°C for 30 sec, followed by a further 5 min extension at 72°C. PCR products were then sequenced for polymorphism detection on an ABI 3730

sequencer in the Laboratory for Biotechnology and Bioanalysis (Washington State University) using a standard protocol. Only one SNP in the promoter of *UTS2* (AAFC03010889.1: *g.9628G>A*) and the INDEL in promoter of *UTS2R* (AAFC03013715.1:*g.2935-36TA*>--) were chosen for genotyping using a PCR-RFLP technique (*Xcm*I for the former and *Bsl*I for the latter mutation, respectively).

Data analysis. The phenotypic data for both BMS and SFD measurements were previously adjusted for year of birth, sex, age (days), live weight (kilograms), or fat depth (inches), as appropriate. The adjusted phenotypes were then used in a subsequent association analysis using the GLM (general linear model) procedure of SAS v9.1 (SAS institute Inc., Gary, NC). Pair-wise comparisons of least squares means were performed using a protected t-test. Additionally, quantitative transmission disequilibrium test (QTDT) (Abecasis et al., 2000) was performed to further test for evidence of within breed, marker associations with the phenotypes, because we found that these markers are not fixed for alternative alleles between Wagyu and Limousin breeds.

Results

Genomic organization of both bovine UTS2 and UTS2R genes. Alignment of a cDNA sequence (CO872879) with a genomic DNA contig (AAFC03010889) revealed that the bovine *UTS2* gene consists of four exons, which corresponds to the transcript variant 2 of the human ortholog (NM_006786). However, alignment between the human transcript variant 1 (NM_021995) and the same bovine genomic DNA contig detected a relic for the exon 1 of the variant, but it seems that its expression in cattle is totally destroyed by an unusual intron splicing site of CT instead of GT (AAFC03010889). In fact, GenBank databases show that the *UTS2* variant 1 also does not exist in mouse (NM_011910) and rat (NM_019160). The *UTS2R* is an intronless gene in mammals, including cattle (BT021614 for cDNA and AAFC03013715 for genomic DNA sequences).

Genetic polymorphisms. Direct sequencing of PCR products on 6 F_1 bulls identified three SNPs in the promoter and two SNPs in intron 2 of the *UTS2* gene: AAFC03010889.1:*g.9408A>C*, *g.9552C>A*, *g.9628G>A*, *g.13294G>A* and *g.13900A>C*, respectively. For the bovine *UTS2R*, one insertion/deletion (INDEL) with two nucleotides (AAFC03013715.1:*g.2935-36TA*>--) was detected in the promoter region, while 13 SNPs were identified in coding and 3"UTR region of the gene, including AAFC03013715.1:*c.6446T>C*, *c.6506C>T*, *c.6593T>C*, *c.6749G>A*, *c.6830T>C*, *c.6842A>G*, *c.7232G>A*, *c.7359C>T*, *c.7466G>C*, *c.7632A>G*, *c.7692C>T*, *c.7714G>A* and *c.7720G>A*, respectively. However, three SNPs in the promoter of the *UTS2* gene had no historical recombination by forming two haplotypes (ACA and CAG) and one of the alleles in remaining SNPs in both genes was rare, accounting for only 1/12 in these 6 F_1 bulls. Therefore, only one SNP in the promoter of *UTS2* (AAFC03010889.1: *g.9628G>A*) and the INDEL in promoter of *UTS2R* (AAFC03013715.1:*g.2935-36TA*>--) were chosen for genotyping on all F_2 progeny.

Association analysis. Associations between genetic polymorphisms in both bovine *UTS2* and *UTS2R* genes with BMS and SFD in a reference population are presented in Table 2 and Table 3. The statistical analysis indicated no association of *UTS2* with BMS, but a suggestive association with SFD (P=0.0948 for the GLM analysis and P=0.0829 for the QTDT test). In particular, animals with *GG* genotypes had 0.088 (P=0.0301) less subcutaneous fat than animals with *AA* genotypes, which account for 0.49 standard deviations for the trait. In the reference population the average SFD was 0.394 inches with a standard deviation of 0.18 inches. For the bovine *UTS2R* gene, the promoter INDEL polymorphism was significantly associated with BMS (P=0.0080 for the GLM analysis and P=0.0018 for the QTDT test, respectively) (Table 2). The homozygous animals with the insertion allele (*II*) were 0.836 and 0.327 marbling scores, respectively higher than the homozygous animals with the deletion allele (*DD*) (P=0.0200) and the heterozygotes (*DI*) (P=0.0169) (Table 2). Overall, the marbling scores for all F_2 progeny averaged 5.916 with a standard deviation of 1 marbling score. Therefore, these differences in BMS between genotypes account for 0.836 and 0.327 standard deviations, respectively. However, the promoter INDEL of bovine *UTS2R* gene had no significant effect on SFD in the reference population (Table 3).

Discussion

In 2005 we reported that a SNP in the bovine *TCF7L2* gene on BTA26 was significantly associated with protein yield, protein percentage, milk yield and fat yield (P < 0.001) in the United States Holstein population (Jiang et al., 2005b). Now this gene has been overwhelmingly investigated in humans as a type 2 diabetes susceptibility gene in more than 25 publications since a first association report was published by Grant and colleagues in 2006. In the present study, we targeted two type 2 diabetes associated genes - *UTS2* and *UTS2R* genes discovered in humans and found they were strong candidate genes for body fat deposition, in particular the muslce fat accumulation in beef cattle (Table 2). These data indicated that cross species candidate gene information transfer is a powerful means to determine the same causal genes that underlie the concordant QTLs in mammals. Certainly, this comparative QTL mapping approach is worth further exploration, because the same orthologous gene may have conserved functions in biological or biochemical pathways, and thus explain the same or similar variations of the concordant QTLs among different species.

Marbling and subcutaneous fat depth are characteristics that have attracted a great deal of publicity and interest for

many years, since they are two of the major quantitative traits that affect carcass quality and production efficiency in beef cattle. However, selection of any carcass traits requires tremendous effort, expense and time. Therefore, identification and utilization of genes responsible for the genetic variation of marbling and fat would provide a powerful means to improve these quantitative traits by marker-assisted selection. Ideally, selection would result in animals with high marbling scores and low SFD. Previous studies have identified the bovine mitochondrial transcription factor A (Jiang et al., 2005a), fatty acid binding protein 4 (Michal et al., 2006) and urocortin 3 (Jiang et al., 2006) as potential candidate genes for fat deposition in cattle. However, the desirable alleles for marbling in these genes were also undesirable alleles for SFD, because they were associated with high marbling as well as high backfat thickness. Therefore, selection of desirable alleles in these genes for high marbling would also lead to an increase in backfat depth. In the present study, the bovine *UTS2* gene was suggestively associated with SFD, but not with BMS, while its receptor was significantly associated with BMS, but not with SFD. Therefore, selection against SFD using the favorable allele in the former gene and selection for marbling using the favorable allele in the latter gene would not result in a negative effect on the other trait. Therefore, these markers could be immediately implemented in beef breeding programs.

Table 1. Primers designed for mutation detection in the bovine *UTS2* and *UTS2R* genes.

Target region	Primer sequences (5'-3')	Size (bp)	Tm
UTS2			
Promoter	F: GCCTTGAGATTGAATTTTTGCTGTG R: AAATTTACTGTCTTGTTGCCTAGTG	676	61°C
Exon 1*	F: TTTTGTACACTAGGCAACAAGACAG R: TGAGACATGCCTTAAGAATCCTCAGA	540	61°C
Exon 1	F: GGGATGATATGAGGTCATAGGATAAT R: TCTGAGACATGCCTTAAGAATCC	409	57°C
Exon 2	F: CTCCTCCAGGGGATCTTCTCAAC R: TAATGCTCTTCTTCCCTCCCCTTG	506	61°C
Exon 3	F: TTTGTGAACTCTGGGGGCTAGAAA R: GGTCCTTGGACCCAGTGAAGATAA	557	61°C
Exon 4	F: ATCCCATGAAACAGCAAGAAAACC R: CAACCACTCATAGTATCTGCAAAACA	404	61°C
UTS2R			
Promoter	F: AGTCACCATCACCAAAATCATCCA R: CGGACTCGGATTCAGATTGTCAGT	676	61°C
Exon 1A	F: GAGAGGCCTTTGAACTTGCACTGT R: TAGACGTACATGGAGGCAGAGGTG	631	61°C
Exon 1B	F: GGCATGGCAGGCAATGTGTA R: ATGCCCAGGATGAGGTAGAGCAC	599	61°C
Exon 1C	F: GTGGTCATCGGGCTGCTCTAC R: CCACTCCCCACAGCCTACCC	531	61°C
Exon 1D	F: CAGCCAGCAAGCCACTGAGAC R: GGGTCCTGCCTCCTTTGACAC	601	61°C

*Putative ancient exon 1.

Table 2. Associations of *UTS2* and *UTS2R* genes with BMS* (in marbling score)

Marker	Genotype	LSM±S.E.	P_{GLM}	P_{QTDT}
UTS2				
g.9628G>A	AA	5.910 ± 0.086^{a}	0.6028	0.5593
	AG	5.933 ± 0.118^{a}		
	GG	5.671 ± 0.238^{a}		
UTS2R				
g.2935-36TA>--	DD	5.236 ± 0.347^{a}	0.0080	0.0018
	D I	5.745 ± 0.107^{a}		
	I I	6.072 ± 0.083^{b}		

*Means within a column without common superscripts are significantly different (P<0.05).

Table 3. Associations of *UTS2* and *UTS2R* genes with SFD* (in inches)

Marker	Genotype	LSM±S.E.	P_{GLM}	P_{QTDT}
UTS2				
g.9628G>A	AA	0.412 ± 0.014^{a}	0.0948	0.0829
	AG	0.402 ± 0.018^{ab}		
	GG	0.324 ± 0.038^{b}		
UTS2R				
g.2935-36TA>--	DD	0.375 ± 0.057^{a}	0.8050	0.6097
	D I	0.394 ± 0.018^{a}		
	I I	0.405 ± 0.014^{a}		

*Means within a column without common superscripts are significantly different (P<0.05).

Acknowledgement

The authors appreciate the assistance of Dr. Michael MacNeil, USDA-ARS, Miles City, MT in providing DNA and phenotypes for this research. Agricultural Research Center, Washington State University, and Merial Ltd. supported this project.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

ULTRASOUND ESTIMATES OF LOIN MUSCLE MEASURES AND BACKFAT THICKNESS AUGMENT LIVE ANIMAL PREDICTION OF WEIGHTS OF SUBPRIMAL CUTS IN SHEEP[1]

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ABSTRACT: The efficacy of live animal, real-time, B-mode ultrasound (US) estimates of carcass traits as (partial) predictors of carcass composition warrants investigation in sheep of varying genetic and environmental backgrounds. Our objectives were to 1) evaluate US estimates of corresponding carcass measures using correlations (r) and statistics established for beef and swine (prediction SE [SEP]; repeatability SE [SER]; and bias [TB]); and 2) estimate variation in weights of subprimal cuts (roast-ready rack, trimmed loin, and boneless leg), after accounting for live BW, explained with US loin muscle and backfat (BF) measures. Wethers (n = 172) from four sire breeds were reared in an extensive system, finished on a concentrate diet, and harvested at a mean weight of 62.9 (SD = 9.5) kg. Before harvest, $12^{th}/13^{th}$ rib transverse US images were captured using an Aloka SSD-500V US device with a 3.5-MHz, 14.5-cm linear array transducer and standoff. Images were interpreted using ImageJ software (v1.36b). After a 24-h chill, carcasses were ribbed, measured for loin muscle area (LMA) and BF, and fabricated. Weights of subprimal cuts were described using linear models with BW and US loin muscle and BF measures as predictors. Prediction models that maximized R^2 and included only significant terms ($P < 0.05$) were identified. For Objective 1, SEP, SER, and TB for BF were 0.14, 0.08, and 0.07 cm, respectively, and r was 0.81. The SEP, SER, and TB for LMA were 1.55, 1.31, and -0.004 cm^2, respectively, and r was 0.75. For Objective 2, the best prediction models for trimmed loin and boneless leg weights included BW and US LMA and BF as predictors. The best prediction model for roast-ready rack weight included BW and US LMA. The BW explained 70.3, 69.9, and 72.9% of variation in trimmed loin, boneless leg, and roast-ready rack weights, respectively; US estimates explained an additional 4.1, 5.6, and 2.3% of variation in these weights. Based on these data, US estimates of carcass measures obtained by a trained technician can be reliable and can augment our ability to predict weights of subprimal cuts in live sheep.

Key Words: Sheep, Ultrasound, Carcass

Introduction

Most U.S. lamb is marketed domestically as high-value retail cuts (Jones, 2004). Increasing the sizes and lean:fat ratios of these retail cuts, without detriment to attributes of sensory quality, may enhance consumer acceptance of lamb (Ward et al., 1995). Ultrasound can afford breeders, producers, and researchers the ability to estimate carcass compositional traits in vivo, and thus contribute knowledge to breeding and marketing decisions.

Despite inherent limitations to the use of ultrasound in sheep (Purchas and Beach, 1981; Edwards et al., 1989; Houghton and Turlington, 1992), low relative cost and ease of portability make ultrasound a practical option for future in vivo estimation of carcass composition. Research efforts, with current ultrasound technologies and in varying populations, to validate ultrasound as a predictive tool will expedite its increased and efficacious use in the sheep industry. The research should be designed to determine whether 1) ultrasound estimates of corresponding carcass measures are reliable; 2) carcass measures to be estimated with ultrasound are reliable indirect predictors of other compositional traits; and 3) ultrasound estimates can augment, or supersede altogether, other easily-obtained predictive measures (e.g., BW, breed, gender).

Thus, our objectives were to 1) assess the accuracy and repeatability of ultrasound estimates of backfat thickness (**BF**) and loin muscle area (**LMA**) at the $12^{th}/13^{th}$ rib location using statistics established for beef and swine certification programs and 2) evaluate the ability of these ultrasound measures to augment live-animal prediction of weights of high-value, subprimal cuts.

Materials and Methods

Wethers (n = 172) that were F_1 progeny from one of four terminal sire breeds and Rambouillet ewes were used in this study. Ewes lambed in March or April 2006, and ram lambs were castrated within 24 h after birth. Ewes and lambs were herded on sagebrush steppe range beginning late April and subalpine range beginning early July. Wethers were weaned on August 1 at a BW of approximately 39 kg, transported to a feedlot at U.S. Sheep Experiment Station headquarters, and finished on a concentrate diet. Wethers were randomly assigned within sire to one of three harvest groups ($n \approx 57$ per group) at targeted mean BW of 54.4, 61.2, and 68.0 kg. The overall mean BW for this study was 62.9 kg (SD = 9.5 kg).

On the day of transport to the Ohio State University for harvest, BW was recorded, and a single, trained technician captured and digitized two, independent ultrasound images at the left-side, $12^{th}/13^{th}$ rib location. The

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

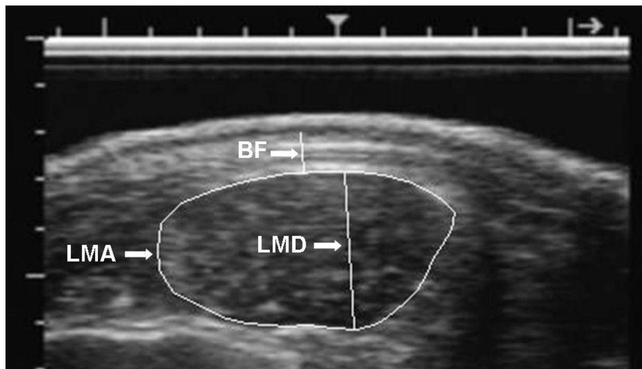

Figure 1. Transverse, 12th/13th rib ultrasound image and technician interpretation of backfat thickness (BF), loin muscle area (LMA), and loin muscle depth (LMD).

ultrasound device was an Aloka SSD-500V (Corometrics Medical Systems, Wallingford, CT) with a 3.5-MHz, 14.5-cm linear array transducer and standoff. Wool was clipped from the scan site, and warm vegetable oil was used as a conductive medium. Settings for the ultrasound device were 90 for overall gain, -25 for near gain, 2.1 for far gain, and focal points F1 and F2. The same technician measured the ultrasound images for BF, LMA, and loin muscle depth (**LMD**) using ImageJ software (v.1.36b; NIH, 2006) after calibration for known pixel dimension and aspect ratio (see Figure 1).

The duration of transport to the abattoir was approximately 48 h, during which time the wethers were provided access to water and alfalfa hay. Upon arrival at the harvest facility, wethers were provided access to water only, rested overnight, and harvested the next morning. After a minimum 24-h chill at approximately 4°C, carcasses were ribbed between the 12th and 13th ribs, and trained university personnel measured left-side BF and traced the left-side loin muscle perimeter on acetate paper. Tracings were transferred onto a digitizing tablet (SummaSketch III; Summagraphics Corp., Fairfield, CT), and LMA was measured (Planimeter Anything; The Logic Group, Austin, TX). Carcasses were fabricated according to Institutional Meat Purchase Specifications (IMPS; USDA, 1996). Weights of roast-ready rack, trimmed loin, and boneless leg (IMPS item numbers 204B, 232, and 234, respectively) were recorded. Table 1 contains summary statistics for BW, ultrasound and carcass measures of BF and LMA, and weights of subprimal cuts.

Statistical Analyses. Ultrasound estimates of BF and LMA were evaluated using statistics established for beef and swine certification programs (Bates and Christians, 1994) and simple correlations (**r**). The certification statistics include prediction SE (**SEP**), repeatability SE (**SER**), and technician bias (**TB**) and are calculated as:

$$SEP = ((\Sigma_j \Sigma_k (Scan_{jk} - Carcass_j - TB)^2 / (2n - 1))^{1/2},$$

$$SER = (\Sigma_j (Scan2_j - Scan1_j)^2 / (n))^{1/2}, \text{ and}$$

$$TB = \Sigma_j \Sigma_k (Scan_{jk} - Carcass_j) / 2n$$

Table 1. Summary statistics for BW, backfat thickness, loin muscle area, and weights of subprimal cuts

Trait	Mean	SD	Minimum	Maximum
BW, kg	62.9	9.5	43.5	95.3
UBF[1], cm	0.67	0.19	0.31	1.34
CBF[2], cm	0.61	0.24	0.20	1.73
ULMA[3], cm^2	15.85	2.02	10.62	21.91
CLMA[4], cm^2	15.81	2.34	9.48	22.46
RRR[5], kg	1.48	0.30	0.82	2.56
TLOIN[6], kg	2.60	0.42	1.77	4.20
BNLSLEG[7], kg	4.36	0.64	2.99	6.35

[1]UBF = Ultrasound estimate of backfat thickness.
[2]CBF = Carcass measure of backfat thickness.
[3]ULMA = Ultrasound estimate of loin muscle area.
[4]CLMA = Carcass measure of loin muscle area.
[5]RRR = Roast-ready rack.
[6]TLOIN = Trimmed loin.
[7]BNLSLEG = Boneless leg.

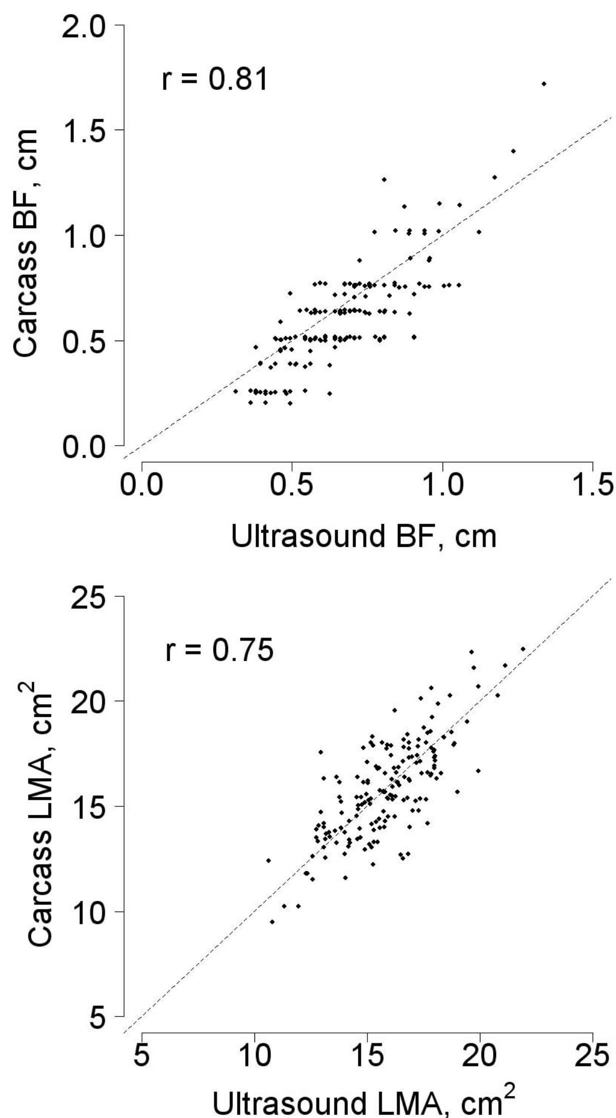

Figure 2. Scatter plots of backfat (BF) and loin muscle area (LMA) data. Dashed line represents unity between ultrasound and carcass measures.

where $Scan_{jk}$ is the kth ultrasound estimate on the jth wether; $Carcass_j$ is the carcass measurement on the jth wether; 2n is the total number of scans; and n is the number of wethers scanned twice.

Weights of subprimal cuts were described using linear models with BW and ultrasound estimates of BF, LMA, and LMD as predictors. Prediction models that maximized R^2 and included only significant ($P < 0.05$) terms were considered the best prediction models.

Results

The plots in Figure 2 illustrate the relationships between ultrasound estimates and carcass measures of BF and LMA. Table 2 contains SEP, SER, TB, and r statistics for LMA and BF. Carcass measures of LMD were not collected. However, SER for LMD was 0.20 cm, and its correlation with carcass LMA was 0.73.

Table 3 contains root mean square error and R^2 statistics for prediction models for weights of subprimal cuts. The best prediction models for weights of trimmed loin and boneless leg included BW, LMA, and BF as predictors. The best prediction model for roast-ready rack included BW and LMA as predictors. As a single predictor, BW explained 70.3, 69.9, and 72.9% of variation in trimmed loin, boneless leg, and roast-ready rack weights, respectively. An additional 4.1 and 5.6% of variation in weights of trimmed loin and boneless leg, respectively, were explained when prediction models included ultrasound estimates of BF and LMA. An additional 2.3% of variation in weight of roast-ready rack was explained when the prediction model included ultrasound estimates of LMA.

Discussion

Our data indicate that ultrasound can be used to accurately predict LMA and BF in sheep, and our predictions are repeatable. Technician bias for LMA approached zero, and TB was less than 1 mm for BF. After accounting for TB, the SEP statistic, assuming normally distributed errors, indicates that 67% of ultrasound estimates of BF and LMA were within 0.14 cm and 1.55 cm^2, respectively, of the carcass measure. Based on SER, 67% of repeat ultrasound estimates of BF and LMA were within 0.08 cm and 1.31 cm^2, respectively, of the initial ultrasound estimate. Because of their accuracy and repeatability, our data can be used to correctly rank animals for each trait and increase the rate of genetic improvement of carcass merit (Robinson et al., 1992).

Our SEP, SER, and TB statistics are well within the validation criteria established for national certification programs for beef and swine (BIF, 2002; Bates and Christians, 1994). However, the U.S. does not have a national certification program for sheep, but we believe that one should be established with sheep-specific validation criteria because trait means and variation differ among species. We could find only one other report of SEP estimates for sheep in the U.S. (Tait et al., 2005). Their SEP for BF ranged from 0.12 to 0.13 cm, compared with our 0.14 cm, and their SEP for LMA ranged from 1.92 to 2.18 cm^2, compared with our 1.55 cm^2.

Table 2. Predictive ability and repeatability of estimates of loin muscle area (LMA) and backfat (BF) derived from ultrasound images

	SEP[1]	SER[2]	TB[3]	r[4]
BF, cm	0.14	0.08	0.07	0.81
LMA, cm^2	1.55	1.31	-0.004	0.75

[1]SEP = Prediction SE.
[2]SER = Repeatability SE.
[3]TB = Technician bias.
[4]r = Simple correlation coefficient (no units).

Table 3. Root mean square error (RMSE, kg) and R^2 statistics of prediction models for weights of subprimal cuts[1]

	Roast-Ready Rack		Trimmed Loin		Boneless Leg	
Model[2]	R^2	RMSE	R^2	RMSE	R^2	RMSE
BW	0.729	0.154	0.703	0.228	0.699	0.353
LMA	0.456	0.219	0.463	0.307	0.520	0.445
LMD	0.400	0.229	0.388	0.327	0.419	0.490
BF	0.304	0.247	0.242	0.364	0.270	0.550
BW, LMA	0.752	0.148	0.732	0.217	0.749	0.323
BW, LMD	0.738	0.152	0.712	0.225	0.715	0.344
BW, BF	0.729[3]	0.155	0.707[3]	0.227	0.699[3]	0.354
BW, LMA, BF	0.753[3]	0.148	0.744	0.213	0.755	0.320
BW, LMD, BF	0.739[3]	0.152	0.720	0.223	0.719[3]	0.343

[1]All terms in prediction models are significant ($P < 0.05$) unless otherwise noted.
[2]BW = Body weight at scanning; LMA = Ultrasound estimate of loin muscle area; LMD = Ultrasound estimate of loin muscle depth; BF = Ultrasound estimate of backfat thickness.
[3]Backfat thickness is not a significant ($P < 0.05$) term in the prediction model.

Most reports on using ultrasound to estimate corresponding carcass measures in sheep are limited to correlations, which are not measures used in the beef and swine certification programs. Correlations, however, are of limited value because trait variability influences their magnitude, and they are not direct measures of precision and accuracy. Correlations among BF and LMA in our study were moderately greater than those reported for sheep with similar trait variability (Banks et al., 2001; Tait et al., 2005).

Figure 2 illustrates a tendency for ultrasound to overestimate measures in lean or light-muscled wethers and

underestimate measures in fat or heavy-muscled wethers. This tendency reduced trait variation for ultrasound measures, compared with carcass measures (Table 1). Similar over- and underestimations from ultrasound have been reported for beef cattle (Greiner et al., 2003) and pigs (Moeller and Christian, 1998).

The data from our study indicate that ultrasound estimates of 12th/13th rib BF and LMA can be used to significantly improve live animal prediction of subprimal cut weights, although the improvements were modest. Despite their magnitude, the improvements reflect variances that are specific for BF and LMA, whereas BW is a composite trait and cannot be used to target specific improvements in carcass merit.

Ultrasound estimates of LMA or LMD seem more valuable than BF as predictors when carcass endpoints are expressed as weights, as in the current study. This is consistent with other reports, but BF was more valuable as a predictor of percentage yield (Edwards et al., 1989; Stanford et al., 1995; Berg et al., 1996). Based on our data, ultrasound measures of LMA explain more variation in weights of subprimal cuts than do measures of LMD or BF. However, LMD may be easier, and perhaps preferable, to measure when a transducer with a lower frequency than ours is used (Stanford et al., 1995). Nevertheless, we believe that, when accurately estimated, LMA, which is a two-dimensional measure from an image that resembles a chop, would yield more information about the animals than would a one-dimensional measure, such as LMD.

In the current study, breed of sire effects were significant ($P < 0.05$) only for weight of boneless leg (data not shown), although models reported did not account for this variation. Breed of sire effects were relatively independent of BF and LMA in these data, because the ultrasound measures accounted for a similar proportion of boneless leg weight variation (approximately 5%), regardless of inclusion of breed of sire effects in the model.

Implications

A trained technician can use ultrasound estimates of BF and LMA in live sheep to make accurate and repeatable predictions of corresponding carcass measures. In addition, these estimates can be used to augment our ability to predict weights of subprimal cuts in live sheep and perhaps increase the rate of genetic improvement of carcass traits.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

FACTORS DETERMINING PRICES OF YEARLING ANGUS BULLS

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ABSTRACT: Regression on historical information commonly available to customers buying bulls was used to determine factors influencing sale prices of yearling Angus bulls. Data included EPD, 112-d performance test results, BW and ultrasound data for 512 bulls sold from 2004 to 2007. Three models were considered: a breeder perception model based on explanatory variables identified using a customer survey, a best fit model which used EPD, phenotypes, year, sire and maternal grandsire (MGS), and a practical model excluding sire and MGS. The breeder perception model included EPD for birth weight, weaning weight, yearling weight, rib-eye area, intramuscular fat, and scrotal circumference, along with phenotypic measurements for test ADG and test weight per day of age. The best fit model used regression selection methods of PROC REG in SAS and included EPD for birth weight, milk, rib-eye area, weaning weight, and yearling weight, along with phenotypic measurements for frame score, ultrasound intramuscular fat, scrotal circumference, end test BW, ADG, year, sire and MGS. The breeder perception, best fit, and practical models accounted for 60%, 79%, and 65% of variation in sale price which corresponded to residual SD of $675, $577, and $630, respectively. Sire and MGS were significant (P = 0.01) factors affecting sale price and together accounted for 38% of variation. Correlations between actual and predicted sale prices were 0.77, 0.89 and 0.81 (P = 0.01) using breeder perception, best fit and practical models, respectively. Variables customers indicated as affecting bull sale prices were similar to those identified using statistical methods except milk EPD and phenotypic measurements of frame score, ultrasound IMF, scrotal circumference and end test BW were significant determinants of sale price. Sire and MGS appeared to be important factors affecting individual sale prices. However, uncertainty of which sires will be in demand in future years suggested the practical model was the most useful for predicting future sale prices of individual Angus bulls.

KEY WORDS: Angus, sale price prediction, sire preference

Introduction

Breeders selling bulls at a bull sale typically distribute a sale catalog which contains phenotypic and published EPD to familiarize customers with available bulls. Customers commonly select bulls based on visual appraisal, phenotypic measurements of performance and EPD (Simms et al., 1994). Studies have shown that estimates of genetic merit and phenotypic measurements influenced sale prices of Merino rams and purebred beef bulls (Dhuyvetter et al., 1996; Miller et al., 1999; Chvosta et al., 2001). The objectives of this study were to determine how factors customers identified as influencing their decision to purchase a bull affected sale prices, and compare this to models that could be developed using catalog information.

Materials and Methods

All data was provided by Yon Family Farms, Ridge Spring, SC. Data included EPD, 112-d post-weaning performance test results, phenotypic BW at various ages, and ultrasound data for 512 bulls from 2004 (n = 113), 2005 (n = 111), 2006 (n = 151) and 2007 (n = 137). Parents of the bulls included a total of 47 sires, 310 dams and 81 maternal grandsires (**MGS**). Only four sires, four dams or 18 MGS were common across all years.

Customers were asked to list the traits they perceived as influencing their decision to purchase a specific bull. Results were compiled and a list was provided by the Angus breeder for analysis. Metric conversion of EPD and phenotypic measurements were performed and converted EPD were designated as "gEPD" and phenotypic measurements were designated as "pMeasurement." Three models were developed to predict individual bull sale prices using information commonly available to customers: a breeder perception model based on explanatory variables identified using a customer survey; a best fit model which used EPD, phenotypes, YEAR, SIRE and MGS; and a practical model which excluded SIRE and MGS. The best fit model was developed using STEPWISE and MAXR regression selection methods of PROC REG in SAS (Version 9.1, SAS Inst. Inc., Cary, NC). The significance level for an explanatory variable to enter and remain in the model was set at 0.10. Sale prices were predicted using the breeder perception, best fit and practical models for each year and for the pooled data set which included data from all years using PROC GLM of SAS. Pearson's correlation coefficients were calculated using PROC CORR between the actual and predicted sale prices.

Results and Discussion

The pooled breeder perception model included EPD for birth weight (**gBWT**), percent intramuscular fat (**gIMF**), rib-eye area (**gREA**), scrotal circumference

(**gSC**), weaning weight (**gWW**) and yearling weight (**gYW**), and phenotypic measurements for 112-d test ADG (**ADG**) and weight per day of age from birth to end of 112-d performance test (**WDA**). The pooled best fit model included gBWT, milk EPD (**gMilk**), gREA, gWW, gYW, and phenotypic measurements for frame score (**FRAME**), ultrasound IMF (**pIMF**), SC (**pSC**), end of 112-d test BW (**EndBW**), ADG, YEAR, SIRE, and MGS. Prediction formulas using pooled data for the breeder perception (Eq. 1), best fit (Eq. 2) and practical models (Eq. 3) are listed below. Numbers contained within parentheses represent the average for that explanatory variable. Prediction equations are shown for an average year. The best-fit model is shown for an average sire and MGS without any corresponding coefficients.

Breeder Perception = \$2765 – 63(gBWT – 1)
+ 1105(gIMF – 0.15) + 3594(gREA – 2) –23(gSC – 0.32)
– 2(gWW – 19) + 7(gYW – 36) – 16(ADG – 2)
+ 1274(WDA – 1) [1].

Best Fit = \$2888 – 96(gBWT – 1) + 33(gMilk – 11)
+ 7047(gREA – 2) + 1(gWW – 19) + 17(gYW – 36)
+ 166(FRAME – 6) + 294(pIMF – 3) + 62(pSC – 35)
+ 2(EndBW – 531) + 98(ADG – 2) [2].

Practical = \$2759 – 72(gBWT – 1) + 8(gMilk – 11)
+ 6941(gREA – 2) – 3(gWW – 19) + 12(gYW – 36)
+ 162(FRAME – 6) + 281(pIMF – 3) + 40(pSC – 35)
+ 2(EndBW – 531) + 135(ADG – 2) [3].

Pooled breeder perception, best fit, and practical models accounted for 60%, 79%, and 65% of the variation observed in sale price. The SD of sale prices after accounting for known factors (i.e., residual SD) was \$675, \$577, and \$630, respectively (Table 1). The breeder perception model used EPD data (Table 2) while the best fit and practical models combined phenotypic measurements and EPD data (Tables 3 and 4). Simms et al. (1994) conducted a survey in which commercial producers ranked individual phenotypic bull performance variables higher than EPD data as factors affecting their decision to purchase a specific bull. Robinson et al. (1992) determined that actual BW and visual appraisal affected bull sale price more than estimated breeding values. However, these authors suggested over time as estimates of genetic potential become more accepted, EPD or estimated breeding values should affect sale price more than their corresponding phenotypic measurements.

Miller et al. (1999) observed that phenotypic measurements and EPD data affected Merino ram sale prices. Higher prices were paid for rams with phenotypically heavier fleece weights and finer wool fibers. Dyuyvetter et al. (1996) determined that a model which contained phenotypic measurements and EPD data accounted for 3% more of the variation observed in bull sale prices compared to a model which only used phenotypic measurements. Phenotypic BWT and gBWT were significant explanatory variables of sale price for Angus bulls, but gBWT was significant for Simmental and Gelbvieh, while pBWT was significant for Charolais bulls only. These researchers concluded phenotypic measurements and EPD data was useful for explaining the variation observed in bull sale price, but effectiveness is breed-dependent. In the current study, pSC was a significant (P = 0.01) source of variation in the best fit model (Table 3), while gSC was not significant in the breeder perception model (Table 2). Customer paid \$62 more (P = 0.01) for every cm increase in pSC when sire or MGS was taken into account (Table 3), but only \$40 when parentage was not considered (P = 0.01; Table 4). In the breeder perception model gIMF was a significant source of variation (P = 0.01) and customers paid \$1105 more (P < 0.01; Table 2) for every one percent increase in gIMF. The pIMF was a significant source of variation (P = 0.01) in the best fit and practical models and customers paid approximately the same amount more (P < 0.01; \$294 and \$281; Tables 3 and 4) for every percent increase in pIMF regardless of ancestry. Chvosta et al. (2001) evaluated a model which contained phenotypic measurements or EPD data, or a combination of both data types for a single breeder or multiple breeders. The model which contained only EPD data accounted for more of the variation observed in sale prices for multiple breeders rather than the single breeder. The same amount of variation was accounted for when the model contained either phenotypic measurements or a combination of phenotypic measurements and EPD data. Collectively, these reports suggest phenotypic measurements and EPD best predict sale prices. Biologically, we would expect EPD information to be more reliable than phenotypic measurements in predicting the likely performance of offspring. These findings suggest bull buyers do not yet share this view.

Sire and MGS were significant (P = 0.01) factors affecting sale price and accounted for 38% of the variation when used instead of EPD and phenotypic data. However, these male ancestors will account for much of the variation in all the EPD of sale bulls. Sire more so than MGS was still significant fitted after individual bull EPD, indicating a buyer preference for particular sires that is not accounted for by published EPD. Similar to Miller et al. (1999), the significance level and size of the effect of sire varied across years. For example, progeny from two sires sold for \$1214 and \$833 more ($P \leq 0.04$) than the average sire (\$1246) in 2004 and customers paid \$206 less (P = 0.03) than the average sire (\$-775) for progeny of a third sire in 2006.

Correlation coefficients between actual and predicted sale prices were 0.77, 0.89 and 0.81 (P = 0.01) using the breeder perception, best fit and practical models, respectively (Table 5). Across years, predicted sale prices were positively correlated with actual sale prices, but the predicted sale prices were more similar to the actual sales when using the best fit model. Robinson et al. (1992) observed a correlation of 76% between actual and predicted sale prices using a model which included BW adjusted for age, actual BW on the day of the sale and estimated breeding values 200-, 400-, and 600-d BW. In the current study, pooled sale prices were positively correlated with all explanatory variables except gBWT (Table 6). However, EndBW and WDA were the only

variables strongly correlated with actual sale price ($r \geq 0.58$, $P \leq 0.03$). In the breeder perception model, customers paid $1274 more for bulls with higher WDA from birth to the end of the 112-d performance test. For example, at 205 d of age, customers paid $6 more per kg BW and at the end of the 112-d test customers paid $3 more per kg BW. In the best fit and practical models, customers paid $2 more for every kg increase of BW at the end of the 112-d bull test and $2 more per kg gained during the bull test (Tables 3 and 4). In general, EPD data (gYW, gWW, gREA and gIMF) and phenotypic measurements collected at 365-d of age (FRAME and pSC) were highly correlated with actual sale price (Table 6). Bull test data EndBW and WDA were collected during the 112-d bull test which ended two mo before the bull sale. Bulls were an average of 404 ± 27 d of age and weighed an average of 531 ± 53 kg at the end of the bull test. On day of sale BW were not available, but if bulls were sold at the end of the 112-d performance test, bulls would have sold for an average $1059, $997, $1245, and $1072 in 2004, 2005, 2006 and 2007 respectively, using reported average cattle prices for the month of December (USDA). Miller et al. (1999) observed a similar effect in which phenotypic measurements collected for fleece weight and fiber diameter near the time of the sale had a greater influence on sale price than predicted genetic merit.

Results indicated variables customers identified as affecting bull sale prices were similar to those identified using statistical methods except in the best fit model, gMilk, FRAME, pIMF, pSC, and EndBW were identified as additional determinants of sale price. Sire and MGS appeared to be important factors affecting individual sale prices. However, the uncertainty of which sires will be in demand in following years suggested the practical model was the most useful for predicting future bull sale prices.

Implications

Phenotypic measurements and EPD data are determinants of individual bull sale prices. However, phenotypic measurements, collected near the time of the sale appear to have greater impact than EPD on sale prices. Ancestry was important as customers seem to use information on the relatives of the bulls when making a purchasing decision. The uncertainty of which sires will be popular next year and the fluctuating cattle market supports using a practical model excluding ancestry to determine relative values of sale bulls.

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Table 1. Multiple correlation coefficient (r^2), average bull sale price and standard deviation (Mean ± SD, $) for breeder perception, best fit and practical models across years and pooled data.

Model	2004	2005	2006	2007	Pooled
Breeder Perception					
r^2	0.46	0.63	0.66	0.61	0.60
Mean	2860	2926	2919	2376	2765
± SD	± 691	± 658	± 613	± 730	± 675
Best Fit					
r^2	0.87	0.88	0.89	0.83	0.79
Mean	2842	2926	2910	2376	2759
± SD	± 584	± 597	± 503	± 668	± 577
Practical					
r^2	0.58	0.71	0.76	0.66	0.65
Mean	2842	2926	2910	2376	2759
± SD	± 610	± 590	± 519	± 692	± 630

Table 2. Average sale price and changes in individual bull sale price ($) for metric converted genetic and phenotypic data[1] used in the breeder perception model across years and pooled data.

Variable	2004	2005	2006	2007	Pooled
Price, $	2860	2926	2919	2376	2765
gBWT, kg	-85[a]	-70[a]	-126[a]	-73[a]	-63[a]
gIMF, %	896	977	2549[a]	1112[b]	1105[a]
gREA, cm^2	7952[a]	4725[b]	7310[a]	4943[b]	3594[a]
gSC, cm	387	24	134	252	-23
gWW, kg	8	12	14	-9	-2
gYW, kg	3	6	6	18[a]	7[b]
ADG, $kg \cdot d^{-1}$	252[a]	295[a]	343[a]	230[a]	-16
WDA, $kg \cdot d^{-1}$	831[a]	894[a]	659[a]	908[a]	1274[a]

[1]gBWT=birth weight EPD; gIMF=intramuscular fat EPD; gREA=rib-eye area EPD; gSC=scrotal circumference EPD; gWW=weaning weight EPD; gYW=yearling weight EPD; ADG=phenotypic 112-d test ADG; WDA=phenotypic BW per d of age from birth to end of 112-d performance test.

[ab]Regression coefficient differed from zero ([a]$P \leq 0.05$; [b]$P \leq 0.10$).

Table 3. Average sale price and changes in individual bull sale prices ($) for metric converted genetic and phenotypic data[1] used in the best fit model across years and pooled data.

Variable	2004	2005	2006	2007	Pooled
Price, $	2842	2926	2910	2376	2888
gBWT, kg	-144[a]	-112[a]	-119[a]	-75[a]	-96[a]
gMilk, kg	-60	28	1	38	33[a]
gREA, cm^2	9897	11228[b]	14222[a]	3930	7047[a]
gWW, kg	73	-25	0.51	-27	1
gYW, kg	-38	41	17	55[a]	17[a]
FRAME	124	205	273	270	166
pIMF, %	136	132	558[a]	-385	294[a]
pSC, cm	106[b]	74	37	11	62[a]
EndBW, kg	3[a]	2[a]	2[a]	2[a]	2[a]
ADG, $kg \cdot d^{-1}$	88	115	52	-50	98[a]

[1]gBWT=birth weight EPD; gMilk=milk EPD; gREA=rib-eye area EPD; gWW=weaning weight EPD; gYW=yearling weight; FRAME=phenotypic frame score; pIMF=phenotypic ultrasound intramuscular fat; pSC=phenotypic scrotal circumference; EndBW=phenotypic end of 112-d test BW; ADG=phenotypic 112-d test ADG.
[ab]Regression coefficient differed from zero ([a]$P \leq 0.05$; [b]$P \leq 0.10$).

Table 4. Average sale price and changes in individual bull sale prices ($) for metric converted genetic and phenotypic data[1] used in the practical model across years and pooled data.

Variable	2004	2005	2006	2007	Pooled
Price, $	2842	2926	2732	2333	2759
gBWT, kg	-65[a]	-69[a]	-113[a]	-51[a]	-72[a]
gMilk, kg	6	3	29[a]	1	8[a]
gREA, cm^2	7815[a]	3225	11492[a]	5626[b]	6941[a]
gWW, kg	-11	13	-3	-18	-3
gYW, kg	15[a]	-4	8	26[a]	12[a]
FRAME	137	352[a]	153	99	162[a]
pIMF, %	243[a]	176[b]	510[a]	18	281[a]
pSC, cm	72[a]	23	46[b]	17	40[a]
EndBW, kg	2[a]	2[a]	2[a]	2[a]	2[a]
ADG, $kg \cdot d^{-1}$	124	166[a]	129[a]	-12	135[a]

[1]gBWT=birth weight EPD; gMilk=milk EPD; gREA=rib-eye area EPD; gWW=weaning weight EPD; gYW=yearling weight; FRAME=phenotypic frame score; pIMF=phenotypic ultrasound intramuscular fat; pSC=phenotypic scrotal circumference; EndBW=phenotypic end of 112-d test BW; ADG=phenotypic 112-d test ADG.
[ab]Regression coefficient differed from zero ([a]$P \leq 0.05$; [b]$P \leq 0.10$).

Table 5. Pearson's correlation coefficients (r; $P = 0.01$) between actual and predicted sale prices for breeder perception, best fit and practical models across years and pooled data.

Model	2004	2005	2006	2007	Pooled
Breeder perception	0.68	0.79	0.82	0.78	0.77
Best fit	0.93	0.94	0.95	0.91	0.89
Practical	0.76	0.84	0.87	0.81	0.81

Table 6. Pearson's correlation coefficients between actual sale price and metric converted genetic and phenotypic data[1] across years and pooled data.

Variable	2004	2005	2006	2007	Pooled
gBWT, kg	-0.14	-0.06	-0.08	0.09	-0.01
gIMF, %	0.25[a]	0.17[b]	0.32[a]	0.14	0.17[a]
gMilk, kg	0.23[a]	0.26[a]	0.40[a]	-0.03	0.12[a]
gREA, cm^2	0.27[a]	0.37[a]	0.34[a]	0.34[a]	0.27[a]
gSC, cm	0.04	0.30[a]	0.21[a]	0.23[a]	0.17[a]
gWW, kg	0.16[b]	0.25[a]	0.28[a]	0.44[a]	0.25[a]
gYW, kg	0.22[a]	0.32[a]	0.32[a]	0.52[a]	0.30[a]
FRAME	0.27[a]	0.47[a]	0.26[a]	0.37[a]	0.34[a]
pIMF, %	0.26[a]	0.14	0.31[a]	0.12	0.08[b]
pSC, cm	0.22[a]	0.39[a]	0.19[a]	0.31[a]	0.29[a]
EndBW, kg	0.58[a]	0.79[a]	0.59[a]	0.73[a]	0.60[a]
ADG, $kg \cdot d^{-1}$	0.42[a]	0.62[a]	0.53[a]	0.59[a]	0.38[a]
WDA, $kg \cdot d^{-1}$	0.45[a]	0.69[a]	0.55[a]	0.68[a]	0.58[a]

[1]gBWT=birth weight EPD; gIMF=intramuscular fat EPD; gMilk=milk EPD; gREA=rib-eye area EPD; gSC=scrotal circumference EPD; gWW=weaning weight EPD; gYW=yearling weight EPD; FRAME=phenotypic frame score; pIMF=phenotypic ultrasound intramuscular fat; pSC=phenotypic scrotal circumference; EndBW=phenotypic end of 112-d test BW; ADG=phenotypic 112-d test ADG; WDA=phenotypic BW per d of age from birth to end of 112-d performance test.
[ab]Correlation coefficient differed from zero ([a]$P \leq 0.05$; [b]$P \leq 0.10$).

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

CHARACTERIZATION OF BRAHMAN, BRANGUS, CHAROLAIS, GELBVIEH AND SIMMENTAL FOR GROWTH TRAITS IN BAJA CALIFORNIA, MEXICO.

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ABSTRACT, The objectives were to estimate genetic parameters of heritability for birth weight BW, weaning weight WW, and yearling weight YW, in a herd located, in Ojos Negros, Baja California, México. It was used the progeny (n=21, n=30, n=33, n=28, and n=32) of heifers and cows of inheritance Brahman B, Brangus Br, Charolais C, Gelbvieh G, and Simmental S, mated to sires B, Br, C, G, and S, respectively. Each trait was analyzed separately by using mixed models, SAS, (1996). The analytical model included: year of birth, age of cow, sex of the calf, birth date as a covariable to adjust a common age as fixed effects**;** sire and the residual as random components. The BW, WW, and YW values (34.67±1.06, 37.82±2.51, 41.50± 2.51, 44.42±1.0, and, 39.83±5.1**;** 174.80±8.52, 206.96±29.30, 216.29±18.65, 225.29±17.87, and 266±8.92**;** 239.97±40.88, 261.98±20.24, 268.30±9.57, 298.50±40.16, and 308.19±29.75 kg) corresponded to the progeny of dams involving inheritance of B, Br, C, G, and S, mated to sires to sires involving inheritance of B, Br, C, G, and S respectively. Female calves were 5 to 7% heavier at birth than female calves. The estimates values of heritability, through the correlation among paternal half sibs were (h^2=0**.**31±0.04, h^2=0**.**21±0.05, and h^2=0=0**.**33±0.03) for BW, WW, and YW, respectively.

Key Words: Genetic parameters, Weights at birth and weaning , Yearling weight

Introduction

Performance attributes of traits of greatest economic value for different breed or breed crosses are important in determining the potential value of alternative germplasm resources for profitable beef production (Cundiff, et al, 1999). The genetic parameters which are functions of (co) variance components, provide information about the genetic nature of traits and are needed to predict direct and correlated responses to selection indexes and determine the method of selection (Van Vleck et al., 1992 . New developments in estimation algorithms (Smith, 1994; Meyer, 1991) allow the analysis of large sets of data and more complicated models. Koots et al.(1994b) summarized published estimates of genetics and phenotypic correlations between a number of traits of interest. Dickerson (1970) suggested that all changes in a commercial cow-calf operation must be evaluated in terms of their effect on profitability of the whole enterprise. The objectives were to estimate genetic parameters of heritability for BW, WW, and YW, in the progeny of heifers and cows of inheritance Brahman, Brangus, Charolais, Gelbvieh, and Simmental S, mated to sires B, Br, C, G, and S, respectively.

Materials and methods

The data available were on observations on birth weight (BW), weaning weight (WW), and yearling weight (YW) on the progeny (n=21, n=30, n=33, n=28, and n=32) of heifers and cows of inheritance Brahman B, Brangus Br, Charolais C, Gelbvieh G, and Simmental S, mated AI to sires B, Br, C, G, and S, respectively. Data came from a beef herd located in Ojos Negros Baja California, Mexico a semi desertic region of North West of the country.

Management

Cows were maintained on warm season pastures and fed (grass and alfalfa) or silage during the winter. Calving was in the spring (March, April and May). At birth all calves were identified, weighed, dehorned (paste), and vaccinated against viral scours. Calves were weaned at approximately 200 d.

Statistical procedure

Data was analyzed by using mixed models SAS, (1989). Each trait was analyzed separately by The analytical model included: year of birth, age of cow, sex of the calf, birth date as a covariable to adjust a common age as fixed effects**;** sire and the residual as random components. The estimates of heritability (direct) for BW, WW, and YW were calculated through the correlation among paternal half sibs as 4[variance of sires]/ [phenotypic variance].

Results and discussion

Least squares unadjusted means for birth weight, weaning weight and yearling weight are given in Table 1. (34.67±1.06, 37.82±2.51, 41.50± 2.51, 44.42±1.0, and, 39.83±5.1**;** 174.80±8.52, 206.96±29.30, 216.29±18.65, 225.29±17.87, and 266.10±8.92**;** 239.97±40.88, 261.98±20.24, 268.30±9.57, 298.50±40.16, and 308.19±29.75 kg) corresponded to the progeny of dams involving inheritance of B, Br, C, G, and S, mated to sires involving inheritance of B, Br, C, G, and S, respectively. Female calves were 5 to 7% heavier at birth than female calves. Thompson (1986) indicated that practical importance of birth weight as a selection tool depends on the age at which animals are marketed. Thomas reported that birth weight is positively correlated with weaning,

yearling, and mature weights. Therefore selection for any of these traits would cause some increase in birth weight.

Strohbehn et al. (1993) reported that a program of selection for low birth weight could lead to declines in weaning and yearling weights, which does not seem desirable. Nevertheless the author indicate that in the 1981 Angus sire evaluation report of 673 sires listed, 59 had below average (BW) but were above average on weaning weight, yearling weight, and maternal breeding value.

Koch, et al. (1982) found that estimates of genetic change from intra-year comparison of sire or dam birth year progeny groups are subject to large random errors because the number per group and the span of generations or birth years are usually small. Also, where comparison involves dams differing in age, genetic change is confounded with age-dam effects, and the validity of the differences is highly dependent on accurate estimates of age-of-dam correction factors.

Birth weight is an effective correlated trait that can be used to reduce calving difficulty CD. However, selection only for reduced CD or BW will lead to lighter postnatal weight. Schemes for simultaneously changing or limiting change in CD, BW, and postnatal weight have been proposed (Dickerson et al., 1974; Mac Neil et al., 1998). Key genetic parameters needed for developing these schemes and for genetic evaluation are the correlation between direct and maternal CD and birth and postnatal weights (Bennett et al., 2001).

Table 5.2 lists estimates of heritability (direct) values (h^2= 0.31±0.04, h^2=0.21±0.05, and h^2=0.33±0.03) for birth weight, weaning weight, and yearling weight, respectively. Consideration of specific non genetic effects, such age of dam, and sex of the calves, is needed to increase accuracy of individual breeding values.

Mac Neil, (2003) as a result of a genetic evaluation of an index of birth weight and yearling weight to improve efficiency of beef production demonstrated that despite a genetic antagonism that compromises selection response for decreased BW and increased postnatal growth, favorable genetic responses can be achieved with the selection index used in his study. The author found that the estimates for direct effects were (0.32±0.04, 0.49±0.05, 0.49± 0.05, 0.30± 0.04, and 0.70± 0.04 for the index, BW, 365-d weight, 200-d weight, and cow weight), respectively.

Implications

Producers must consider birth weight in their breeding programs. These results show large differences among breeds for birth weight, weaning weight, and yearling weight. Lowest birth weights and weaning weights involved calves from Brahman dams. The highest weights at birth, at weaning, and yearling weights involved inheritance of Continental breeds as Simmental, Gelbvieh, and Charolais.

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Table 1. Least squares means for birth weight, weaning weight and yearling weight of progeny involving Brahman, Brangus, Charolais, Gelbvieh, and Simmental inheritance.

Item	Birth weight, kg	Weaning weight, kg	Yearling weight, kg
Brahman	34.67±1.06	174.80±8.52	239.97±40.88
Brangus	37.82±2.51	206.96±29.30	261.98±20.24
Charolais	41.50± 2.51	216.29±18.65	268.30±9.57
Gelbvieh	44.42±1.00	225.29±17.87	298.50±40.16
Simmental	39.83±5.10	266.10±8.92	308.19±29.75

Table 2. Estimates of heritability and their standard errors for birth weight, weaning weight, and yearling weight of progeny involving Brahman, Brangus, Charolais, Gelbvieh, and Simmental inheritance.

	Traits		
Item	BW	WW	YW
Heritability	$h^2=0.31\pm0.04$	$h^2=0.21\pm0.05$	$h^2=0.33\pm0.03$

Heritability values were estimated through the correlation among paternal half sibs.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECT OF RAM BREED ON PRE-WEANING GROWTH PERFORMANCE OF CROSSBRED LAMBS[1]

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ABSTRACT: The objective of this study was to determine the effects of ram breed on the pre-weaning performance of crossbred lambs. Silverdale (Suffolk X Texel) ewes were allocated to one of three ram breeding groups (Silverdale ram; Blackface ram, Suffolk-Hampshire breeding; or White Dorper ram). Ewes were bred in October and lambed late February and into March. Lambs were weighed at birth and then again every 30 d leading to weaning at 90 d. Birth data were analyzed using ANCOVA (Statistix8, 2003) as a 3X2X3 factorial (3 ram breeds, 2 genders, 3 birth types – single, twins, triplets), fitting dam age as a covariate and blocking on year. Age-adjusted weaning data were analyzed similarly, but birth type was replaced with number of lambs reared by dam (single, twins, triplets). There was a significant interaction between ram breed and gender in the birth weight analysis; furthermore, there was a significant interaction between ram breed and the number of siblings reared by the ewe. Silverdale-sired (4.7 kg) and White Dorper-sired (4.7 kg) ram lambs were significantly lighter at birth compared to Blackface-sired (5.3 kg) ram lambs ($p<0.05$). Within ewe lambs born, Silverdales (5.0 kg) were heavier than White Dorpers (4.5 kg; $p<0.05$); however, Blackface (4.6 kg) were not significantly different from either Silverdale or White Dorper at birth. At weaning, Blackface-sired and White Dorper-sired lambs raised as singles were significantly heavier at 90 d compared to all other lambs raised as twins and triplets with the exception of Silverdale triplet raised lambs ($p<0.05$). Considering multiple births are the desired production scenario, there does not appear to be a sire breed advantage among Silverdale, White Dorper and Blackface rams; thus, one must consider the potential benefits of a lighter birth weight sire breed if an equivalent weaning weight can be achieved relative to a heavier birth weight sire breed at 90 d.

Key Words: Sheep, Breeds, Growth

Introduction

Because of the emphasis on growth and carcass, lamb producers have preferred Blackface rams, predominantly of Suffolk breeding (Leymaster and Jenkins, 1993). Producers complain these rams have a short productive lifespan under range conditions (Meyer et al., 2001). Other breeds exist in the United States and abroad that may be equivalent in growth characteristics to Blackface breeding (Suffolk and Hampshire). For instance, the Dorper breed was imported into the United States as a potential easy-keeping hair breed and/or terminal meat sire (Snowder and Duckett, 2003). Furthermore, Superior Farms (Hermiston, OR) developed a composite breed consisting of Suffolk-Texel genetics in hopes of developing a terminal meat sire. Little is published on the performance of this composite ram breed.

There appears to be a need for the development of a terminal sire to be used on western range and farm flocks, producing superior quality slaughter lamb. Sheep producers desire a more durable breed of ram that possess the growth and carcass qualities of the Suffolk, yet can withstand harsh production environments. Thus, the objective of this study was to determine the effects of ram breed on the pre-weaning performance of crossbred lambs.

Materials and Methods

In August 2003, 102 mature, crossbred ewes representing Suffolk-Texel (Silverdale) breeding were obtained from Superior Farms of Hermiston, OR and delivered to California State University, Chico's Agricultural Teaching and Research Center located in Chico, CA. For three breeding seasons (2003-2005), ewes were randomly assigned to one of three ram mating groups: Silverdale, Blackface (Suffolk-Hampshire breeding), or White Dorper (South African hair sheep). The Silverdale mating group generated purebred, composite Silverdale lambs (n=65); the Blackface generated F1 Blackface-Silverdale lambs of approximately 75% Suffolk-Hampshire breeding and 25% Texel (n=79); and the White Dorper generated F1 White Dorper-Silverdale lambs (n=88). Initial data collection began with the first lamb crop being born March 2004. Following each breeding season, ewes were managed as a single group on irrigated pasture. As lambing approached, ewes were supplemented with increasing amounts of corn and almond hulls. Upon lambing, ewe and lamb spent approximately 24 hours in a lambing jug. Lambs were individually identified, weighed and given an iodine navel treatment. For the first week after lambing, ewe and lambs were kept in pens near the lambing shed. Where appropriate, lambs in excess of twins were grafted upon ewes with singles or ewes with lost lambs. At the end of the first week, tails were docked; subsequently, male lambs were castrated. Ewes and lambs were maintained as a single management group on irrigated pasture without supplementation through June of each year. Lambs were processed every 30 d culminating in weaning at approximately 90 d of age.

Birth data were analyzed using ANCOVA (Statistix8, 2003) as a 3X2X3 factorial (3 ram breeds, 2 genders, 3 birth types – single, twins, triplets), fitting dam age

[1]Funded in part by the California State University Agricultural Research Initiative and Superior Farms.

as a covariate and blocking on year. Age-adjusted weaning data were analyzed similarly, but birth type was replaced with number of lambs reared by dam (single, twins, triplets).

Results and Discussion

There was a significant interaction between ram breed and gender in the birth weight analysis; furthermore, there was a significant interaction between ram breed and the number of siblings reared by the ewe. Silverdale-sired (4.7 kg) and White Dorper-sired (4.7 kg) ram lambs were significantly lighter at birth compared to Blackface-sired (5.3 kg) ram lambs (Figure 1; p<0.05). Within ewe lambs born, Silverdale-sired (5.0 kg) were heavier than White Dorper-sired lambs (4.5 kg; p<0.05); however, Blackface (4.6 kg) were not significantly different from either Silverdale or White Dorper at birth (Figure 1). The differences reported for birth weight among ram lambs was likely due to hybrid vigor and environmental conditions. Interestingly, the same was not observed in the ewe lambs.

Snowder and Duckett (2003) reported a similar trend in birth weight among Dorper-sired and Suffolk-sired lambs. Similarly relative to Dorper performance, Notter et al. (2004) reported lower Dorper birth weights relative to Dorset-sired lambs. Neither study reported a significant breed by gender interaction. Meyer et al. (2001) reported higher birth weights for Suffolk-sired lambs (2 kg) compared to Dorper-sired lambs. Another study by Leymaster and Jenkins (1993) reported similar birth weights for Texel-sired and Suffolk-sired lambs. Of interest, the Silverdale is a composite of Suffolk-Texel breeding.

At weaning, Blackface-sired and White Dorper-sired lambs raised as singles were significantly heavier at 90 d compared to all other lambs raised as twins and triplets with the exception of Silverdale triplet raised lambs (Figure 2; p<0.05). Snowder and Duckett (2003) and Meyer et al. (2001) reported a 2-3 kg advantage in weaning weight of Suffolk-sired lambs compared to Dorper-sired lambs. The former reported that the advantage seen at a 77-d weight was no longer significant at 118-d weight. Notter et al. (2004) reported comparable weaning weights of Dorper-sired lambs relative to Dorset. Also, Leymaster and Jenkins (1993) reported a .5 kg advantage of Suffolk-sired lambs compared to Texel-sired lambs. The advantage in weaning weight observed within singles was likely due to hybrid vigor in this study.

Implications

Considering multiple births are the desired production scenario, there does not appear to be a sire breed advantage among Silverdale, White Dorper and Blackface rams as a terminal sire; thus, one must consider the potential benefits of a lighter birth weight sire breed if an equivalent weaning weight can be achieved relative to a heavier birth weight sire breed at 90 d.

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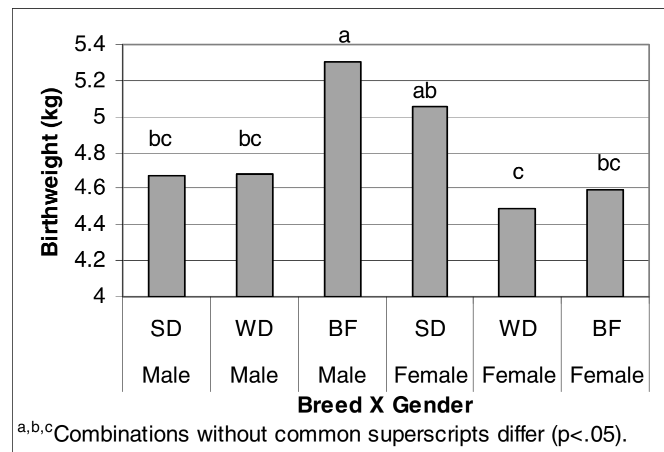

Figure 1. Breed (SD = Silverdale; WD = White Dorper; BF = Blackface) by gender effects on lamb birth weight.

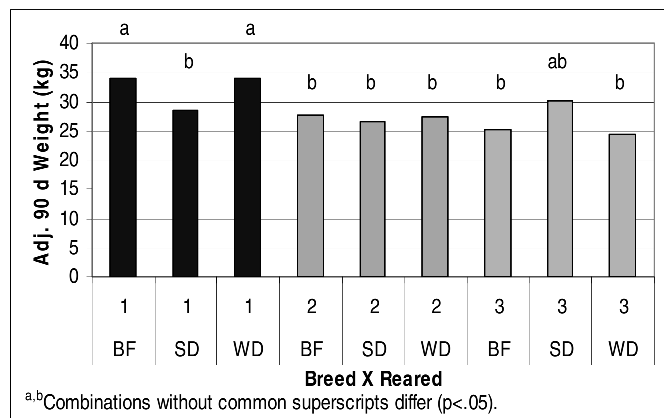

Figure 2. Effect of breed (SD = Silverdale; WD = White Dorper; BF = Blackface) X reared (number of siblings raised to weaning by ewe) on adjusted 90 d lamb weaning weight.

Proceedings, Western Section, American Society of Animal Science

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EFFECT OF RAM BREED AND FINISHING DIET ON CARCASS TRAITS OF CROSSBRED WETHER LAMBS[1]

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ABSTRACT: The objective of this study was to determine ram breed and finishing diet effects on carcass traits of crossbred wether lambs. Weaned, crossbred lambs approximately 180 d of age were randomly assigned to one of two finishing diets (grain-based or forage-based) across the three ram biological types (Silverdale, a Suffolk-Texel cross; White Dorper; and Blackface, Suffolk-Hampshire breeding). Lambs were harvested at a constant backfat of 0.4 cm as determined by real-time ultrasound (Aloka 500) between the 12th and 13th ribs. A random sample (n=54) of wether lambs was taken and processed at the CSU, Chico Agricultural Teaching and Research Center. Carcass measurements included carcass weight (kg), dressing percent (%), loin eye area (cm^2), loin depth (cm), and backfat (cm). Data were analyzed using ANCOVA (Statistix8, 2003) as a 3X2 factorial (3 ram breeds and 2 diets), blocking on year and fitting trial start weight as a covariate. There was no significant interaction between ram breed and diet. Ram breeds were similar in carcass merit for all traits except carcass weight. While dressing percents were similar among the breeds, Blackface-sired lambs produced heavier carcass weights (3.0 kg advantage) compared to Silverdale-sired lambs ($p<0.05$) at similar backfats. White Dorper-sired lambs were not significantly different from either breed. Additionally, forage-finished lambs produced carcasses that were lighter (5.7 kg), dressed lower (6.9%), and possessed smaller (3.1 cm^2), shallower loin eyes (0.5 cm) with less backfat (0.3 cm) compared to grain-finished lambs ($p<0.05$). Blackface-sired lambs appear to have a slight advantage in pounds produced on the rail compared to the other breeds studied. Furthermore, grain-finishing practices appear to produce a carcass more in-line with industry standards relative to carcass merit.

Key Words: Sheep, Carcass Conformation, Breeds

Introduction

A significant percentage of the domestic lamb supply marketed annually does not meet the specifications required by the meat packing industry, resulting in discounts to the seller. Because of the variation in production environments found across the United States requiring various biological types, the lamb packing industry harvests lambs of varying growth rates and maturity at a target, constant live weight (Dickerson et al., 1972). Many lambs are too small or too large, are under-finished or too fat, have inferior conformation, or are lacking the desired eating characteristics demanded by the consumer. Today's market is about narrow product variability, consistency and predictability.

Because of the emphasis on growth and carcass, lamb producers have preferred blackface rams, predominantly of Suffolk breeding (Leymaster and Jenkins, 1993). Producers complain these rams have a short productive lifespan under range conditions (Meyer et al., 2001). There appears to be a need for the development of a terminal sire to be used on western range and farm flocks, producing superior quality slaughter lamb. Sheep producers desire a more durable breed of ram that possess the carcass qualities of the Suffolk, yet can withstand harsh production environments. Thus, the objective of this study was to determine ram breed and finishing diet effects on carcass traits of crossbred wether lambs.

Materials and Methods

In August 2003, 102 mature, crossbred ewes representing Suffolk-Texel (Silverdale) breeding were obtained from Superior Farms of Hermiston, OR and delivered to California State University, Chico's Agricultural Teaching and Research Center located in Chico, CA. For two breeding seasons (2003-2004), ewes were randomly assigned to one of three ram mating groups: Silverdale, Blackface (Suffolk-Hampshire breeding), or White Dorper (South African hair sheep breed). The Silverdale mating group generated purebred, composite Silverdale lambs (n= 12); the Blackface generated F1 Blackface-Silverdale lambs of approximately 75% Suffolk-Hampshire breeding and 25% Texel (n= 19); and the White Dorper generated F1 White Dorper-Silverdale lambs (n= 23). Initial data collection began with the first lamb crop being born March 2004. Following each breeding season, ewes were managed as a single group on irrigated pasture. As lambing approached, ewes were supplemented with increasing amounts of corn and almond hulls. Upon lambing, ewe and lamb spent approximately 24 hours in a lambing jug. Lambs were individually identified, weighed and given an iodine navel treatment. For the first week after lambing, ewe and lambs were kept in pens near the lambing shed. Where appropriate, lambs in excess of twins were grafted upon ewes with singles or ewes with lost lambs. At the end of the first week, tails were docked; subsequently, male lambs were castrated. Ewes and lambs were maintained as a single management group on irrigated pasture without supplementation through June of each year. Lambs were processed every 30 d culminating in weaning at approximately 90 d of age. Lambs were then maintained on irrigated pasture through August.

[1]Funded in part by the California State University Agricultural Research Initiative and Superior Farms.

Weaned, crossbred lambs approximately 180 d of age were randomly assigned to one of two finishing diets (grain-based or forage-based) across the three ram biological types (Silverdale, a Suffolk-Texel cross; White Dorper; and Blackface, Suffolk-Hampshire breeding). The grain-based diet consisted primarily of corn supplemented with alfalfa hay at a rate of 39% while the forage-based diet consisted primarily of alfalfa hay supplemented with almonds hulls at a rate of 18%. Lambs were maintained on a raised floor facility in diet by breed treatment groups during the finishing phase of the trial. Animals in all treatment groups had ad libitum access to feed and water.

Lambs were harvested at a constant backfat of 0.4 cm as determined by real-time ultrasound (Aloka 500) between the 12th and 13th ribs. A random sample (n=54) of wether lambs was taken and processed at the CSU, Chico Agricultural Teaching and Research Center. Carcass measurements included carcass weight (kg), dressing percent (%), loin eye area (cm^2), loin depth (cm), and backfat (cm). Data were analyzed using ANCOVA (Statistix8, 2003) as a 3X2 factorial (3 ram breeds and 2 diets), blocking on year and fitting trial start weight as a covariate.

Results and Discussion

There was no significant interaction between ram breed and finishing diet. Ram breeds were similar in carcass merit for all traits except carcass weight (Table 1). While dressing percents were similar among the breeds, Blackface-sired lambs produced heavier carcass weights (3.0 kg advantage) compared to Silverdale-sired lambs ($p<0.05$) at similar backfats. Based on ultrasound monitoring of backfat, Silverdale lambs reached market readiness at lighter live weights compared to the Blackface. The difference reported for carcass weight between the Blackface-sired and the purebred Silverdale was likely due to hybrid vigor. The difference may be a function of differences in physiological maturity reflected in carcass characteristics (Snowder et al., 1994). White Dorper-sired lambs were not significantly different from either breed for carcass weight. Furthermore, no differences ($p>.05$) were detected for the remaining carcass traits.

Leymaster and Jenkins (1993) reported heavier carcass weights at fixed backfat levels for Suffolk-sired lambs (3.7 kg advantage) compared to Texel-sired lambs. A similar difference was found in this study between Blackface-sired lambs and the Silverdale-sired lambs. Of interest, the Silverdale is a composite of Suffolk-Texel breeding. Furthermore, Snowder and Duckett (2003) reported similarities in carcass merit between Suffolk-sired and Dorper-sired lambs.

Additionally, forage-finished lambs produced carcasses that were lighter (5.7 kg), dressed lower (6.9%), and possessed smaller (3.1 cm^2), shallower loin eyes (0.5 cm) with less backfat (0.3 cm) compared to grain-finished lambs (Table 2; $p<0.05$). While both diet groups were monitored for market readiness based on a targeted backfat of .4 cm determined by ultrasonography, there were significant differences between the two feeding regimes. It is important to note this was a target; however, the forage-finished group's backfat did not differ significantly ($p>.05$) from the .4 cm target. As was observed in this study, Murphy et al. (1994) and McClure et al. (2000) found that lambs finished on a concentrate diet were fatter compared to lambs finished on alfalfa. Furthermore, Borton et al. (2005) reported that industry-desired lambs can be achieved through forage-finishing systems; however, forage finishing required longer finishing periods due to lower average daily gains. In addition, there were concerns regarding less palatable lamb meat associated with forage finishing.

Implications

Of those breeds studied, none appear to have a clear carcass advantage other than in carcass weight. Blackface-sired lambs appear to have a slight advantage in pounds produced on the rail compared to the other breeds studied. Furthermore, grain-finishing practices appear to produce a carcass more in-line with industry standards relative to carcass merit.

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Table 1. Effect of sire breed on lamb carcass traits.

Sire Breed	Carcass Wt. (kg)[1]	Dressing %	Loin Eye Area (sq. cm)	Loin Depth (cm)	Backfat (cm)
Blackface	29.81[a]	53.0[a]	18.10[a]	3.72[a]	.46[a]
	(.60)	(.82)	(.66)	(.15)	(.04)
Silverdale	26.76[b]	50.0[a]	17.06[a]	3.64[a]	.52[a]
	(.80)	(1.10)	(.98)	(.22)	(.05)
White Dorper	28.30[ab]	52.0[a]	16.83[a]	3.40[a]	.52[a]
	(.55)	(.70)	(.61)	(.13)	(.03)

[1]Standard error reported in parentheses.
[a,b]Breeds within column (carcass traits) without common superscripts differ ($p<.05$).

Table 2. Effect of finishing diet on lamb carcass traits.

Diet	Carcass Wt. (kg)[1]	Dressing %	Loin Eye Area (sq. cm)	Loin Depth (cm)	Backfat (cm)
Grain-Based	31.15[a]	55.1[a]	18.86[a]	3.82[a]	.62[a]
	(.46)	(.58)	(.49)	(.11)	(.03)
Forage_Based	25.45[b]	48.2[b]	15.80[b]	3.35[b]	.38[b]
	(.55)	(.76)	(.68)	(.15)	(.04)

[1]Standard error reported in parentheses.
[a,b]Diets within column (carcass traits) without common superscripts differ ($p<.05$).

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ESTIMATES OF GENETIC PARAMETERS FOR WEIGHT IN THE PROGENY OF NUBIAN, FRENCH ALPINE, SAANEN, TOGGENBURGH, AND SPANISH GOATS MATED TO BOER SIRES

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ABSTRACT, The objectives of this study were to compare the performance of the progeny of goats involving inheritance of Nubian(N), French Alpine (A), Saanen (S) Toggenburgh (T), and Spanish (SP) (n=160), and to estimate genetic parameters for growth traits. Traits analyzed were weight at birth BWT and weaning WWT, and average daily gain (ADG) from birth to weaning. Separate analysis for each trait used least squares mixed model SAS (1992). The analytical model included: breed of dam, age of dam, sex of the kid, season of parturition as fixed effects; sire, sire x breed of dam interaction and the residual as random components. The overall mean values for weight at birth and weaning were: 1.99 and 12.89 kg respectively. The average values for weight at birth were (2.12±.0.07, 2.11 ±.06, 2.04±.05, 1.95±.06, 2.10±.05 and 1.98±.07, 1.97±.06, 1.93±.05, 1.83±.05, 1.96±.06) for males and females kids, respectively. The average values for weaning weight were (13.99±.0.37, 13.29 ±.0.33, 13.25±0.34, 12.67±0.31 and 13.51±0.43, and 12.50±0.29, 12.48 ±0.30, 11.98 ±0.29, 12.68± 0.41 and 12.60±0.32 kg) for male and female kids, respectively. The estimated ADG from birth to weaning was 181±0.32 g. The average values for daily gain were: 187±0.36 and 175±0.43 g for male and female kids, respectively. Estimates of heritability direct values were (h^2 =0.20±0.03, h^2=0.15±0.03 and h^2=0.25±0.05) to BWT, WWT, and ADG, respectively.

KEYWORDS: Genetic parameters, Weight, Boer goat

Introduction

The first step in a breeding program consists in selecting among existing breeds or strains and their cross combinations (Dickerson, 1969). Selection within the best adapted breed or new breed combinations is the logical second stage in developing the most efficient genotype. Boer is one breed that can major contribution to increasing goat productivity worldwide Devendra and Burns (1983).The performance of Boers in Southern Africa, have high growth rates and large mature sizes (Van Niekirk and Cassey, 1988). Although the Boers may have potential as terminal sires, much of the interest by goat industry is focused on pure breed Boer productivity. Accurate estimates of variance components are important because prediction error variances for predicted genetic values as estimated values move away from the true values (Henderson, 1975; Schaeffer, 1984). The objective of this study was to document the performance of the progeny of dams involving inheritance of N, A, S, T, and SP goats in different proportion when mated by natural service to Boer sires for weight at birth BWT, weaning weight WWT, average daily gain from birth to weaning ADG, and survival rate of kids at birth and weaning.

Materials and Methods

Data came from a commercial goat farm in Imperial Valley, California. The origin of the herd was from Texas, a total of 2000 goats involving inheritance of Spanish, Nubian, Toggenburgh, Saanen and Alpina in different proportion. In 1997 were aqquired 31 sires Boer to an average age of eleven months. The analyzed data involved 2 years from 2001 to 2002. A total of 160 goats (40 of each genotype) were randomly chosen and mated naturally to Boer sires September to December.

Management

Management was reasonably constant, from August to April goats were maintained on irrigated pastures of bermuda (Cynnodon, spp. L.) or warm season pastures and fed (grass and alfalfa) during the winter when parturition, goats were confined, a mixed block of minerals was provided through the year. At birth, all kids were identified, weighed, and vaccinated against viral scours.

Statistical analyses

The data were analyzed by least squares mixed model procedures. SAS, Version 6.2 1992. Separate analyses were conducted for the analyzed traits. The analytical model included: breed of dam, age of dam, sex of kids and season of parturition as fixed effects; sire, interaction sire x breed of dam and the residual as random components. Phenotypic variances and fractions of variance due to genetic, dam and residual effects with sire model were also estimated.

Results and Discussion

Birth weight

Least squares means for birth weight are presented in Table 1. As shown the overall mean value for weight at birth was 1.99 kg. The average values for birth weight were (2.12±.0.07, 2.11 ±.06, 2.04±.05, 1.95±.06, and 2.10±.05

and 1.98±.07, 1.97±.06, 1.93±.05, 1.83±.05, 1.96±.06) for males and females kids from dams involving inheritance of N, A, S, T, and SP goats in different proportion mated by natural service to Boer sires.

Weaning weight

Table 1. Also shown weaning weight at 60 d. The average WWT was 12.89 kg. The average values for WWT were (13.99±.0.37, 13.29 ±.0.33, 13.25±0.34, 12.67±0.31 and 13.51±0.43, and 12.50±0.29, 12.48 ±0.30, 11.98 ±0.29, 12.68± 0.41 and 12.60±0.32 kg) for male and female kids, respectively. The estimated ADG from birth to weaning was 181±0.32 g. The unadjusted survival rate percentages at birth and weaning was (87.37 and 64.97%) respectively. Male kids were consistently heavier, and grew faster than female kids. A difference of 0.26 kg in weight at birth increased 2.80 kg at weaning.

Average daily gain

Least squares means for ADG of kids from birth to weaning are also presented in Table 1. The estimated ADG from birth to weaning was 181±0.32 g. The ADG ranged from 187 to 175, for male and female kids, respectively. The ADG ranged from 178 to 197 and 175 to 180 g among male and female kids, respectively. The ADG values (197, 186, 186, 178, and 190 and 175, 175, 167, 180, and 177 g) corresponded to male and female kids as progeny of dams involving inheritance of N, A, S, T, and SP in different proportion, mated by natural service to Boer sires, in that order.

Survival

Least squares means for survival at birth and weaning ranged from 87.37 to 64.97%, respectively. The estimated average values (91.17, 87.13, 87.14, 83.09, and 88.03 and 66.33, 64.87, 63.47, 64.44, and 65.76) corresponded to the progeny, male and female kids at birth and weaning from dams involving inheritance of N, F, A, S, T, and SP goats mated naturally to Boer sires. Gregory et al.(1993) reported effects of breed groups in calves with two years old dams were important (P<.01) for calving difficulty CD percentage and survival S percentage at birth 72 hr and at weaning independent of breed group effects on BWT (P<.01) for CD percentage; (e.g., males=62% and females= 46% adjusted to a common BWT). The negative linear and quadratic regressions (P<.01) of S percentage at birth, 72 hr and weaning on birth reflect the importance of BWT on calf survival percentage and the curvilinearity of the effect.

Heritability

Estimates of heritablity and their standard errors of the analyzed traits are presented in Table 2. As shown the estimates of heritability direct values were (h^2=0.07±0.04, h^2=0.20±0.03, h^2=0.15± 0.03 and h^2=0.25±0.05) to P, BWT, WWT, and ADG, respectively. The estimated direct heritability value (h^2=0.20±0.05) for BWT in this study suggest a moderate value for this trait. Hanford et al. (2003), and Van Wyk et al. (1993) reported heritability direct values (h^2=0.27±0.02 and h^2=0.16) for BWT and WWT respectively. Van Wyk et al. (1993) also reported estimates of maternal heritability values (h^2=0.43 and h^2=0.13) for BWT and WWT, respectively. Mousa et al. (1999) estimated variances due to direct genetic effects of heritability (h^2=0.09 and 0.17) for BWT and WWT. Notter (1998) reported estimates values (h^2=0.19) for WWt 60 d. Van Wyk et al. (1993) estimated heritability values (h^2=0.13 and h^2=0.18) direct and maternal, respectively for ADG.The estimated direct value (h^2=0.13) by Van Wyk et al. (1993) was half than the estimates (h^2=0.25±0.05) for ADG in this study. The maternal heritability estimates suggest that genetic maternal effects are not important for weights or gain at older ages. When the variances of maternal effects are near zero, the covariance between direct and maternal effects (ram) has little meaning. It suggests , being an antagonism between direct genetic and maternal genetic effects (Robison, 1981) especially for WWT, in agreement with the direct-maternal correlation of –0.39 from the analysis of (Maria et al., 1993).

Implications

The experimental results from this study constitute a knowledge resource which may be useful in the planning of effective breeding schemes to accomplish defined objectives in specific production situations. The importance of fertility and viability levels are important in determining final productivity, the number of young born per year is an important factor, in this respect the goat performs particularly well. In most cases the pattern of estimated heritability of traits was what might reasonably be expected in agreement with previous reports.

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Table 1. Least squares means for prolificacy, weight at birth and weaning, average daily gain from birth to weaning, and survival rate of kids at birth and weaning of the progeny of dams involving inheritance of Nubian, French Alpine, Saanen, Toggenburgh, and Spanish goats mated by natural service to Boer sires.

Breed group	**Birth Weight kg**		**Weaning weight kg**		**Average daily gain g**		**Survival at birth %**	**Survival at weaning %**
	Males	Females	Males	Females	Males	Females		
Overal Mean	2.06±0.06	1.93±.06	13.34±0.36	12.44±0.32	187±0.30	175±0.31	87.37	64.97
Nubian	2.12±0.07	1.98±0.07	13.99±0.37	12.50±0.29	197±0.33	175±0.29	91.17	66.33
Alpine	2.11±0.06	1.97±0.06	13.29±0.33	12.48±0.30	186±0.31	175±0.29	87.13	64.87
Saanen	2.04±0.05	1.93±0.05	13.25±0.34	11.98±0.29	186±0.31	167±0.34	87.14	63.47
Toggenburgh	1.95±0.06	1.83±0.05	12.67±0.31	12.68±0.41	178±0.29	180±0.30	83.09	64.44
Spanish	2.10±0.05	1.96±0.06	13.51±0.43	12.60±0.32	190±0.27	177±0.35	88.33	65.76

Table 2. Estimates of heritablity direct values and SE from single-traits analyses.

Trait	**h^2a**	**SE**
Birth weight	0.20	±0.03
Weaning weight	0.15	± 0.03
	0.25	±0.05

h^2_a = direct heritability; se=standard errors

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PREVALENCE OF *SALMONELLA* AND *E. COLI* O157:H7 IN LARGE HERD DAIRIES IN SOUTHWESTERN UNITED STATES AND NORTHERN MEXICO

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ABSTRACT: Dairy cattle can harbor both *Salmonella* and *E. coli* O157:H7 . Salmonellosis outbreaks have been reported in southwestern U.S. dairies, resulting in decreased milk production and a higher rate of mortality. However, cattle can harbor and shed *E. coli* O157:H7 without experiencing morbidity. A study was conducted to determine the prevalence of *Salmonella* and *E. coli* O157:H7 in two Southern New Mexico large herd dairies and one Northern Mexico large herd dairy, all of which were in close proximity of the U.S.-Mexico border. Four pens (lactation pens) on each dairy were selected and fecal grab samples were collected from 7-15 cows per pen. Samples were collected in March, May, July, August, September and November. *Salmonella* was cultured using a double enrichment followed by plating on brilliant green agar supplemented with novobiocin. *E. coli O157:H7* was cultured using an immuno-magnetic separation technique. On all three dairies, the highest (P = 0.05) incidence of *Salmonella* was in July, August and September. However, prevalence in November was still greater (P = 0.05) than March and May. *Salmonella* prevalence was greater (P = 0.05) in the Mexico dairy compared to the combined U.S. dairies during August. Shortly before this collection, record rainfall occurred in the area of the Mexico dairy. Based on interviews with the dairy managers, salmonellosis was not evident at any time during the collection period. The prevalence of *E. coli* O157:H7 was generally low in all dairies. However, the Mexico dairy had a higher (P = 0.05) prevalence of *E. coli* O157:H7 in March and the U.S. dairies were (P = 0.07) higher in August. In conclusion, data confirm cattle can harbor and shed both *E. coli* O157:H7 and *Salmonella*.The greatest prevalence occurred in summer months which correspond with the period of greatest rainfall in the area.

KEYWORDS: *Salmonella*, *E. coli,* Dairy cattle

Introduction

Outbreaks of infective bacterial pathogens, especially *Salmonella* and *E. coli* O157:H7, are of potentially serious concern for dairy managers (Herriott et al., 1998). Diseases such as salmonellosis are caused by fecal-oral contamination from carrier animals to herd mates and can rapidly spread through high-density dairy herds (You et al., 2006). Such infections are not only of economical consequence to dairy producers, but may be linked to human infection as well (Padungtod and Kaneene, 2006).

Early detection of pathogenic shedding by dairy cattle may be challenging, as many carriers of causative agents do not exhibit outward symptoms of infection (Hume et al., 2004). By the time a visually detectable number of animals become clinical, a large portion of the herd, if not the entirety, may have received exposure to contamination. Rather than relying solely on the poor probability of early detection of an infectious outbreak of *Salmonella* or *E. coli* O157:H7, some studies have suggested that outbreaks may be predicted based on events such as seasonal changes and related precipitation levels (Edrington et al., 2004; Martinez-Urtaza et al., 2004).

A study was conducted to determine the prevalence of *Salmonella* and *E. coli* O157:H7 in two large-herd dairies in southern New Mexico and one in Northern Mexico along the U.S.-Mexico border.

Materials and Methods

Fecal samples were obtained from mature, lactating Holstein dairy cows located at three different dairy farms along the U.S.-Mexico border; one in Ciudad Juarez, Mexico and two near Las Cruces, NM, U.S. Samples were obtained from each farm for March, May, July, August, September, and November 2006. Four lactation pens were selected at each dairy and fecal samples were collected from seven cows per pen at the U.S. dairies and 15 cows per pen at the Mexico dairy. The method of collection was fecal grab via gloved rectal palpation, after which the glove was inverted and fecal mater retained within the glove throughout shipping to avoid cross-contamination. After collection, samples were refrigerated for 24-h and transported on ice to the Food and Feed Safety Research Unit, Southern Plains Agricultural Research Center in College Station, TX for analysis. Each fecal sample was tested for presence or absence of *E. coli* O157:H7 using the immuno-magnetic separation technique (Elder et al., 2000) and for *Salmonella* using brilliant green agar with novobiocin plating (Edrington et al., 2004). Data generated from sample analysis were statistically evaluated using the GLM procedures function of SAS (SAS Institute, Cary, NC). A Dairy by month interaction was detected ($P <$ 0.05) for prevalence of *Salmonell*a . Therefore dairies were compared within each month. A dairy by month interaction was not ($P >$ 0.010) detected for prevalence of *E. coli* O157:H7. The means are presented by month across the three dairies.

The study conformed to the New Mexico State University Institutional Animal Care and Use Committee approval protocol.

Results and Discussion

Percentage of samples testing positive for *Salmonella* (Table 1.) was higher in the U.S. dairies than the Mexico dairy for the months of March ($P = 0.03$), July ($P = 0.06$), September ($P = 0.06$), and November ($P = 0.06$), but was higher in the Mexico dairy in August ($P = 0.01$). The spike in the percentage of observed cases in the Mexico dairy during August corresponds to a higher level of rainfall in that location (Table 2). These data are consistent with the findings of previous studies that sited water runoff in areas of concentrated fecal contamination as a major factor in the spread of bacterial infection (Martinez-Urtaza et al., 2004; McDonald et al., 2006).

Table 1.Prevalence of *Salmonella* in lactating dairy cattle isolated from feces collected between March and November on two dairies in the Southwest, and one Northern Mexico dairy[1]

Month	Mexico	US Dairy-1	US Dairy-2	Std.Error[2]
March	0.0[a]	12.5[b]	41.8[c]	6.5
May	11.7	10.6	16.7	4.8
July	13.2[c]	55.4[e]	30.8[e]	10.9
Aug	91.7[a]	69.6[b]	24.1[c]	5.8
Sept	40.0	67.9	69.6	11.9
Nov	30.0	46.8	50.0	8.7

[1]Dairies were located in Southwestern New Mexico and Northern Mexico. A dairy by month interaction was detected ($P < 0.05$) for prevalence of *Salmonella*. Therefore dairies were compared within each month.
[2]n = 4 per dairy.
[a,b,c,d,e]Differing superscripts signify differences between means.

Table 2. Approximate total monthly precipitation in cm and monthly average high temperature in °C for Las Cruces, NM and Ciudad Juarez, Mexico during fecal sample collection[1]

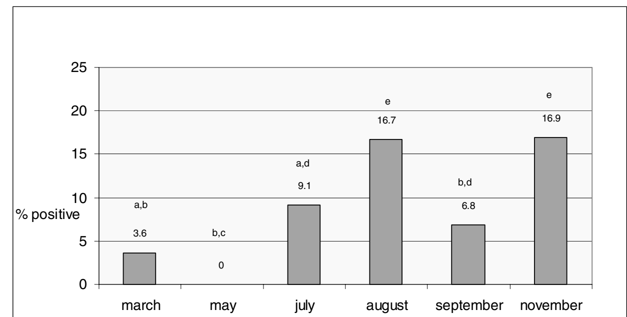
	Site			
	Juarez		Las Cruces	
Month	Precip.[2]	Temp.[3]	Precip.[2]	Temp.[3]
March	0.00	23.8	0.00	21.5
May	2.26	33.5	0.02	33.4
July	8.05	34.8	5.74	36.1
August	17.17	31.4	7.11	31.5
September	12.67	27.9	10.18	28.0
November	0.15	21.3	0.00	20.9

[1]National Weather Service.
[2]in cm.
[3]in °C.

For the months of March, July, September, and November, percentages of samples testing positive for *Salmonella* were the same or lower for the Mexico dairy compared to its U.S. counterparts, despite little variation in total monthly rainfall among the three locations. This supports previous observations that multiple factors can contribute to the variability in the incidence of pathogenic contamination and infection rate between dairy herds (Edrington et al., 2004). Previous work has implicated such influences as diet composition, level of environmental adaptation, herd density, ambient temperature, and a number of management practices (Chaucheyras-Durand et al., 2006; Shanks et al., 2006; Clegg et al., 1983)

The percentage of samples testing positive for *E. coli* O157:H7 was highest($P = 0.05$) in August and November (Figure 2), and the values generally followed the trend of precipitation levels with an apparent lag effect of around one month. This is consistent with a previous report recognizing that clinical and asymptomatic carrier animals can shed *E. coli* O157:H7 for up to 43 days after infection (Shere et al., 2002). That is, heavy downpours initiate the increase in transfer of pathogenic microbes from infected to non-infected animals, but the resulting increase in fecal shedding facilitates a prolongation of herd-wide infection for several weeks after the causative precipitative episode.

Figure 1.Average percent of *E. coli* O157:H7 in lactating dairy cattle isolated from feces between March and November on two dairies in the Southwest and one in Northern Mexico[1,2]



Most conservative Standard Error = 2.6; n = 4.
[1]Dairies were located in Southwestern New Mexico and Northern Mexico. A dairy by month interaction was not ($P > 0.10$) detected for prevalence of *E. coli* O157:H7. The means are presented by month across three dairies.
[2]Bars with different superscripts differ ($P < 0.05$)

Implications

Data collected in this study lend support to two claims by authors of previous studies: many different variables contribute to the onset of *Salmonella* and *E. coli* O157:H7 outbreaks in dairy herds (Edrington et al., 2004), and one leading factor in outbreaks is water runoff associated with rainfall (Martinez-Urtaza et al., 2004; McDonald et al., 2006). General data trends followed expectations based on precipitation levels, but a small number of unexpected recorded values did not seem to be attributable to rainfall alone. Further research is being planned to explore the magnitude of the role certain other variables such as ambient temperature and humidity play in the spread of bacterial infections in dairy herds.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

LOW-INPUT PASTURE BACKGROUNDING SYSTEM IS MORE PROFITABLE THROUGH HARVEST THAN HIGH-INPUT DRYLOT SYSTEM.

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ABSTRACT: Calves are commonly backgrounded for ≥ 45 d on the ranch of origin; however, few comparisons of on-ranch backgrounding programs exist. This study compared a low-input pasture backgrounding system (**PAST**) to a high-input drylot system (**DLOT**) of the same duration each year (42 - 45 d) to evaluate performance and profit during the backgrounding (**BACKGRD**; weaning to 42 - 45 d) and finishing (**FINISH**; end BACKGRD to harvest) phases. Over 3 yr, 250 calves (236 kg avg. initial BW; 133 steers and 117 heifers) were randomly assigned to PAST or DLOT treatments during BACKGRD. The DLOT calves were fed a corn/wheat midds-based pelleted ration (restricted max. intake 3.0% BW) plus alfalfa hay (0.68 to 1.13 kg/d), and PAST calves were supplemented with a 32% CP range cube (0.57 kg/d; 3×/wk). After BACKGRD, only steers were finished at a commercial feedlot where they were managed as a single group. During BACKGRD, DLOT calves gained more weight ($P < 0.01$) and had a higher final value ($P = 0.03$), but feed and total costs were more than 4-fold higher ($P < 0.01$). Net income during BACKGRD was $45 greater ($P < 0.01$) for PAST than DLOT. During FINISH, initial BW and value were similar ($P \geq 0.37$) among DLOT and PAST steers. The PAST steers had greater ADG ($P < 0.01$; 1.27 vs. 1.07 kg/d) through interim weight (74-94 days on feed; **DOF**) than DLOT steers, but subsequent ADG was similar ($P = 0.68$). There were no differences ($P \geq 0.13$) in interim BW, DOF, total ADG, carcass characteristics, or proportion of steers treated for sickness. However, DLOT steers had greater death loss ($P = 0.02$; 7.6% vs. 0.0%) and lower feed cost ($P = 0.04$; $221 vs. $238/steer). Although the average price received for carcasses sold was not different ($P = 0.11$), PAST steers garnered $111 more gross income during FINISH than DLOT ($P < 0.01$; $946 vs. $833/carcass), and had a net return advantage ($P < 0.01$) of $103/hd. The low-input PAST backgrounding system was more profitable than the DLOT system during both the backgrounding and finishing phases.

Key Words: Backgrounding, Beef Calves, Feedlot

Introduction

Price premiums for "VAC-45" calves marketed through Superior Livestock Auction video sales increased every year from 2000 to 2004, with annual average premiums ranging from $3.66 to $7.91/45.4 kg (King and Seeger, 2005). Justification for such premiums are supported by the analysis of New Mexico Ranch to Rail data by Waggoner et al. (2005), which showed that steers weaned 41 d or more before feedlot entry generated greater net income during finishing than steers backgrounded 21 to 40 d, or less than 20 d. Those findings also support the premise that backgrounding programs of 45 d or more improve finishing profit potential. Results of a 2005 Cattle-Fax membership survey revealed that 74% of respondents "weaned" calves for at least 45 d prior to shipping (Anonymous, 2005). However, studies evaluating backgrounding calves have typically focused on programs less than 40 d (Prichard and Mendez, 1990; Roeber et al., 2001; St. Louis et al., 2003), and controlled experiments evaluating the impact of divergent backgrounding systems on performance and profit through harvest are not available. Therefore, this study compared a low-input pasture backgrounding system to a high-input drylot system of the same duration (42 to 45 d) to evaluate performance and profit during the backgrounding and finishing phases.

Materials and Methods

Over 3 yr, 250 calves (236 kg avg. initial BW; 133 steers and 117 heifers) were used to compare two backgrounding systems at the New Mexico State University Corona Range Livestock Research Center (**CRLRC**) located 13 km east of Corona, NM (avg. elevation = 2000 m; avg. annual precipitation = 380 mm). All animal handling and experimental procedures were in accordance with guidelines established by the New Mexico State University Animal Care and Use Committee. Calves originated from the CRLRC spring-calving British cross cowherd, and were born in February, March, or April.

Steer calves were castrated at branding (early May). At branding and 16 to 21 d prior to weaning all calves were vaccinated against bovine respiratory syncytial virus, infectious bovine rhinotracheitis, bovine viral diarrhea, and parainfluenza 3, and were administered a 7-way clostridial vaccine.

Backgrounding Phase. All BW were measured unshrunk, and a 4% pencil shrink was applied. At weaning (d 0), calves were weighed, assigned a market price, and randomly assigned to one of two treatments: 1) high-input drylot backgrounding system (**DLOT**) or 2) low-input pasture backgrounding system (**PAST**). Each treatment was replicated within year. Following vaccination and measuring weaning BW, calves were transported to their

respective pen/pasture. The same two native range pastures (minimum 4.1 ha/hd) and drylot pens (minimum 17.8 m^2/hd) were used each year. Free choice access to water and a loose mineral mix (38% NaCl, 12% Ca, 8% P, 2% K, 2% Mg, 2500 ppm Mn, 1000 ppm Cu, 1000 ppm Zn, 13 ppm Se, and 125,000 IU/kg Vitamin A; Hi-Pro Feeds, Friona, TX) was provided.

Native range pastures were not grazed during the summer growing season prior to stocking for backgrounding. Average CP, NDF, and ADF content (% DM) of forage samples collected from each pasture at the end of the backgrounding period was 8.3, 67.2, and 42.5, respectively. Forage availability exceeded cattle needs during all three years.

On d 0 - 6 following weaning, PAST calves were trained to hand-delivery of protein supplement by enticement with alfalfa hay (max. 0.75 kg/hd), plus protein supplementation with a 32% CP range cube fed at 0.57 kg/d. Beginning d 7, protein supplement delivery frequency was reduced to 3×/week. Calves were fed between 1000 and 1200 h each day feed was delivered.

On d 0, DLOT calves were fed 4.54 kg/hd alfalfa hay (92.4% DM; 17.3% CP, 45.8% NDF, and 38.1% ADF of DM). Beginning d 1, DLOT calves were offered 2.25 kg/hd of a corn/wheat midds-based backgrounding pellet (Table 1), plus alfalfa hay. Pellets and hay were offered in feed troughs (300 × 60 cm) allowing access from both sides. Linear trough space exceeded 30 cm/hd. Pellet intake was increased by 0.68 kg/hd when all troughs in a pen were completely empty at 0700, and pellet offering was restricted to 3.0% BW/d. Pellets were increased to 4.54 kg/hd by d 7. Hay was reduced to 0.68 to 1.13 $kg \cdot hd^{-1} \cdot d^{-1}$ by d 13, and maintained at that amount throughout the backgrounding phase.

All calves were weighed on a single day each year near the mid-point (d 19 or 21) and at the end of the backgrounding phase (d 42 to 45). The day final backgrounding BW was measured marked the end of the backgrounding phase. The backgrounding treatment period was 45 d in yr 1, 44 d in yr 2, and 42 d in yr 3. All steers were place in a common drylot pen and fed alfalfa hay to appetite for 5 to 9 d prior to shipping. Therefore, steers remained at the CRLRC for 46 to 54 d post-weaning.

Each year, weaning price and final backgrounding price was individually applied to each calf based upon prices in the New Mexico Weekly Weighted Average Feeder Cattle Report (USDA CV LS795) for the week of the beginning and end of the backgrounding phase. No premium for backgrounding was applied. Purchased feed cost varied by year, with delivered price/ton ranging from \$204 to \$213 for backgrounding pellets, \$244 to \$262 for range cubes, and \$130 to \$165 for alfalfa hay. Feed costs were applied as weight of feed delivered to each pasture/pen times unit feed cost. A grazing fee was charged to PAST calves at $\$0.132 \cdot hd^{-1} \cdot d^{-1}$. Time spent delivering feed to calves was recorded for each pasture/pen to calculate labor cost, which was charged at \$6.00/h.

Finishing Phase. Heifers were not included in the finishing phase. Steers were fed at a commercial feedlot (Double A Feeders, Clayton, NM) where they were entered into the New Mexico Ranch to Rail Program. Final BW and price of steers from the backgrounding phase was used as the initial BW and price of steers for the finishing phase.

Steers were received at the feedlot on a single day in mid-November each year, and were managed according to standard procedures in place at the feedlot at the time of finishing. Steers were diagnosed as morbid based on subjective visual appraisal by feedlot staff. Upon arrival, all steers were administered a growth-promoting implant and preventive pharmaceuticals based on the judgment of feedlot management at receiving. Steers were housed in pens of varying sizes, but all pens allowed more than 9.3 m^2/hd and 40 cm/hd linear bunk space.

Steers were processed for secondary application of growth-promoting implants in late January or early February, thus days on feed (**DOF**) to secondary processing date ranged from 74 to 94. At that time, steers were weighed (interim BW) and individually assigned to marketing groups using the ultrasound technology and computer software of the Cattle Performance Enhancement Co. (CPEC, Oakley, KS). Once the optimum market date for each steer was estimated, steers were assigned to marketing groups harvested between March and early July. Cattle were harvested at a commercial facility (National Packing Co., Liberal, KS). Hot carcass weight (**HCW**) was collected at slaughter, and longissimus muscle area, fat thickness, calculated yield grade, and marbling score were evaluated by an independent data collection service (Cattle Trail LLC, Johnson, KS) following chilling.

At the completion of finishing, steers were sold on an individual carcass basis through the National Beef Grid. Premiums and discounts were applied using HCW and USDA quality and yield grade.

Statistical Analysis. The effect of backgrounding system on performance, carcass, and financial data was evaluated using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with pen/pasture as the experimental unit. The model included replicate, year, and treatment. Chi-square in the FREQ procedure of SAS (SAS Inst. Inc., Cary, NC) was utilized to evaluate the categorical distribution of USDA quality grade and yield grade, morbidity, and death loss.

Results and Discussion

Backgrounding Phase. There were no differences ($P \geq 0.56$) in weaning BW, price, or value between PAST and DLOT (Table 2). Between d 0 and interim backgrounding BW, PAST had higher ADG ($P < 0.01$). This likely occurred because PAST calves experienced less environmental and nutritional change following weaning. From interim BW to the end of backgrounding, DLOT calves had greater ADG ($P < 0.01$), which was expected because the preconditioning ration provided DLOT calves a higher plane of nutrition than pasture forage supplied the PAST calves. Overall, DLOT calves had greater ADG ($P < 0.01$) during backgrounding, resulting in heavier final backgrounding BW ($P = 0.03$) for DLOT calves.

Final backgrounding price was higher for the PAST calves ($P = 0.04$) because they were lighter, but the final value was \$6.90/hd less ($P = 0.03$) for PAST than DLOT calves. The higher value of DLOT calves was offset by a

$52.76 difference ($P < 0.01$) in total costs. Feed and labor cost were 5-fold and 2-fold greater ($P < 0.01$), respectively, for DLOT than PAST. Consequently, net income during backgrounding was $44.59 greater ($P < 0.01$) for PAST calves. These results support the findings of St. Louis et al. (2003) that showed lower feed cost and greater net return ($43.17) for a 30-day ryegrass pasture backgrounding program compared to a 30-day drylot backgrounding program. A final price premium of $5.00/45.4 kg would have been required for the DLOT system to be profitable; however, the PAST backgrounding system was profitable without a premium.

Finishing Phase. Initial BW, price, and value of DLOT and PAST steers were similar ($P \geq 0.37$; Table 3), even though BW of steers and heifers combined collected at the end of backgrounding was different. The PAST steers had greater ADG ($P < 0.01$) through interim BW, but subsequent ADG was similar ($P = 0.68$). Higher ADG to interim BW among PAST steers supports the findings of Choat et al. (2003) who reported greater feedlot ADG from 15 to 70 DOF among steers previously wintered on native range with supplement compared to contemporary steers wintered on irrigated wheat pasture that entered the feedlot heavier. However, there were no differences ($P \geq 0.12$) in interim BW, total ADG, estimated final BW, DOF, calculated yield grade, or any measured carcass characteristics in this study. Additionally, the distributions of USDA quality and yield grade were similar ($P \geq 0.30$; data not shown).

The proportion of steers treated for sickness and medicine cost/hd were similar ($P \geq 0.14$); however, DLOT steers had greater death loss ($P = 0.02$). Even though morbidity was not different, the 7.6 percentage unit difference in death loss (all due to BRD complex) indicates that DLOT steers likely experienced some degree of suppressed immune function as compared to PAST steers.

The DLOT steers had lower feed cost ($P = 0.04$), but average price received for carcasses sold was not different ($P = 0.11$). Gross income was $111 greater ($P < 0.01$) for PAST steers because they had no mortalities and numerically higher carcass weight and carcass price. Consequently, PAST steers garnered $103/hd more net income ($P < 0.01$) than DLOT steers. To achieve the same finishing phase net income for DLOT and PAST steers, price of DLOT steers at the beginning of the finishing phase would need to be reduced by $17/45.4 kg.

Implications

Backgrounding programs that conform to "VAC-45" marketing requirements can vary in intensity and cost. However, the additional gain achieved with higher-input systems may not offset higher costs; and stress associated with dietary change and confinement immediately following weaning may impact subsequent death loss. Low-input pasture backgrounding systems can be more profitable than drylot systems of the same duration during the backgrounding and finishing phases.

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Table 1. Drylot backgrounding pellet[1]

Ingredient	% of Diet (as-fed)
Corn, ground	34.7
Wheat middlings	32.0
Soybean hulls	15.0
Cottonseed meal	5.8
Cottonseed hulls	5.0
Molasses	5.0
Calcium carbonate	1.5
Potassium Chloride	0.5
Salt, vitamins, trace minerals[a]	0.5
DM analysis	
CP, %	15.8
NE_m, Mcal/kg	1.83
NE_g, Mcal/kg	1.10

[a]Includes Rumensin-80 at 0.0125 %.

TABLE 2. Backgrounding performance and profitability of mixed steers and heifers backgrounded in a drylot or pasture

Item	Backgrounding System		SE	*P*
	Drylot	Pasture		
Number of head	125	125		
Performance[1]				
Weaning BW, kg	235	236	1.1	0.56
Interim BW, kg[2]	244	251	1.3	<0.01
Final BW, kg[3]	263	257	1.3	0.03
ADG, d 0 to Interim, kg/d	0.43	0.71	0.03	<0.01
ADG, Interim to Final kg/d	0.84	0.30	0.04	<0.01
Total ADG, kg/d	0.64	0.50	0.02	<0.01
Financial				
Weaning Price, $/45.4 kg	109.31	109.08	0.34	0.65
Weaning Value, $	564.88	566.16	1.96	0.66
Final Price, $/45.4 kg	104.39	105.35	0.27	0.04
Final Value, $	602.79	595.89	1.72	0.03
Feed Cost, $	60.84	11.91	0.09	<0.01
Drylot Pellet	54.52	0		---
Hay[4]	6.31	0.61		---
Range Cube[5]	0	5.55		---
Grazing Fee[6]	0	5.70		---
Labor[7]	5.93	2.10	0.04	<0.01
Total Cost, $	66.77	14.01	0.10	<0.01
Net Income, $	(28.87)	15.72	1.71	<0.01

[1]A 4% pencil shrink was applied to all BW.
[2]Interim BW collected d 21 in yr 1 and 3, and d 19 in yr 2.
[3]Final BW collected on d 45, 44, and 42 during yr 1, 2, and 3, respectively.
[4]Hay fed to PAST steers during initial week to train steers to range cubes.
[5]Range cubes (32% CP) provided to PAST steers at 0.57 kg/d; delivered 3×/wk.
[6]Grazing fee charged to PAST steers $\$0.132 \cdot hd^{-1} \cdot d^{-1}$.
[7]Labor cost based on $6.00/h.

TABLE 3. Feedlot performance, carcass characteristics, and profitability of steers backgrounded in a drylot or pasture

Item	Backgrounding System		SE	*P*
	Drylot	Pasture		
Number of steers	66	67		
Performance				
Initial BW, kg[1]	274	270	1.7	0.27
Interim BW, kg[2]	361	363	3.1	0.78
Final BW, kg[3]	493	502	6.2	0.34
Days on Feed	168	173	2.9	0.26
ADG, d 0 to Interim, kg/d	1.08	1.27	0.03	<0.01
ADG, Interim to Harvest, kg/d	1.50	1.51	0.02	0.68
Total ADG, kg/d	1.33	1.35	0.01	0.32
% Treated for sickness[4]	47.6	34.3		0.14
% Death loss[4]	7.6	0.0		0.02
Carcass				
Hot Carcass Weight, kg	309.9	316.6	4.1	0.29
Fat Thickness, cm	1.35	1.39	0.04	0.59
Longissimus Area, cm^2	80.8	79.6	1.3	0.54
Marbling Score[5]	472	481	6.5	0.35
Calculated Yield Grade	2.90	3.06	0.06	0.12
Financial				
Initial Price, $/45.4 kg[1]	105.94	106.65	0.53	0.38
Initial Value, $	636.36	631.78	3.02	0.32
Medicine cost, $	23.32	23.01	3.54	0.33
Feed Cost, $	220.53	238.18	4.68	0.04
Total Cost, $	932.60	941.40	7.90	0.46
Carcass Price, $/45.4 kg	133.48	135.67	0.82	0.11
Gross Income, $	834.27	945.64	15.54	<0.01
Net Income, $	(98.33)	4.68	10.64	<0.01

[1]Initial BW and Price = Final backgrounding BW and Price of steers; 4% pencil shrink applied to BW.
[2]Interim BW occurred at 74, 77, and 94 DOF during yr 1, 2, and 3, respectively; 4% pencil shrink applied.
[3]Final BW is an estimate calculated as carcass weight ÷ average dressing % of marketing group.
[4]Chi-square analysis.
[5]Marbling score: Small 00 = 500.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

RELATIONSHIP OF DAM'S BODY WEIGHT, MILK COMPONENTS, AND MILK ENERGY DENSITY TO REINDEER CALF GROWTH RATE

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ABSTRACT: Reindeer are an important livestock species in Alaska, although the level of reproductive management in reindeer herds is currently low. None-the-less, rapid genetic improvements can be achieved through selective application of AI. Current efforts to develop AI in reindeer require identification of desirable traits worthy of selection (e.g., rapid calf growth rate). Objectives of this trial looked at the relationship between dam BW at parturition, calf birth weight and growth rate (measured as ADG) and the associations between reindeer milk components and calf ADG. Twelve reindeer cows with calves were grouped according to whether the calves were born early (EC; n=6) or late (LC; n=6) in the calving season. Calving dates ranged from April 6 to 11 for EC and from April 23 to 30 for LC. Milk samples were collected from cows at 10, 40 and 70 days-in-milk (**DIM**). Calf BW was recorded coincident with milk sampling and ADG was calculated for each period and for the entire 70d study. Milk samples were sent to Rocky Mountain DHIA (Logan, UT) for component analysis. Milk energy density was estimated and correlation coefficients were calculated for ADG with dam BW at parturition, milk components and milk energy. Calf ADG did not differ between groups and averaged 0.66 ± 0.06 kg (range 0.53 to 0.89 kg). Dam BW correlated with ADG at 70 DIM (r=.57; p=.04). In the EC group, ADG was correlated with milk energy (r=.92; p=0.01), %fat (r=.91; p=0.01) and %total milk solids (r=.93; p=0.001) at 10 DIM. Milk components appear to be more important to EC calves than LC calves in early life. Reindeer calves are precocious and nibble grass soon after birth. From this study, it appears that milk components play a larger role in early born calves when snow cover prevents access to grass.

[1]Key Words: Reindeer Milk, Calf Growth

Introduction

The history of reindeer domestication is nearly as long as that of cattle and sheep (Diamond, 1997). Reindeer have been an important livestock species in Alaska since their importation during the last decade of the 19th century. They have been raised in extensive free-ranging systems on the Alaskan Seward Peninsula for over 100 years, providing a renewable source of subsistence meat and hides for native Alaskans.

In light of their tractable nature, reindeer are readily adaptable to intensive farming using traditional agricultural techniques. This characteristic has fostered the development of a small reindeer industry along the road system, resulting in improved quality and accessibility of reindeer meat as well as the ability to tap into agrotourism. However, a much greater in-depth knowledge of reindeer husbandry and reproductive management is necessary if intensive farming is to succeed. Even though reindeer are tractable, rutting reindeer bulls remain aggressive, dangerous to handle, destructive to facilities and potentially dangerous to herd-mates (Blake et al, 2007). In addition, seasonal rutting activity takes a serious toll on the animal's condition, depleting as much as 35% of their mass (Barboza et al., 2004). Maintaining adequate male stock behind a fence currently requires a disproportionate amount of capital investment and a lot of skill to bring the animals through the highly vulnerable post-rut phase. Collectively, these factors make reindeer bulls costly, challenging to manage in traditional agricultural settings, and poor candidates for agrotourism.

The use of AI reduces the need to maintain as large a stock of breeding males, in addition to offering the opportunity to promote rapid genetic selection of desirable characteristics. However, we first need to identify traits for selection. The objectives of this trial were to relate dam BW at parturition to calf ADG and to examine associations between reindeer milk components, estimated milk energy and calf growth rate.

Materials and Methods

Animals and Breeding Management

The University of Alaska Fairbanks (**UAF**) Institutional Animal Care and Use Committee approved all experimental protocols associated with this study (protocol #05-41). Twelve halter trained female reindeer from the R.G. White Large Animal Research Station,

This research was funded by USDA and the Alaska Agricultural and Forestry Experiment Station, paper # 2007-004. Corresponding author - M.P. Shipka at ffmps@uaf.edu.

Institute of Arctic Biology, UAF (64°50' N), were divided into 2 groups based on calving date. Females calving within the first 2 wk of April were placed in the early calving group (**EC**: n=6). Those calving within the last 2 wk of April were placed in the late calving group (**LC**: n=6).

Sample Collection and Analysis

Dam BW and calf birth weight were collected within 24 hours of parturition and following milk sample collection at 10, 40, and 70 days-in-milk (**DIM**). Reindeer were milked by hand with the female halter tied to a corral wall. A minimum of 15 mL milk was collected into 30 ml vials containing a preservative supplied by Rocky Mountain DHIA (RM DHIA). Samples were immediately placed in a cooler, refrigerated within 45 min of collection and express shipped to the RM DHIA Laboratory (Logan, UT) within 48 hrs of collection. The milk samples were immediately analyzed for fat, protein, lactose and solids-not-fat (SNF). Total milk solids were calculated by the addition of SNF + fat. One calf (weak from birth) was euthanized at 27d of age and this cow-calf pair eliminated from the study.

Milk Energy Calculation and Statistical Analysis

Energy content per unit of milk was calculated using 2 formulae. The first was validated for bovine (Tyrrell and Reid, 1965) where energy (kcal/lb) = 41.84(%fat) + 22.29(%SNF) – 25.58. The second formula was validated for cervine (red deer, Landete-Castillejos et al., 2003) where energy (cal/g) = 0.345 + 8.339(fat) + 5.407(protein). Comparison of the 2 methods produced virtually the same milk energy estimate per unit volume (r=.99), therefore, energy estimates derived from red deer were used for further comparison.

Calf ADG was calculated and Pearson Product Moment correlation coefficients were determined using Sigma Stat (Systat Software Inc., Point Richmond, CA.) Correlations were determined for ADG vs %fat, ADG vs %protein, ADG vs %lactose, ADG vs %SNF, ADG vs %total milk solids, and ADG vs calculated milk energy for EC and LC groups at each sample period (10, 40, and 70 DIM).

Results and Discussion

Median calving date for the EC group was April 9 (range: April 6 to 11) and for the LC group was April 29 (range: April 23 to 30). Despite the 20 day difference in calving date, there was no difference in calf birth weight (***P*** = 0.83) or sex ratio. The ratio of Males:Females was 2:4 in both groups (Table 1). Calf ADG did not differ between groups and averaged 0.66 ± 0.06 kg (range 0.53 to 0.89 kg) overall (Table 1), however, the ADG of the two groups did differ at earlier sample periods (Figure 1). Dam BW was not significantly correlated with calf ADG at 10 (r = -0.22) or 40 (r = 0.11) DIM but was correlated with ADG at 70 DIM (r=.57; p=.04). Mean milk component percentages for samples collected at 10, 40, and 70 DIM for both groups are presented in Table 2.

Several studies have examined the change in reindeer milk components and composition during lactation under a variety of conditions in reindeer (Gjostein et al., 2004; Holand et al., 2002; Jacobsen et al., 1981; Luick et al., 1974) and other cervidae (Landete-Castillejos et al, 2004; Landete-Castillejos et al, 2000). Changes in milk composition over the first 70 DIM in this study are

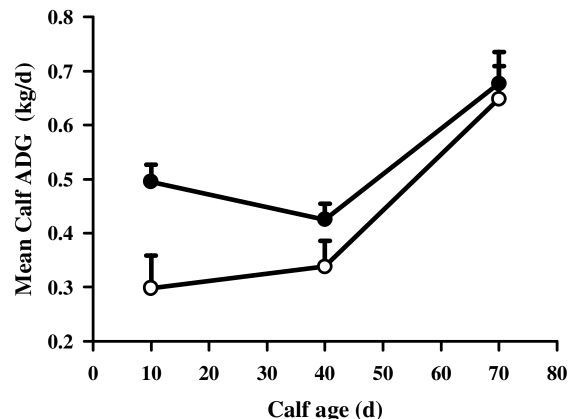

Figure 1. Mean average daily gain (ADG) for reindeer calves born early (EC ●) or late (LC ○) in April.

similar to previous reports. Milk fat, protein, solids-not-fat and total milk solids tended to increase while lactose remained level or fell slightly over the course of the study period. To the best of our knowledge, this is the first study to examine the association between reindeer milk components and calf ADG over the first 70 DIM. The only significant, positive correlations were found between calf ADG at 10 DIM and %milk fat (r = 0.91; p = 0.01) and %total milk solids (r = 0.93; p = 0.001) for EC cows only (Table 3). Estimated milk energy at 10 DIM was also significantly correlated with calf ADG (r = 0.91; p = 0.01) in the EC group. Very young reindeer calves will nibble grass shortly after birth (pers. comm. Kawerak Reindeer Herders and Ginny Kratzer, Four Tracks Reindeer Farm). However, in this study the EC calves were born in pastures completely covered with snow. By the time the LC calves were born new grass was emerging as the snow melted in late April. The ready availability of new grass coupled with the precocious nature of reindeer in general may have provided a selective advantage to calves in the LC group. The EC calves, born 10 to 24 d earlier, had no access to new grass and were completely dependent on their dam's milk for nourishment.

Among the LC calves, ADG at 70 DIM was negatively correlated with %total milk solids (r = -0.87; p = 0.05) and estimated milk energy (r = -0.85; p = 0.07). When both groups of calves were considered together, ADG at 70 DIM was negatively correlated with %protein (r = -0.60; p = 0.05). These negative associations are a bit more difficult to understand. One possible explanation may be that larger calves are suckling reindeer cows other

than their dams. Such activity has been observed on numerous occasions in cow-calf pens during late lactation (pers. obs.). We have no data quantifying the impact of suckling from multiple cows or cows other than their dam. Nevertheless, if older calves spent less time suckling their own dam, she would be in the process of drying off, a time potentially associated with changes in milk composition that wouldn't be expected to affect calf growth.

Information from this study indicates that milk components are more important to early born reindeer calves compared to reindeer calves born later in the calving season. Reindeer calves are precocious and nibble grass soon after birth. From this study, it appears that milk components play a big role in neonatal calves when snow cover prevents access to grass.

Acknowledgments

The authors wish to thank the staff at the R.G. White Large Animal Research Station for the able assistance in reindeer handling and milking. Special thanks to Jason Jack, Bill Hauer, Chris Terzi and Don Harbauer.

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Table 1. Dam BW (kg) at calving and calf birth weight (kg), calf sex and calf ADG at 10, 40, and 70 days-in-milk (DIM) for reindeer calves grouped into early born (EC) and late born (LC) categories

Group	Dam BW at Calving (kg)	Calf Sex	Calving Date	Calf Birth Wt (kg)	ADG (kg/d)		
					10 DIM	40 DIM	70 DIM
EC	162.73	M	6-Apr	7.39	0.56	0.53	0.89
	118.64	F	7-Apr	7.27	0.45	0.43	0.58
	105.00	F	11-Apr	7.27	0.52	0.42	0.59
	104.55	F	8-Apr	7.16	0.42	0.41	0.66
	113.64	M	10-Apr	7.05	0.60	0.45	0.81
	116.36	F	11-Apr	5.23	0.42	0.31	0.53
Mean EC	120.15	2M:4F		6.89	0.50	0.43	0.68
LC	131.82	F	30-Apr	6.82	0.30	0.25	0.57
	125.45	F	29-Apr	7.39			
	125.00	F	29-Apr	7.05	0.41	0.39	0.66
	136.36	M	25-Apr	6.59	0.38	0.49	0.88
	140.45	M	29-Apr	7.16	0.07	0.23	0.58
	120.00	F	23-Apr	6.82	0.33	0.33	0.55
Mean LC	129.85	2M:4F		6.97	0.30	0.34	0.65
Overall	124.63	4M:8F		6.93	0.41	0.39	0.66

Table 2. Mean values (%) for reindeer milk components at 3 stages of lactation (10, 40, and 70 DIM) for 2 groups of reindeer cows that calved early (EC) or late (LC)

DIM	Group	%Fat	%Protein	%Lactose	%SNF[a]	%TS[b]
10	EC	11.5 ± 0.6	7.0 ± 0.2	4.7 ± 0.1	13.0 ± 0.2	24.5 ± 0.5
	LC	12.6 ± 0.9	7.1 ± 0.3	4.4 ± 0.1	12.7 ± 0.3	25.3 ± 0.9
40	EC	12.6 ± 0.4	7.7 ± 0.1	4.2 ± 0.1	13.0 ± 0.2	25.6 ± 0.4
	LC	13.9 ± 0.6	7.6 ± 0.2	4.2 ± 0.1	13.1 ± 0.2	27.0 ± 0.6
70	EC	14.4 ± 0.8	8.3 ± 0.2	3.7 ± 0.1	13.2 ± 0.3	27.6 ± 0.7
	LC	13.3 ± 1.2	8.4 ± 0.3	3.9 ± 0.1	13.4 ± 0.2	26.7 ± 1.3

[a]Solids-not-fat
[c]Total milk solids

Table 3. Pearson Product Moment correlation coefficients and ***P***-values for correlations between calf ADG and milk components at 3 stages of lactation (10, 40, and 70 DIM) for reindeer calves grouped into early born (EC) and late born (LC) categories[a]

DIM	Group	%Fat		%Protein		%Lactose		%SNF[b]		%TS[c]	
		r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
10	EC	**0.91**	**0.013**	-0.00	0.10	-0.26	0.626	-0.24	0.643	**0.93**	**0.001**
	LC	0.57	0.319	-0.41	0.496	0.25	0.686	-0.41	0.496	0.36	0.548
40	EC	-0.38	0.457	-0.59	0.223	-0.15	0.782	-0.49	0.326	-0.56	0.245
	LC	0.22	0.725	-0.83	0.085	0.44	0.458	-0.85	0.072	0.05	0.933
70	EC	-0.12	0.817	-0.39	0.441	0.22	0.671	-0.16	0.768	-0.209	0.692
	LC	-0.83	0.083	-0.79	0.112	0.57	0.321	-0.76	0.133	-0.87	0.053

[a] Significant correlations have been bolded
[b] Solids-not-fat
[c] Total milk solids

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

IMPACT OF EQUINE ANTHELMINTICS ON DUNG BEETLE REPRODUCTION

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ABSTRACT: Dung beetles, belonging to the family Scarabaeidae, refer to those beetles which feed on the feces of animals thus benefiting the environment by increasing water infiltration, removing the breeding habitat for pests, and improving nutrient recycling. The objective of this study was to evaluate toxicity of the equine anthelmintics, ivermectin versus moxidectin, on dung beetle reproduction. Manure was collected from a control group consisting of six horses, unexposed to any equine anthelmintics for a period of ninety days. The horses were randomly divided into two groups and dewormed with IVOMEC™ (ivermectin) or QUEST™ (moxidectin). Following treatment, fresh manure was collected on days 1, 7, 14, and 21 from the two groups. Adult dung beetles, *Onthophagus gazella*, were separated into thirty different containers and fed treated or untreated manure according to the container they were restricted to. Containers contained a mixture of soil and four to five breeding pairs of adult dung beetles. After a ninety-day period, offspring were collected and counted to determine the effects of the equine anthelmintics on dung beetle reproduction. An analysis of variance was performed using a general linear model to show the survivability of dung beetle offspring and produced the following results. On day 1, IVOMEC and QUEST had low survivability compared to the Control ($P<0.05$). On days 7 and 14 IVOMEC had low survivability compared to QUEST and Control ($P<0.05$), but QUEST and Control did not differ ($P>0.05$). On day 21, there was no dung beetle survivability difference between IVOMEC, QUEST, and Control ($P>0.05$). These results indicated that the equine anthelmintic, moxidectin, was less toxic than ivermectin on dung beetle reproduction.

Key Words: Anthelmintic, Equine, Dung Beetle, Onthophagus gazella spp.

Introduction

Dung beetles occur on every continent except for Antarctica. There are 4,500 species worldwide with the majority of them found in Africa where they evolved with herbivores such as buffalo and elephants. There are over 90 species of dung beetles in North America with varying importance as to their ability to bury dung. It has been reported that one *Onthophagus gazella* beetle can consume and bury over 6.8 kg of manure during its two-month lifespan. By decomposing dung, dung beetles remove non-point source pollution, which reduces pasture fouling and nutrient runoff into waterways. The physical burying of dung aerates the soil allowing for increased water infiltration. Each extra inch of water absorbed adds 27,255 gallons per acre of water to the soil, reducing both flooding and drought. Dung beetles are beneficial to pasture and range management through their removal of breeding habitats for pests, such as flies and parasitic worms. By burying and consuming dung, the dung beetle improves nutrient recycling by exposing the dung to soil microbes and earthworms.

Upon the introduction of non-native domesticated herbivores, onto continents such as the America's, parasite and fly populations were suppressed using chemical intervention. Pharmaceutical companies developed anthelmintics for livestock, of which some were found to be toxic to dung beetle larvae, thus reducing and/or altering dung beetle populations. One chemical, ivermectin, known to be toxic to dung beetle larvae is in the Avermectin family of drugs and is marketed for horses under the trade names of ZIMECTRIN and IVOMEC or EQVALAN. Ongoing research is being conducted for effective athelmintics, which do not have such grave consequences to the life cycle of the dung beetle. One such available equine anthelmintic is moxidectin, commercially available as QUEST.

The objective of the present study was to evaluate the impact of the two equine anthelmintics, IVOMEC and QUEST, on the survivability of dung beetle larvae. The dung beetle species chosen for the experiment was *Onthophagus gazella***.**

Materials and Methods

For the purpose of investigating the effects of equine anthelmintics on dung beetle reproduction, fresh manure was collected from three groups of horses over a period of twenty-one days. Group One, the Control group, consisted of six horses unexposed to any

anthelmintics for a minimum of ninety days. Group Two, the IVOMEC group, consisted of three horses unexposed to any anthelmintics for ninety days and then administered IVOMEC paste on Day One of the experiment. Group Three, the QUEST group, consisted of three horses unexposed to any anthelmintics for ninety days and then administered QUEST gel on Day One of the experiment. Manure was collected on Day 1, 7, 14, and 21 for the IVOMEC and QUEST groups. Collected manure samples were stored in 1-gallon plastic freezer bags and weighed approximately 2, 250 grams. The bags were labeled according to sample type and day of collection. A total of six bags would be collected for the Control group and three bags each for the IVOMEC and QUEST groups on days 1, 7, 14, and 21. The bags of manure were placed in a freezer, as they were collected.

Thirty 5-gallon containers were used to house the dung beetles and contained a mixture of two parts topsoil, one part sand, and one part peat moss. The soil mixture was kept moist by adding distilled water. To ease the collection of the dung beetles at the end of the experiment, a plastic cup covered with saran wrap was imbedded in the soil until the top of the cup was level with the soil mixture in the containers. Six containers were labeled as the Control group and three containers each for each treatment and day. The containers were uniformly arranged in a temperature and light controlled room with an average temperature of 78 degrees and a set amount of light for 14 hours on and 10 hours off.

The species of dung beetle used in the experiment, *Onthophagus gazella*, were easily sexed by the presence (males) or absence (females) of horns on the head. The dung beetles were evenly distributed into the thirty different containers. Each container contained 150 grams of manure and 60 grams of distilled water. Every four days the manure was removed and weighed and exchanged with fresh manure. After a period of fifty-two days, the adult dung beetles were collected into the plastic cups and disposed of according to protocol. The offspring were fed for an additional four weeks to allow adequate growth for observation and records.

The recorded data was subject to two Analyses of Variance (ANOVA) using a General Linear Model (GLM). For the first ANOVA, factors that were modeled included day (1,7,14,21), treatment (IVOMEC, QUEST, Control), and the interaction between treatment and day. The second analysis performed was an ANOVA using a GLM for the duration of the study (Day 0-21) modeling the treatment (IVOMEC, QUEST, Control).

Results and Discussion

The study set out to test the validity of the claims made by the equine anthelmintic manufacturers. The expected results were that anthelmintics in the avermectin family, such as IVOMEC, would be toxic to dung beetle larvae throughout the course of the experiment and that anthelmintics in the moxidectin family, such as QUEST would not be toxic to dung beetle larvae. The results of the experiment found that IVOMEC residues in dung were toxic to dung beetle larvae on Day 1, Day 7, and Day 14 after administration of the equine anthelmintic. IVOMEC was shown to be non-toxic to dung beetle larvae on Day 21 after administration. The results concluded that residues following QUEST administration were as toxic to dung beetle larvae as IVOMEC residues on Day 1 of administration. Day 7, Day 14, and Day 21 showed positive results for dung beetle reproduction and survivability following treatment with QUEST. An ANOVA was performed using a GLM modeling the interaction between each treatment (*C*=Control, *I*=IVOMEC, *Q*=QUEST) and corresponding day. For the following data refer to Table 1.

Day 1: The interaction plot calculated a P-value of P=0.001 indicating strong evidence that there was a difference in at least two of the comparing treatments.

$(\mu_I - \mu_C) < 0,\ \mu_I < \mu_C\ \ 4.5 \pm 2.75$

The margin of error was 2.75. The greater the margin of error, the greater the variability between the recorded number of offspring in each group (same treatment and day).

$(\mu_Q - \mu_C) < 0,\ \mu_Q < \mu_C\ \ 4.5 \pm 2.75$

$(\mu_Q - \mu_I) = 0,\ \mu_Q = \mu_I\ \ 0.0 \pm 3.18$

The results for Day 1 indicate that the offspring collected were greater for the Control group than for both the QUEST and IVOMEC groups.

Day 7: P=0.012.

$(\mu_I - \mu_C) < 0,\ \mu_I < \mu_C\ \ 4.5 \pm 3.58$

$(\mu_Q - \mu_C) > 0,\ \mu_Q > \mu_C\ \ 0.5 \pm 2.58$

$(\mu_Q - \mu_I) > 0,\ \mu_Q > \mu_I\ \ 5.0 \pm 4.13$

The results for Day 7 indicate that the offspring collected were greater for the Control and QUEST group than the IVOMEC group.

Day 14: P=0.003

$(\mu_I - \mu_C) < 0,\ \mu_I < \mu_C\ \ 4.83 \pm 2.86$

$(\mu_Q - \mu_C) = 0,\ \mu_Q = \mu_C\ \ 0.175 \pm 2.52$

$(\mu_Q - \mu_I) > 0,\ \mu_Q > \mu_I\ \ 4.67 \pm 3.30$

The results for Day 14 indicate that the offspring collected were greater for the Control and QUEST group than the IVOMEC group.

Day 21: P=0.367 The large P-value indicates that a difference cannot be concluded in at least two of the comparing treatments.

$(\mu_I - \mu_C) < 0,\ \mu_I < \mu_C\ \ 1.83 \pm 5.92$

$(\mu_Q - \mu_C) > 0,\ \mu_Q > \mu_C\ \ 1.83 \pm 5.92$

$(\mu_Q - \mu_I) > 0,\ \mu_Q > \mu_I\ \ 3.67 \pm 6.83$

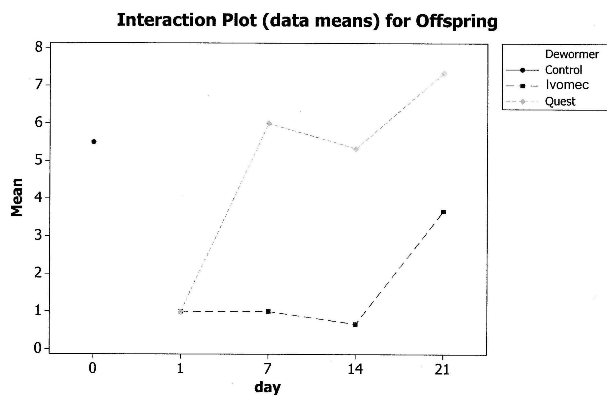
The results for Day 21 indicate that there is no statistical difference between the numbers of offspring collected.

Table 1. Comparison for each day modeling treatment.

Treatment	Day 1	Day 7	Day 14	Day 21
Control	5.5[a]	5.5[a]	5.5[a]	5.5[a]
IVOMEC	1.0[b]	1.0[b]	1.0[b]	3.5[a]
QUEST	1.0[b]	6.0[a]	5.5[a]	7.5[a]

[a, b,] Different letters in rows indicate significant difference (P<.05).

The second analysis performed was an ANOVA using a GLM for the duration of the study (Day 0-21) modeling each treatment (IVOMEC, QUEST, Control). Refer to the interaction plot below.



Implications

The experiment showed that some equine anthelmintics for horses are safer to dung beetle larvae than others. These results could be used to educate the broader community about dung beetles and potentially redistribute or introduce dung beetles to areas identified as requiring population enrichment. The numerous benefits that dung beetles provide to the ecosystem have been dramatically reduced by the use of anthelmintics in livestock. These benefits, such as healthy soil and water quality are the cornerstones of diversity and health for plants, animals, and ultimately our survival.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

BEEF COW PERFORMANCE IN RESPONSE TO EARLY THROUGH MID-GESTATIONAL NUTRIENT RESTRICTION AND PROVISION OF SUPPLEMENTARY RUMINALLY UNDEGRADABLE PROTEIN

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ABSTRACT: Twelve triparous and 24 diparous cows (500 ± 7.6 kg initial BW) were individually fed native grass hay plus 1 of 3 supplements from d 45 through d 185 of gestation to evaluate effects of gestational dietary treatment on BW, BCS, and ultrasonographic LM area, fat within the LM, and fat depth over the 12th rib. Dietary treatments were native grass hay plus a soybean meal-based supplement formulated to achieve 0.51 kg/d of BW gain (C), 70% of NE_m provided by C (NR), and 70% of NE_m provided by C plus a ruminally undegradable protein (RUP) supplement (6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fishmeal; DM basis) designed to provide duodenal essential AA flow equal to that of cattle fed C (NRP). Data were analyzed as a split-plot in a randomized complete block (parity) design. Dietary treatment x d of gestation interactions were noted ($P < 0.05$) for all variables except for fat depth over the 12th rib. Cows fed C had significantly greater BW, BCS, and LM area than NR from d 73 through 185 (final BW = 584 vs. 521 kg). Body weight and LM area of NRP cows were intermediate until d 115; NRP cows had significantly greater BW and LM area than NR cows thereafter, but BW and LM area of NRP cows did not differ from C cows throughout the experiment. Body condition score of NRP cows was similar to both C and NR throughout the experiment. Fat within the LM was greatest for C on d 157; fat within the LM was similar among dietary treatments at all other collection dates. Average DMI by NR was 3.1 kg/d less than C cows, and NRP cows consumed 2 kg/d less DM than cows fed C. Differences in beef cow performance during early to mid-gestation were attributable to differences in plane of nutrition; however, cows fed a RUP supplement designed to balance intestinal supply of essential AA were able to withstand this period of nutrient restriction. Provision of supplemental protein balanced for intestinal supply of essential AA may be an effective nutritional management strategy to increase production efficiency of pregnant beef cows consuming limited amounts of forage.

Key Words: beef cows, nutrient restriction, supplementation

Introduction

Drought is a recurring meteorological phenomenon in Wyoming and surrounding areas (NWS, 1988-1989; USGS, 2004), which results in arid and semi-arid conditions, causing a significant reduction in rangeland forage production (Derner et al., 2007). Furthermore, occurrence of precipitation early in the growing season followed by dry conditions can cause a rapid decline in forage quality by advancing phenological maturity of rangeland forage plants (Ganskopp and Bohnert, 2001). Spring-calving beef cows grazing rangelands affected by those conditions are likely to experience nutrient restriction during early to mid-gestation. Feed intake restriction is often practiced in experimental settings to emulate nutrient restriction that may occur in production settings (Dunn and Moss, 1992). As an initial step toward separating physiological effects of protein from energy in ruminants, Scholljegerdes et al. (2005a) used a ruminally undegradable protein (**RUP**) supplement consisting of 6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fishmeal (DM basis) to balance intestinal supply of essential AA in cattle consuming restricted quantities of forage. However, production responses by pregnant beef cows fed restricted amounts of forage plus the aforementioned RUP supplement have not been determined. Our objectives were to evaluate effects of gestational plane of nutrition and RUP supplementation on BW, BCS, and ultrasonographic measurements of LM area, intramuscular fat within the LM, and fat depth over the 12th rib.

Materials and Methods

General

The University of Wyoming Animal Care and Use Committee approved all procedures for the following study. All 3- and 4-yr old, lactating Angus x Gelbvieh cows from the University of Wyoming beef herd were estrous synchronized to be bred via AI on June 2, 2006. Cow-calf pairs were then allowed to graze a 1,800 ha pasture on the University of Wyoming's McGuire Ranch located approximately 56 km northeast of Laramie at an elevation of 2,203 m. Dominant native forage species within the pasture (Weston et al., 2005) include Sandberg's bluegrass (*Poa secunda*), western wheatgrass (*Pascopyrum smithii*), and prairie junegrass (*Koeleria pyramidata*). On d 33 of gestation, cows were separated from their calves, then cows were evaluated for pregnancy via palpation per rectum by a licensed veterinarian using ultrasound technology. Calves were permanently separated from 50 of the cows (18 triparous and 32 diparous) that were diagnosed pregnant. Forty-two (18 triparous and and 24 diparous) of the most uniform cows were then transported to the University of Wyoming Livestock Center located in Laramie, WY. Cows were pen-fed (6 cows/pen) native grass hay (6.2% CP, DM basis) plus supplemental protein to provide a diet with 10% CP until initiation of experimental diets. Cows were reconfirmed pregnant by a licensed veterinarian on d 40 of

gestation. On d 45 of gestation, 36 of most uniform cows (12 triparous and 24 diparous) were then selected to be individually fed native grass hay plus 1 of 3 supplements from d 45 through 185 of gestation.

Dietary Treatments

The control (**C**) diet consisted of native grass hay plus a soybean meal-based supplement formulated for pregnant replacement heifers (590 kg mature BW) to achieve 0.43 kg/d of BW gain (NRC, 2000), which we estimated would be comparable to daily gain of 0.51 kg/d of BW gain for non-lactating cows pregnant with their second and third calf. The other dietary treatments were 70% of NE_m provided by C (**NR**), and 70% of NE_m provided by C plus a RUP supplement (6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fish meal; DM basis; Scholljegerdes et al., 2005a) designed to provide duodenal essential AA flow equal to that of cattle fed C (**NRP**). Basal flows of total essential AA were predicted for each treatment using the equation reported by Scholljegerdes et al. (2004; total essential AA flow to the small intestine, g/d = [0.055 × g of OM intake] + 1.546). The quantity of RUP supplement delivered was adjusted for more extensive ruminal degradation of RUP supplement in cattle fed restricted amounts of forage (Scholljegerdes et al., 2005b). Using actual mineral content of each dietary ingredient, the C supplement was fortified to ensure that cows fed C would consume the same amount of mineral per unit BW as cows fed the RUP supplement.

The feeding protocol followed procedures outlined by Whitney et al. (2000) as modified by Lake et al. (2005). Briefly, supplement was offered daily at 0600 and 1600 in equal allotments. Supplement was always consumed within 20 min, after which one half of the daily hay allotment was offered. Cows were allowed to consume hay for the remainder of each 2-h feeding period. Refusal of hay was seldom more than 1 kg by a single cow at any given feeding time. Feed offered daily was adjusted for biweekly changes in BW and increased NE_m requirements as gestation proceeded.

Sampling and Laboratory Analyses

Cow BW was the average of 2 consecutive-d BW recorded every 14 d from d 45 to 185 of gestaion. Cow BCS was recorded every 28 d as the average of 3 trained technicians. Area of the LM, percentage i.m. fat within the LM, and fat covering the 12th rib were collected on d 45, 101, 157, and 185 of gestation via ultrasound using the "New" Aloka SSD-500 with a 17.2-cm transducer (Aloka Co., Ltd, Wallingford, CT). Images were collected on Beef Image Analysis (BIA) software (Designer Genes Technologies, L.L.C.).

Grab samples of supplement and hay were collected every 2 wk throughout the experiment. Feed samples were ground through a Wiley Mill (Thomas Hill and Sons, Philadelphia, PA) to pass a 1-mm screen. Ground samples were analyzed for DM (AOAC, 1990), N (LECO model FP-528 Nitrogen Determinator, LECO, St. Joseph, MO), NDF (ANKOM 200 fiber analyzer, ANKOM Technology Fairport, NY), and IVDMD (ANKOM Daisy[II] Incubator, ANKOM Technology Fairport, NY). Diet composition is reported in Table 1.

Table 1. Ingredient and diet composition

	Dietary treatment[1]		
	C	NR	NRP
Ingredient[2], % as fed			
Native grass hay	86.6	86.6	77.7
Soybean meal	8.3	8.3	7.4
Molasses	1.2	1.2	1.1
DiCal	2.4	2.4	-
Limestone	1.4	1.4	-
Premix[3]	0.2	0.2	-
Fishmeal	-	-	9.5
Feather meal	-	-	3.4
Blood meal	-	-	1.1
Diet composition			
DM, %	92.3	92.3	92.6
CP, % of DM	10.0	10.0	17.1
NDF, % of DM	62.7	62.7	58.8
IVDMD, %	47.2	47.2	50.9
DMI[4], kg/d	10.0	7.0	8.1

[1]Dietary treatments consisted of native grass hay plus soybean meal-based supplement formulated to achieve 0.51 kg/d of BW gain (C), 70% of NE_m provided by C (NR), and 70% of NE_m provided by C plus a RUP supplement (6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fish meal; DM basis).
[2]227g of 110,000 IU of vitamin A/kg, 27,500 IU of vitamin D/kg, and 660 IU of vitamin E/kg was added to 907 kg (as fed) of each protein supplement mixture.
[3]68.3% KCL, 27.6% $FeSO_4$, 3.1 ZnO, 0.6% MnO, 0.4% $CuSO_4$
[4]Actual DMI from d 48 through 163 of gestation

Statistical Analyses

Data were analyzed as a split-plot in a randomized complete block (parity) design using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The MODEL statement included fixed effects of dietary treatment, parity, sampling period, in addition to all possible interactions. The random effect of cow within dietary treatment × block (specified in the RANDOM statement) accounted for the correlations among repeated observations on the same cow. Means for dietary treatment, sampling period, and the dietary treatment × sampling period interaction were separated using the LSMEANS option.

Results and Discussion

Intake

Dietary treatment x d of gestation interactions were noted ($P < 0.05$) for all variables except for fat depth over the 12th rib (Table 2). Average DMI was 6.9, 8.0 and 9.6 kg/d during the first trimester (d 45 through 91) then was increased to 7.1, 8.2, and 10.4 kg/d from d 92 to 163 of gestation for NR, NRP, and C, respectively. Compared with C cows, DMI was 3.1 kg/d less for NR and 2 kg/d less for NRP from d 45 through 163 (Table 1). Greater DMI by

NRP vs. NR cows was largely attributable to greater intake of RUP by NRP cows. Hay intake by NRP cows also was 3.9% greater than NR. Total feed offered was adjusted for bi-weekly BW change, and BW of NRP cows averaged 6.1% more than BW of NR over the course of the feeding period.

Body Weight

Cows fed C had significantly greater BW than cows fed NR from d 73 through 185. Body weight of cows fed NRP was intermediate until d 115 of gestation; NRP cows had significantly greater BW than NR cows thereafter, but BW of NRP cows did not differ from C cows throughout the experiment. Total BW change from d 45 to 101 of gestation by C and NR cows in the present experiment was comparable to values previously reported by our laboratory in which Miller et al. (2004) conducted a similar experiment with multiparous cows. Those authors reported that C cows gained 22.2 kg from d 45 to 101 of gestation whereas NR cows lost 12.8 kg over the same period. Although the magnitude of change in BW from d 45 to 101 of NR in the present experiment was 4.6 kg more than that reported by Miller et al. (2004), due to much greater variance, change in BW for NR cows in the present experiment was not statistically significant indicating that the NR cows maintained BW. In summarizing 27 yr of data collected at the High Plains Grassland Research Station located in Cheyenne, WY, Garrelts (2006) noted that average BW gain by 3- and 4-yr old March-calving cows nursing calves while grazing rangelands from mid-June through mid-October was approximately 30 kg during dry years. Some of the 3- and 4-yr old cows experienced minimal gain, and it was not uncommon for mature cows in the data set of Garrelts (2006) to experience BW loss during dry years. Cows that have not reached mature BW should be expected to support growth of their bodies in addition to growth and development of their fetuses (NRC, 2000). Despite NR cows in the present experiment not consuming adequate nutrient intake to accomplish growth, pregnancy was maintained because maternal and placental systems compensate for maternal undernourishment to provide the fetus with adequate nutrients (Bassett, 1986; 1991). Cows fed NRP on the other hand, began to gain BW between d 129 and 143 of gestation. The ability of NRP cows to achieve BW similar to C cows may be related, in part, to intake of digestible energy. Using IVDMD plus DMI values in Table 1, intake of digestible DM would be 3.3, 4.1, and 4.7 kg/d for NR, NRP, and C cows, respectively. Differences in dietary IVDMD are corroborated by in vivo estimates of total tract OM digestibility reported by Scholljegerdes et al. (2004; 2005a), although the magnitude of increased IVDMD for the NRP treatment was nearly twice that of differences in total tract OM digestibility (Scholljegerdes et al., 2005a). Adjusting dietary IVDMD of NRP to be of the same magnitude reported by Scholljegerdes et al. (2005a), however, would only decrease DDM intake by 0.1 kg/d. Hence, differences in beef cow performance during early to mid-gestation were attributable to differences in plane of nutrition. It is also possible that cows fed NRP were utilizing AA from the RUP to support or maintain protein deposition because cows fed NRP had similar BW as C cows throughout the experiment.

Body Condition Score

Similar to BW, cows fed C had significantly greater BCS than NR cows from d 73 through 185. Body condition score of NRP cows was similar to both C and NR throughout the experiment. Maintenance of BCS for NR was consistent with maintenance of BW throughout the experiment. Alternatively, cows fed C and NRP were expected to gain BCS according to relationships published in the NRC (2000). Changes in BW over the course of the experimental period included the weight of the gravid uterus (Ferrell et al., 1976; Prior and Laster, 1979). Thus, we concur with Miller et al. (2004) who suggested that apparent associations between changes in BW and BCS in pregnant are likely confounded by weight of the gravid uterus.

Ultrasonic Measurements

Cows fed C had significantly greater LM area than NR from d 101 through 185; LM area of cows fed NRP were intermediate on d 101 of gestation. Cows fed NRP had significantly greater LM area than NR cows on d 157 and 185 of gestation, but BW and LM area of NRP cows did not differ from C cows throughout the experiment. Differences in LM area among dietary treatments are consistent with differences in BW, which was in agreement with results of Miller et al. (2004). The ability of NRP cows to finish the experimental feeding period with an LM area similar to C cows provides further evidence for the suggestion that cows fed NRP were utilizing AA from the RUP to support protein deposition.

Intramuscular fat within the LM was greatest for C on d 157 of gestation; i.m. fat within the LM was similar among dietary treatments at all other collection dates. Cows fed C began and finished the feeding period with greater fat thickness covering the 12th rib than NR cows. Similar to BCS, fat thickness over the12th rib of NRP cows did not differ from either C or NR at d 185 of gestation. Maintenance of LM area and fat cover over the 12th rib for NR cows was consistent with maintenance of BW and BCS over the 140-d feeding period. Likewise, differences in fat cover over the 12th rib among cows with differing BCS in the present study were comparable to our previous reports (Miller et al., 2004; Lake et al., 2005).

Implications

Provision of supplemental protein balanced for intestinal supply of essential AA may be an effective nutritional management strategy to increase production efficiency of pregnant beef cows consuming limited amounts of forage.

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Table 2. Effects of early to mid-gestation nutrient restriction and dietary supplementation with ruminally undegradable protein on body weight and composition of beef cows

	Day of gestation												*P*-value		
Measurement	45	59	73	87	101	115	129	143	157	171	185	SEM[2]	Diet	Day	Diet × Day
BW, kg															
C	495.5^{aJ}	495.2^{aJ}	509.0^{aI}	516.9^{dH}	522.5^{aG}	535.2^{aF}	540.2^{aE}	565.4^{aD}	568.4^{aC}	579.0^{aB}	584.0^{aA}	12.7	0.096	<0.001	<0.001
NR	503.1^{aBC}	484.5^{aD}	486.7^{aD}	486.7^{eD}	485.7^{bD}	493.2^{bC}	492.4^{bC}	510.0^{bB}	512.6^{bB}	520.1^{bA}	521.5^{bA}				
NRP	501.4^{aE}	490.6^{aF}	499.6^{aF}	505.8^{deE}	507.8^{aE}	522.3^{abD}	526.9^{abD}	547.9^{aC}	554.8^{aB}	569.5^{aA}	573.6^{aA}				
BCS, 1-9 scale															
C	5.34^{aB}	-	5.73^{dA}	-	5.82^{aA}	-	5.89^{aA}	-	5.96^{aA}	-	6.01^{aA}	0.22	0.058	0.073	0.001
NR	5.20^{aA}	-	5.23^{eA}	-	5.05^{bA}	-	5.14^{bA}	-	5.10^{bA}	-	4.96^{bA}				
NRP	5.32^{aA}	-	5.16^{eA}	-	5.13^{bA}	-	5.39^{abA}	-	5.50^{abA}	-	5.39^{abA}				
LM area, cm^2															
C	63.03^{aB}	-	-	-	63.87^{dB}	-	-	-	66.90^{aA}	-	67.74^{dA}	2.1	0.146	<0.001	0.049
NR	60.58^{aA}	-	-	-	59.10^{eA}	-	-	-	60.32^{bA}	-	60.26^{eA}				
NRP	59.94^{aB}	-	-	-	61.16^{deB}	-	-	-	64.39^{abA}	-	65.55^{dA}				
i.m. fat, %															
C	3.93^{aC}	-	-	-	4.43^{aB}	-	-	-	4.80^{aA}	-	4.49^{dB}	0.31	0.223	<0.001	0.001
NR	3.54^{aB}	-	-	-	3.54^{bB}	-	-	-	3.98^{bA}	-	3.74^{eB}				
NRP	3.78^{aA}	-	-	-	3.99^{abA}	-	-	-	4.00^{bA}	-	3.92^{deA}				
12th rib fat, cm															
C	0.56^{aA}	-	-	-	0.46^{dA}	-	-	-	0.48^{dA}	-	0.56^{aA}	0.08	0.080	0.926	0.320
NR	0.33^{bA}	-	-	-	0.30^{dA}	-	-	-	0.33^{dA}	-	0.28^{bA}				
NRP	0.30^{bA}	-	-	-	0.43^{dA}	-	-	-	0.41^{dA}	-	0.41^{abA}				

[1]Diets consisted of native grass hay plus a soybean meal-based supplement formulated to achieve 0.51 kg/d of BW gain (C), 70% of NE_m provided by C (NR), and 70% of NE_m provided by C plus a RUP supplement (NRP).

[2]n = 36 cows per dietary treatment.

[a,b,c]Means within a column lacking a common superscript differ ($P < 0.05$).

[d,e]Means within a column lacking a common superscript differ ($P < 0.10$).

[A,B,C,D,E,F,G,H,I,J]Means within a row lacking a common superscript differ ($P < 0.05$).

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

Effect of Copper Supplementation on Artificial Insemination Conception Rate of Angus Cows and Feedlot Performance of Angus Bulls

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SUMMARY

Copper (Cu) oxide boluses were administered in two trials to evaluate their effects on conception to Artificial Insemination (AI) in Angus cows (2 to 12 yrs) and heifers (7 mo of age) and feedlot performance in Angus bulls. Trial 1; 68 Black Angus cows/heifers ranging from 1-12 y of age were blocked by age (0, 1 or multiple pregnancies) and randomly assigned to one of three treatment groups (Trt); Trt 1) Control-no Cu supplementation, Trt 2) 1 Cu bolus on d 180, Trt 3) Cu boluses, d 0 and d 180. Blood plasma samples were taken and analyzed for Cu concentrations in both trials on days 0 (Cu administration), 7, 14, 28, 56, and at 56-day intervals therafter. Sampling began when current calves were weaned and continued until the next calf was weaned. Cows and heifers were synchronized and AI on d 190 - 200. Pregnancy was determined 56 d post AI using an ALOKA 500 ultrasound with a 7 MHz trasducer rectally. There were no differences ($P > 0.05$) in conception among treatments. Copper supplementation resulted in heavier ($P < 0.05$) calf birth weights than in unsupplemented cows. In Trial 2; 20 yearling Angus bulls were randomly assigned to one of two Trt; Trt 1) Control, Trt 2) Cu bolus on d 0 (weaning and placed on feed). Treatment 2 had a greater ($P < 0.01$) average daily gain (ADG) than Trt 1. Results show that Cu did not increase conception to AI but may increase birth weights in fetuses and ADG in fed Angus bulls. The increase in calf birth weights was unexpected and can not be fully explained. Additional research is needed to better understand the increase in birth weights and determine potential production concerns with supplementing Cu.

INTRODUCTION

In order to optimize performance in livestock, nutritional balance must occur. Deficiency of various trace minerals can hinder performance and prevent the animal from reaching its optimum potential. Balanced nutrition is especially important in reproduction whether it is by natural mating or by artificial insemination (AI) (Field and Taylor, 2003).

Copper (Cu) deficiency is a widespread problem in cattle (Suttle, 1986; McDowell, 1992) and can be caused by low intake of Cu levels or high intake of Molybdenum (Mo) and Sulfur (S) (Ward and Spears, 1999). Concentrations of S and Mo are the major dietary factors influencing copper requirements (NRC, 1985) because S and Mo form physiological complexes that tie up Cu and render it nutritionally unavailable to animals. This kind of situation can occur in pastureland where there is a deficiency in dietary Cu and thus, require supplementation in order to maximize performance.

It is common to see livestock operations in west Texas have sheep and cattle together under range conditions. Sheep are more susceptible to Cu toxicity than cattle (Maynard et al., 1979). In fact, if the Mo level is low, forage with a normal Cu content of 8 to 11 ppm can produce toxicity in sheep (NRC, 1985). The requirement for beef cattle is 10 ppm in their diet to supply their daily requirement (NRC, 1996). Research has shown that in cattle grazing pastures containing 3 to 20 ppm Mo, Cu concentrations in the range of 7 to 14 ppm were inadequate. Therefore, inadequate levels of dietary Cu in cattle can be toxic to sheep under some conditions. Some manufactured supplements contain 25 to 35 ppm Cu which is well above the recommended maintenance requirement for adult of 4.6 to 7.4 ppm Cu (NRC, 1985). A supplementation dilemma occurs when trying to adequately supplement cattle and not subject the sheep to Cu toxicity.

Supplemental Cu can be beneficial in performance of feedlot cattle (Ward and Spears, 1999). Research has shown that ADG tended (P=0.11) to increase with Cu supplementation compared with the unsupplemented control (Arthington et al., 2003). Other studies have shown that gestating cattle may need greater amounts of Cu to ensure adequate Cu stores in the livers of their offspring (Ward et al., 1995) and that supplementation improves AI pregnancy rate (Ahola et al., 2004).

In order to further understand the role that Cu plays on reproduction in beef cattle and performance in fed cattle, more research is needed.

MATERIALS AND METHODS

Animal Management

Trial 1 was conducted at the Angelo State University Ranch north of San Angelo, Texas on Highway 87. Black Angus cows ranging in age from one year of age to 12 years of age were assigned to one of three Cu supplementation treatments. Cows were blocked by age and the blocks were multiparous cows (3-12 years of age), single-parous cows (2 years of age), and heifers (1 year of age). Cows were randomly assigned to 3 treatments with equal numbers of each age group in each treatment.

Treatments were as follows; treatment 1—Control (no Cu supplement), treatment 2—1 capsule of supplemental Cu, treatment 3—2 capsules of supplementation. Treatment 2 received their dose on d 180 of the study and Treatment 3 received a dose on d 0 and d 180. The supplemental dose is a 25g Cu bolus

(Animax; Stanton, England). Each bolus contains 100s of tiny Cu oxide wires in a gelatin capsule. Once in the rumen, the gelatin capsule dissolves allowing the Cu wires to disperse throughout the digestive tract and dissolve slowly over a time giving a constant flow of Cu to the animal. The bolus was deposited into the rumen via "Balling Gun."

All three treatments were placed together to prevent treatment x location interactions. The cattle were placed together on pastureland and either wheat or oat fields. They were fed mineral free choice, which does not contain Cu, in order to meet other nutrient requirements. Forage samples were taken in the fields and the pastures and were analyzed by Dairy One Forage Laboratory for Cu content (Dairy One; Ithaca, New York).

Blood samples on the cattle were also taken. The blood samples were collected on days 0, 7, 14, 28, 56, and at 56-day intervals after day 56. The blood was collected via caudal veinapuncture into heparinized blood collection tubes. The tubes were transported on ice to the Angelo State University Management, Instruction, and Research Center to be centrifuged at 2500 rpm for 30 minutes at 5˚C immediately following bleeding. Plasma was separated into collection vials, frozen, and stored. Blood plasma samples were shipped frozen for analysis to CEPS Central Analytical Laboratory (University of Arkansas Poultry Science Center; Fayetteville, AR)

All treatments received an intramusclar shot of PGF2α (ProstaMate, St. Joseph, MO) for estrus synchronization. Estrus was synchronized using the two shot method described by Wilson (2000). Heat watch patches were mounted just above the tail head for mount detection and estrus determination. Approximately 8-12 hours after estrus, each cow was artificially inseminated. Cows were then exposed to a bull the following estrus cycle as a backup to the artificial insemination. Conception rates on all three treatments were assessed. Birth weights were recorded and assessed on the offspring of the treatments to see the effect that Cu supplementation has on the growing fetus.

Trial 2 was conducted using 18 Angus bulls on full feed. They were randomly selected into two treatment groups. Treatment 1 received the supplementation and treatment 2 did not (Control). Blood samples were collected at the same time and by the same method as the cows in Trial 1. Weight gain and ADG were recorded.

Statistical Analysis

The experimental design is a randomized complete block with parity serving as the block. Individual cow or bull served as an experimental unit. Single point data (wt gain, calf birth weights, weaning weights and average daily gain) were analyzed using the general linear models (GLM) of SAS (SAS Institute, Cary NC). Conception rates were analyzed with Chi-square and plasma Cu concentrations were analyzed as a repeated measures. Treatments were considered different at $P \leq 0.05$.

RESULTS AND DISCUSSION

Forage Analysis

Forage samples that were analyzed for dietary Cu content showed no differences in levels of Cu in any of the pastures that the cattle were exposed to during the study. The forage samples had Cu levels ranging from 9-11 ppm (Table 1). The current NRC (1996) recommendations for dietary copper levels in cattle recommend 10 ppm in the total consumed feed.

Trial 1

None of the blood plasma Cu levels analyzed were below 0.85 ppm, which is above the 0.60 ppm that indicates Cu deficiency in beef cattle (NRC, 1996). There were no plasma Cu level differences among treatments in this study (Table 2). However, there was a difference in plasma Cu levels among age. Heifer calves had lower Cu plasma concentrations ($P < 0.05$; Table 3) than both first-calf heifers and cows regardless of treatment. This may be indicative of higher Cu maintenance requirements for growing heifer calves than older cattle. The latest edition of the Nutrient Requirements for Beef Cattle by the NRC (1996), states that growing pregnant heifers require higher levels of energy, protein, and minerals such as calcium (Ca) and phosphorus (P). Though not mentioned in the requirements, there could be a higher requirement for Cu in growing pregnant heifers as well, but this increase may not be substantial enough to make a physiological difference. Providing nutrients to meet animal requirements is especially important when pushing young females into reproductive productivity and maintaining reproductive efficiency in older females (NRC, 1996).

Cows and heifers were given a numbers upon parturition associating when she conceived and then analyzed by treatment groups and by age. If they conceived by first service AI they were given a 1, second service AI a 2, cleanup bull a 3, and if they never came into drug induced estrus a 4. When calving data was analyzed by treatment, there were no differences. The percentage of cattle conceiving either first or second service AI were 82% in treatment 1, 84% in treatment 2, and 89% in treatment 3. Treatment 3 had the highest percentage of cattle that conceived AI however; it was not different than the other two treatments. These data support previous research by Muehlenbein et al. (2001), where no effect of Cu supplementation on 60-d pregnancy rates was observed when compared to unsupplemented cows. Previous research by Olson et al. (1999) showed negatve effects of Cu (trace mineral) supplementation on pregnancy rate observed in 2-yr old beef cows. These findings are different when compared to a study conducted by Ahola et al. (2004) where they found that Cu supplementation improved pregnancy rate to AI compared with cows not supplemented. Also in contrast to the results of this experiment, Stanton et al. (2000) reported a greater pregnancy rate to mass insemination in cows supplemented with Cu (trace minerals) than in cows not supplemented. However, based on liver Cu concentrations reported by Stanton et al. (2000), cows appeared to be deficient in Cu, whereas the cattle in this experiment did not show

defficient levels of Cu in plasma concentrations. This could be a factor in why no supplemental differences in conception rates were shown in this study.

When calving data was analyzed by age, heifer calves as an age group regardless of treatment had lower conception rates ($P < 0.05$). This may not be relevant to Cu levels. First of all, with heifers, it is not known if the animal is even reproductively sound because there has not been an opportunity previous to this study proving that she is capable of reproducing. Second, one animal may not sexually mature as fast as another, therefore, she may not conceive as quickly as other cattle. Age of puberty differs among breeds of cattle as well as females within the same breed (NRC, 1996). The onset of puberty can also be affected by weight and low body condition score (Field and Taylor, 2003). Underfeeding as well as deficiency in some minerals can delay puberty in heifers (NRC, 1996). Birth weights of the calves were recorded and compared among treatments (Table 4). Birth weights of calves in treatment 3 were higher than in treatment 1 ($P = 0.0013$) and in treatment 2 as well. Copper concentrations of 170d old calves of cattle fed Cu supplemented diet were higher ($P < 0.05$) than calves of cattle fed non-Cu-supplemented diets (Gengelbach et al., 1994). Ward et al. (1995) stated that gestating cattle may need greater amounts of copper to ensure adequate copper stores in the livers of their offspring. This indicates that substantial Cu stores in the livers of gestating cows also provides substantial Cu stores in the livers of their offspring which can be beneficial to growth performance in their offspring. Ward et al. (1997) showed that Cu supplementation increased dry matter intake (DMI) during the receiving and growing phases and increased ADG and gain: feed ratios during the finishing phase. In a study that evaluated the effect of Cu bolus administration before weaning (Arthington et al., 1995), weaning weights were heavier in bull calves and tended to be heavier in heifer calves that received supplemental Cu compared with unsupplemented controls. These studies by Ward et al. (1997) and Arthington et al. (1995) indicate that Cu supplementation has a physiological effect on growth. If Cu supplementation increases growth rate and Cu supplementation in gestating females increases the amount of Cu in their offspring, it is easy to see how Cu supplementation can increase birth weights in calves whose dams were supplemented with Cu. Although not originally hypothesized in this study, this finding could be very important and should be considered before producers supplement pregnant cattle with Cu. Increasing birth weights of calves in Cu supplemented dams could cause dystocia problems (trouble giving birth), especially in smaller cows and heifers that are susceptible to this condition.

Trial 2

Blood results in Trial 2 were much like the ones in Trial 1. There were no differences in Cu plasma levels between supplemented and nonsupplemented bulls ($P > 0.05$; Table 5). There was a significant difference of amount of total gain ($P < 0.0001$) and ADG ($P < 0.0001$) between supplemented and nonsupplemented bulls (Table 6). Ward et al. (1997) showed that Cu supplementation increased DMI during the receiving and growing phases and increased ADG and gain: feed ratios during the finishing phase. Heifer ADG tended ($P = 0.11$) to increase with Cu supplementation compared with the unsupplemented control in another study (Arthington et al., 2003). Arthington et al. (1995) found that Cu bolus supplemented weaned bulls had heavier weaning weights than controls when supplemented before weaning. Also, Cu supplementation at 10 or 40 mg/kg of DM improved ADG and daily feed intake (Engle et al., 2000). Conversely, Engle and Spears (2000) indicate that as little as 20mg/kg of supplemental Cu can reduce performance in finishing steers. Gengelbach et al. (1994) and Muehlenbein et al. (2001) found no significant differences in body weight changes for first-calf heifers exposed to mineral treatments. Interestingly, most literature that has findings in contrast to the findings of this study was trials that added dietary Cu supplementation to a feed ration. Adding dietary supplements to a feed ration can have negative effects on palatability. If the dietary additive decreases palatability then there would be a decrease in DMI, feed: gain ratio, and ADG. In this study, bulls were supplemented will a Cu oxide bolus that gives a continual and steady release of Cu for an extended period of time, thus having no negative effects on palatability.

IMPLICATIONS

Cows and heifers supplemented with Cu boluses showed higher percentages of first or second service AI conception rates but did not show any statistical differences between treatments. Copper bolus supplementation in gestating cows and heifers can increase birth weights in their calves at parturition. Careful consideration should be used when deciding to use Cu boluses in gestating first-calf heifers due to the risk of dystocia. Copper bolus supplementation in fed cattle can improve ADG and growth rate. Additional research is needed to determine effects on feed: gain ratio.

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Table 1: Dietary copper content of forage samples collected in fields and pastures at the Angelo State University Ranch

Pastures	Cu[a]
1[b]	10
2[b]	9
3[b]	9
4[c]	9
5[d]	11
6[d]	11

[a] Copper values in column are expressed as ppm
[b] Grass pasture land
[c] Oat field
[d] Wheat fields

Table 2: Plasma copper levels (ppm) in Angus cows and heifers receiving no copper supplementation or supplementation using a continuous release copper bolus

	Treatments[a]			
	1	2	3	SE[b]
Bleeding 1 d 0	1.25	1.05	1.11	0.060
Bleeding 2 d 7	1.18	1.13	1.23	0.061
Bleeding 3 d 180	1.08	1.10	1.29	0.078
Bleeding 4 d 236	1.13	1.12	1.19	0.047

[a] Treatments = 1 Control; 2 bolus on d 180, 3 bolus on d 0 and d 180
[b] SE = Standard error of the least squares mean

Table 3: Plasma copper (ppm) levels in Angus cows and heifers regardless of copper supplementation

	Age[a]			
	HC	H	C	SE[b]
Bleeding 1, d 0	1.12[cd]	1.27[d]	1.03[c]	0.055
Bleeding 2, d 7	1.25[c]	1.24[c]	1.05[d]	0.049
Bleeding 3, d 180	1.14[c]	1.33[d]	0.99[c]	0.063
Bleeding 4, d 236	1.05[c]	1.23[d]	1.15[d]	0.037

[a] HC = heifer calves; H = heifers (single-parous cows); C = cows (multiparous cows)
[b] SE = Standard error of the least squares mean
[cd] Means in the same row with differing superscripts are different (*P* < 0.05)

Table 4: Mean birth weights in Angus cows and heifers receiving no copper supplementation or supplementation using a continuous release copper bolus

	Treatments[a]			
	1	2	3	SE[c]
Birth Weights[b]	35.76 [d]	37.98 [d]	41.43 [e]	1.24

[a] Treatments = 1 Control; 2 bolus on d 180, 3 bolus on d 0 and d 180
[b] Average Birth Weights expressed in kg
[c] SE = Most conservative standard error of the least squares mean
[de] = means in the same row with differing superscripts are different (*P* < 0.05)

Table 5: Plasma copper levels (ppm) in weaned Angus bulls receiving no copper supplementation or 1 copper continuous release bolus

	Treatments[a]		
	1	2	SE[b]
Bleeding 1	0.94	1.16	0.083
Bleeding 2	1.19	1.10	0.197
Bleeding 3	1.03	1.09	0.023
Bleeding 4	1.45	1.14	0.207

[a] treatment 1 = copper continuous release bolus on d 0; treatment 2 = no copper bolus
[b] SE = standard error of the least squares mean

Table 6: Body weight and body weight gains in Angus bulls either receiving or not receiving copper bolus supplementation

	Treatments[a]		
	1	2	SE[b]
Initial wgt, kg	365.15	311.52	11.32
Final wgt, kg	579.04	484.39	15.36
Gain	213.89[d]	172.88[c]	5.25
ADG	1.81[d]	1.46[c]	0.04

[a] treatment 1 = copper continuous release bolus on d 0; treatment 2 = no copper bolus
[b] SE = standard error of the least squares mean
[cd] Means in the same row with differing superscripts are different (*P* < 0.05)

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

THE EFFECT OF PROTEIN LEVEL ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF TEXAS RAMBOUILLET EWES

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SUMMARY

Aged Rambouillet ewes, 5 to 7 years old, are usually culled Texas. Some producers have chosen to feed their aged ewes high energy diets, a feedlot practice, before they send them to harvest. This practice may prove to be profitable as the ewes will gain extra weight and bring more money at sale time. Research on the feedlot performance of aged ewes is very limited. The purpose of this research is to compare protein level on feedlot performance (rate and efficiency of gain) and carcass composition in aged Rambouillet ewes. A total of 28 ewes were blocked by weight and BCS and randomly assigned to a pen. The pens measured 3.048 m by 9.144 m. Ewes were placed in one of 14 pens with two ewes per pen. Pens were allocated to one of three different treatments consisting of WH, SBH, and GR. These treatments resulted in varying amounts of protein. Ewes were weighed every 28 days and kept on trial for 84 days. Carcass characteristics were measured after carcasses were chilled for 24 hours. The trial consisted of 28 ewes in 14 different pens, four pens on WH, five pens on SBH, and five pens on GR. Performance was greater (P<0.05) for ewes on GR for total gain, ADG as well as BCS and BCS change. Feed efficiency was also better (P<0.05) for GR as compared to WH and SBH. Ewes on GR had greater (P<0.05) fat depth at the twelfth rib than SBH or WH and SBH ewes were fatter than WH with no differences (P>0.05) across treatments in carcass weights or dressing percents. Upon evaluation of the economic data, the feeding of aged ewes in a down market appears to be unprofitable and actually resulted in a loss. However, if the market remained steady, profit could be gained by feeding aged ewes. This only shows that further focus of commercial operations is needed to determine the actual profitability of feeding aged ewes.

INTRODUCTION

Sheep production in West Central Texas is a large constituent of the agricultural economy of the area. The West Texas region is considered the top sheep producing region in the nation (USDA-AMS, 1997). According to the United States Department of Agriculture – National Agriculture Statistics Service (USDA-NASS), the West Central region of Texas had an inventory of 857,000 head of sheep in 2003. This comprises 76 percent of all sheep in Texas (USDA-NASS, 2003). In 1997 sheep, lamb, and wool sales totaled 97 million dollars in Texas. Most of this was from the sale of red meat, especially that of lambs. Yet, a part of this red meat also comes from the sale of older ewes.

The termination of wool subsidies made sheep producers turn their focus from wool production to red meat production. Sheep operations in West Central Texas make most of their money from the sale of feeder lambs to feedlots (Personal communication, A.H. Denis, Denis Ranch, Vancourt, TX). On a per farm basis, there is a thin margin between profit and loss on sheep operations. Therefore, any extra income to a sheep producer from any venue, such as sales of cull ewes fed to a higher weight, can be the difference between profit and loss.

Aged ewes, 5 to 7 years old, are usually ewes that the producer has decided not to breed anymore. The ewes are usually sent to harvest facilities after their last lamb is weaned, as an effort to minimize the cost of maintenance for the operation. Some producers have chosen to feed their aged ewes high energy diets, a feedlot practice, before they send them to harvest. This practice may prove to be profitable as the ewes will gain extra weight and

Research on the feedlot performance of aged ewes is very limited. Therefore, little is known about how aged ewes perform in feedlot situations. The purpose of this research is to compare protein level on feedlot performance (rate and efficiency of gain) and carcass composition in aged Rambouillet ewes.

MATERIALS AND METHODS

Animals and Feeding

This study was conducted at the Angelo State University Management, Instruction, and Research Center (MIR Center), located in Tom Green County north of San Angelo, Texas. A total of 28 Rambouillet ewes averaging 53 kg were used for this trial. The ewes were blocked by weight and BCS and assigned to 14 pens of two ewes per pen. Pens were allocated to one of the three different treatments consisting of four pens on WH, five pens on SBH, and five pens on GR, which was prepared at the MIR Center, (Table 1) containing varying levels of protein. All treatments met or exceeded NRC requirement for maintenance in ewes (NRC, 1985a).

Ewes had *ad libitum* access to feed and fresh water for the 84d trial. Feed refusals were removed and weighed each time a new batch of feed was placed in the feeders so that feed efficiency could be calculated, including ADG, gain:feed ratio, cost of gain, and profit. Feed efficiency was calculated by dividing the kg of gain by kg of feed. Percent of maintenance CP and percent of maintenance TDN were calculated for each diet. Ewes were kept in pens measuring 3.048 m by 9.144 m. Upon arrival ewes were tagged and weighed and treated with an anthelmintic.

Data Collection

Ewes were individually weighed on day zero, to get the initial weight, 28, 56, and 84, for final weight, to determine feedlot performance for each treatment. At initial and final weigh days, ewes were evaluated and given a BCS on a scale of zero to five, zero being extremely emaciated and five being excessively obese. Evaluation was done by palpation method as described in the Sheep Production Handbook (American Sheep Industry Association, 1996). At d 84 of the trial, ewes were harvested following normal commercial conditions at Rancher's Lamb of Texas Inc., and carcasses were spray chilled at $2^{o}C$ for 20 to 24 hours. Carcasses were then evaluated for backfat thickness at the twelfth rib. Dressing percent was also calculated by taking the hot carcass weight (HCW) and dividing it by the live weight and multiplying it by 100.

Statistical Analysis

The trial was a completely randomized block design with a pen of two ewes being the experimental unit. The General Linear Model procedure (SAS Inst. Inc., Cary, NC) was used to determine the effect of protein level on feedlot performance and carcass characteristics. Analysis of variance and Fisher's protected LSD test was used to determine statistical significance at a predetermined $\alpha = 0.05$.

RESULTS AND DISCUSSION

Feed Analysis

Chemical analysis of all three feed treatments was conducted by Dairy One Inc., Ithaca, NY. Although the CP levels in SBH were lower than WH, TDN values were different with WH being the lowest followed by SBH and then GR. Table 2 shows the percent of maintenance CP and TDN which the ewes ingested for each treatment. Maintenance CP and TDN levels were obtained from the NRC (1985a).

Performance Data

Ewes were blocked by weight and initial BCS. No differences (P>0.05) were found for weight and initial BCS (Table 3). The total number of ewes on trial was 28 in 14 different pens. The 14 pens were four pens on WH, five pens on SBH, and five pens on GR. Table 3 shows the least square means of final body weight (BW), gain, ADG, final BCS, and BCS change. No differences (P>0.05) were observed for the mean final weights. Although the ewes on GR gained more than the other treatments, they averaged a lighter initial weight numerically, therefore, the final weights tended to average to the same weight. Both total gain and ADG were significantly higher (P<0.05) for GR when compared to WH and SBH. Ewes on GR increased an average of 19.7 kg, which was 15 kg and 10.6 kg more than WH and SBH, respectively. This result agrees with Fluharty and McClure (1997), Hinds et al. (1965), and Hudson et al. (1967) who found increases in ADG and final weight in growing lambs when they increased the recommended NRC protein requirement. The final BCS and BCS change were also Table 4 displays the intake and feed efficiency least square means for the three treatments. All three treatments significantly differed (P<0.05) from each other in intake. A difference in intake greatly differed between WH and GR from 199.6 to 393.2 kg per pen, respectively, a difference of 193.6 kg. A significant difference (P<0.05) was seen in efficiency when GR was compared to WH and SBH. GR ewes gained 0.10 kg per kg of feed consumed, while WH and SBH ewes only gained 0.03 kg and 0.06 kg, respectively, per kg of feed consumed. Fluharty and McClure (1997) also found an increase (P<0.01) in dry matter intake, but observed no difference in feed efficiency when protein level was increased in lamb rations. In another study, done by Braman et al. (1973), lambs and steers fed protein supplements had significantly higher feed efficiencies. Lana et al. (1997) observed no improvement in ADG or feed efficiency. The effects of protein increases in a ration are greatest when a ration is low in protein and another is high in protein and energy is readily available (Zinn and Owens, 1993). Small increments in protein make very little difference.

Carcass Data

In this study fat depth, at the twelfth rib, hot carcass weight, and dressing percent were observed (Table 5). The fat depth of GR ewes was significantly different (P<0.05) from that of SBH and WH. WH and SBH fat depth measurements were not significantly different (P>0.05), but tended to increase as protein level increased. No differences (P>0.05) were found in hot carcass weight and dressing percent measurements, only a tendency for weight to increase as protein level increased. Overall protein had only a slight effect on carcass composition other than fat depth. This agrees with findings of Braman et al. (1973) on steers and lambs and Prior et al. (1977) with cattle.

Economic Data

Table 5 shows the cost of the WH, SBH, and GR feeds. The average price of WH was $70 per 909.1 kg (USDA-NASS, 2003) which equated to $0.08 per kg. The prices for SBH and GR were obtained from the financial records of the MIR Center and calculated to $0.12 and $0.14 per kg, respectively. The highest protein, GR, was $61.94 per 909.1 kg more than lowest protein WH.

Table 6 shows the least square means of the economic data for this trial. Ewes were sold at the harvest facility for $0.55 kg of carcass weight. No differences (P>0.05) were found between any of the treatments for carcass value. Total feed cost, cost of gain, and profit were not statistically tested, only calculated on averages. Table 6 shows that all treatments were at a loss. Yet, since the smallest cost of gain is $1.70, and the ewes only bring $0.55 kg, this seems to imply that feeding out aged ewes is non-profitable. However, at the time these ewes were bought the price of slaughter ewes was high because supply was low, and when ewes were sold prices were low. The average price per slaughter ewe is $0.66 per kg or $33.00 for a 50 kg ewe. If the market had remained steady from purchase to sale the profit margin would have been positive.

IMPLICATIONS

Results from this trial show increased gain rates in aged ewes and more weight on the higher protein treatment. In addition, the fat depth at the twelfth rib increases with increasing protein level. Overall, this trial showed a loss of money occurs when feeding out aged ewes; however, if market conditions remain steady the cost of gain should be profitable. Further research is needed to determine the actual profitability of feeding aged ewes on an actual operation situation where the operator does not have to purchase the ewes.

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Table 1. Ingredients and nutrient density for WH, SBH, and GR fed *ad libitum* for 84 d.

	Treatment[a]		
Item	WH	SBH	GR
	% as fed		
Ingredients			
Sorghum grain	-	-	45
Soybean hulls	-	100	22.5
Alfalfa pellets	-	-	17
Cottonseed meal	-	-	10
Cane molasses	-	-	3
Mineral premix	-	-	2.5
Wheat hay	100	-	-
	DM		
Nutrient Density			
Crude Protein(CP), %	15.4	14.3	17
NE_g, Mcal/kg	0.6	0.7	1.2
Neutral Detergent Fiber, %	51.4	59.8	34.3
Acid Detergent Fiber, %	28.7	40.2	25.7
TDN, %	59	63	76

[a]WH = wheat hay, SBH = soybean hulls, GR = grain ration

Table 2. Percent of maintenance crude protein and total digestible nutrients which pens of Texas Rambouillet ewes were ingesting per treatment.

Item	Treatment[a]		
	WH	SBH	GR
N	4	5	5
% of maintenance CP	166	258	405
% of maintenance TDN	109	195	311

[a]WH = wheat hay, SBH = soybean hulls, GR = grain ration

Table 3. Least square means of initial weight and BCS, and the effect of protein level on feedlot performance of pens (two ewes/pen) of Texas Rambouillet ewes.

Item	Treatment[a]			
	WH	SBH	GR	SE[b]
n	4	5	5	
Initial wt (kg)	113	108	100.2	6.8
Initial BCS(avg/animal)	2.25	1.97	1.96	0.17
Final BW, kg	122.4	126.2	139.8	8.5
Total Gain, kg	9.4[c]	18.22[c]	39.4[d]	4.5
ADG. kg/d	0.1[c]	0.22[c]	0.46[d]	0.06
Final BCS(avg/animal)	1.72[c]	2.83[d]	3.28[d]	0.18
BCS change(avg/animal)	0.53[c]	0.85[d]	1.31[d]	0.21

[a]WH = wheat hay, SBH = soybean hulls, GR = grain ration.
[b]Standard error of estimate.
[c,d]Means in the same row with uncommon superscripts differ $P < 0.05$.different ($P<0.05$) for SBH and GR from WH.

Table 4. Least square means of intake, feed efficiency, and the effect of protein level on carcass characteristics of pens (two ewes/pen) of Texas Rambouillet ewes.

Item	Treatment[a]			
	WH	SBH	GR	SE[b]
n	4	5	5	
Intake, kg	199.6[e]	308[f]	393.2[g]	31.6
Efficiency, kg gain/kg feed	0.03[e]	0.06[e]	0.10[f]	0.015
Fat depth[c]. cm	0.45[e]	0.57[e]	1.07[f]	0.05
Hot carcass weight, kg	52.2	55.5	61.9	4.3
Dressing percent[d]	42.8	44.9	48.0	2.21

[a]WH = wheat hay, SBH = soybean hulls, GR = grain ration.
[b]Standard error of estimate.
[c]Fat depth measurement at the twelfth rib (avg/animal).
[d]Dressing percent = hot carcass weight/live weight.
[e,f,g]Means in the same row with uncommon superscripts differ $P < 0.05$.

Table 5. Analysis of treatment cost.

Item	Treatment[a]		
	WH	SBH	GR
Price per 909.1 kg	$70.00	$106.00	$131.94
Price per kg	$ 0.08	$ 0.12	$ 0.14

[a]WH = wheat hay, SBH = soybean hulls, GR = grain ration

Table 6. Least square means of economic data (U.S. dollars) for pens (two ewes/pen) of Texas Rambouillet ewes.

Item	Treatment[a]			
	WH	SBH	GR	SE[b]
n	4	5	5	
Purchase price[c], $	107.66	107.66	107.66	
Carcass value[d], $	66.83	68.93	76.37	5.27
Total feed cost[e], $	15.06	37.57	55.49	
Cost of gain kg[f], $	2.19	2.63	1.70	
Profit[g], $	-55.89	-76.30	-86.78	

[a]WH = wheat hay, SBH = soybean hulls, GR = grain ration.
[b]Standard error of estimate.
[c]Average price of ewes at beginning of trial.
[d]Average value ewes were sold for.
[e]Average cost of ration per ewe.
[f]Cost of ration per weight gain in kg.
[g]Profit = (carcass value) – (purchase rice + tot feed cost)

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

USE OF N-ALKANES TO ESTIMATE SEASONAL INTAKE, DIGESTIBILITY, AND DIET COMPOSITION OF SMALL RUMINANTS GRAZING IN CALIFORNIA CHAPARRAL

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ABSTRACT. Grazing of livestock oriented to reduce the fire hazard associated with the accumulation of fuel load in Chaparral is a strategy to assist human settlements at-risk. To successfully implement a prescribed grazing regime in chaparral, dry matter intake (DMI), digestibility (DMD), and diet composition (DC) of herbivores must be determined. This study was conducted to determine seasonal variation in: DMI, nutrient content, and DMD of plants selected by sheep and goats grazing on chaparral using the alkane method. Six male Kiko goats (average weight 22.9±2.7 kg) and six whether Targhee sheep (average weight 39.6±0.66 kg) were used during fall, spring and summer to assess DMI and DC. Animals were orally dosed with n-alkane control release capsules using intra-ruminal controlled release devises. N-alkane profiles in fecal and plant samples were used to estimate DC and DMI. Overall DMI was 0.9, 1.1, and 1.3 kgd^{-1} for goats and 0.8, 1.2, and 0.8 kgd^{-1} for sheep in fall, spring and summer, respectively. DMD, crude protein (CP), and metabolizable energy (ME) contents of the selected diets by goats in fall and summer were higher ($P<0.05$) than that of sheep. In spring, DMD, CP and ME were higher ($P<0.05$) in diets selected by sheep compared to diets selected by goats. The proportions of browse in the consumed diet were 78, 87, and 95% by goats and 73, 59, and 82% by sheep, in fall, spring and summer, respectively. Main chaparral species consumed by goats and sheep during all periods were *A. fasciculatum*, *Quercus*, spp., *C. cuneatus* and grass. *A. fasciculatum* was an important ($P<0.05$) species for goats in spring and sheep in summer. Sheep consumed more *Quercus* spp. ($p<0.05$) in fall and grass in spring than goats did. This study indicates that, faced with similar opportunities for choice, sheep and goats select fairly similar species, but in different proportions at different seasons. CP and ME in chaparral species consumed by goats and sheep were deficient in all seasons; thus, feed supplementation is required to ensure productivity and health of small ruminants grazing chaparral and predict efficacy of their use in vegetation management programs to reduce fuel load in this type of vegetation.

Keywords: sheep, goats, intake, diet composition.

Introduction

Chaparral is a broad-leaved sclerophyllous vegetation type (between 1 and 4 meters high), adapted to the prevailing Mediterranean-type climate of winter rain and summer drought (Hanes, 1977). The Chaparral plant community, one of the most fire-prone and fire-dependent plant communities in the world, covers more than 4.5 million ha of California's rangelands. The steady accumulation of fuel, the Santa Ana winds, the extreme whether conditions, the water-repellent soils, steep terrain, and the increased urbanization, residential construction and occupation of rural landscapes of California expose people to the destructive potential of a wildfire, (Keeley, 2002). Large and intense wildfires in Chaparral have resulted in catastrophic damage to property and resources, and the tragic loss of lives. Government agencies recognize that fire hazards associated with accumulation of woody biomass caused by the combination of removal of grazing and browsing, interruption of natural fire cycles over many decades, and aggressive invasive weeds are at a stage where only an integrated vegetation management program can promise long-term solutions. Herbicides, prescribed burn and mechanic control are the vegetation management tools currently used. Herbicides are considered a risk for wildlife diversity and have serious implications for water quality and safety. Prescribed fire may be an effective tool, but it use is limited by air quality regulations and proximity of urban development. Mechanical methods are expensive, and the benefits are usually short lived. Use of livestock grazing in vegetation management has the potential to be an ecologically and economically sustainable management tool for fuel load reduction. In order to successfully apply a prescribed grazing regime, feed intake and diet composition of herbivores must be determined. The aims of this study were to estimate the seasonal composition, intake, and digestibility of diets selected by sheep and goats free grazing in California chaparral, and to evaluate the quality of that diet during the year. This information is required in order to know aspects of selectivity and optimal timing of grazing, as well as to assess the potential impact of grazing on the plant community and to assure adequate livestock production.

Materials and Methods

Study Area. The study was conducted in a chaparral community (1.7 ha) situated at the Hopland Research Extension Center (**HREC**), University of California (39° 00’N latitude and 123° 4’W longitude) at an elevation of 2900 m. The climate is typically Mediterranean, with mild winters and hot dry summers.

The minimum and maximum temperatures (°C) throughout the experimental periods were 12.8-23.2, 18-30.6, and 10.2-18.5 in spring, summer and fall, respectively, whereas the mean precipitation (mm) was 155.6 in fall and 27.7 in spring.

Animals and Management. Six castrated male Kiko goats (average weight 22.9±2.7 kg) and six wether Targhee sheep (average weight 39.6±0.66 kg) were used. Animals were given a 10-days adjustment period to become familiarized with the vegetation (other chaparral areas than the experimental). Animals were dewormed prior to the experiment. Animals were placed on the experimental area on October 23st, 2000 (fall), May 3th, 2001 (spring) and July 21th, 2001 (summer), and were allowed to graze continuously until November 22nd, 2000, June 3rd, and August 21st, 2001. N-alkanes (C_{32} and C_{36}) were dosed using an intraruminal Controlled Release Capsule (**CRC**) (Nufarm, NZ). The CRC was placed into the rumen through the oesophagus at the beginning of each measurement period. The CRC were design and calibrated to release C_{32} and C_{36} at 50 mg/day for approximately 20 days. Between monitoring periods, animals were at a lower elevation (700 ft) in a mixture of chaparral and grass vegetation (25% chaparral, 75% grass) and were supplemented with 0.5 kg of alfalfa pellets before starting the corresponding experimental period.

Plant Collection. For each experimental period, leaf and stem from plant species were randomly collected across the paddock based on either their availability or intake by sheep and goats. Considering that n-alkane concentrations in plant species vary with the season, individual plant species were collected at different times during each experimental period. Plant samples were dried for 48 h, at 55°C and ground at 1-mm sieve for subsequent n-alkanes and quality analyses.

Fecal Collection. According to manufacturer's specifications rumen n-alkane equilibrium was achieved within 7 days of dosing. Thus, fecal samples were taken daily from the 8th through the 23rd day after dosing the alkanes in every season. Fecal samples were put into sealed plastic containers, dried for 4 days at 50°C, and ground through a 1-mm sieve for subsequent n-alkane analysis.

Chemical Analyses. Fecal and plant samples were analyzed for ash, and crude protein (**CP**) according to AOAC (1990). Neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**) following Van Soest et al. (1991). In vitro gas production after 24 h of incubation was used as an index of digestibility and energy feed value (**ME**), as suggested by Menke and Steingass (1988). Concentrations of n-alkanes were determined by gas chromatography (Dove and Mayes, 1996).

Estimation of Diet composition and Intake. N-alkane profiles in fecal and plant samples were used to estimate diet composition and intake (Dove and Mayes, 1996). A least-squares optimization procedure was used to determine diet composition (Salt et al., 1994). The intake of each individual animal (sheep and goat) was calculated from the pair of n-alkanes C_{31} (naturally present in the diet) and C_{32} (dosed) as follows (Brosh et al., 2006; Dove and Mayes, 1996):

$$I\ (kgd^{-1}) = [(CRC\ C_{32}\ release\ rate) * (Fecal\ C_{32})]^{-1} * [(Fecal\ C_{32}) * (Diet\ C_{31}) - (Fecal\ C_{31}) * (Diet\ C_{32})]\ ;$$

where: I = daily DM intake (kg); CRC C_{32} = release rate of C_{32} dosed (g); Fecal C_{31}, Fecal C_{32}, = Concentrations of C_{31} and C_{32} in feces; Diets C_{31}, Diet C_{32}, = Concentration of C_{31} and C_{32} in diet ($mgkgDM^{-1}$).

Statistical Analysis. The similarity within and among the n-alkane profiles of plants were tested by a multivariate analysis of variance (MANOVA) using SAS (1999) in each sampling period. Diet composition and intake data were analyzed by analysis of variance (ANOVA) with a mixed model maximum likelihood approach of repeated measure data with a 2 x 3 factorial arrangement (SAS, 1999). A compound symmetry covariance structure was fitted to all response data, as indicated by Akaike's Information Criterion.

Results and Discussion

Diet composition. Fecal n-alkane concentrations were corrected using published recovery rates reported for sheep (Dove and Olivan, 1998) and goats (Brosh et al., 2003). Composition of diets consumed by sheep and goats differed between animal species (P<0.001) and season (P<0.001), and changes in the seasonal contribution of the different plant species were clearly evident between sheep and goats (Figure 1). Interactions were detected among animal species, plant species and season (P<0.001).

In this study, browse was the most important dietary constituent of goats and sheep in all seasons (P<0.001). Shrub constituted 78, 87, and 95% of the goats' diet and 73, 59 and 81.5% of the sheep's diet in fall, spring and summer, respectively. *A. fasciculatum*, *Quercus* spp, *C. cuneatus*, and *E. californicum*, considered among the most dominant chaparral species, contributed 24, 24.4, 18.5, and 11.5% in goats and 19.4, 36.5, 10.6, and 3.8% to the total shrub consumption during all seasons, respectively. Similar results have been reported by other researchers with goats and sheep grazing in chaparral (Brennecke and Pittroff, 2003; Sidahmed et al., 1983). Additionally, *Arctostaphylos* spp. and *B. pilularis*, other dominant shrub in the experimental area were minor component of the diet of both species in all seasons (Figure 1). Similar results were reported by Sidahmed (1981b; 1983) and Brennecke and Pittroff (2003). These findings could be associated with the presence of secondary compounds in these species. Previous studies in Arizona chaparral indicated that goats consumed less manzanita species than other shrubs (Pond and Schmutz, 1984).

In fall, goats consumed mostly *C. cuneatus* (29.5%) and grass (21.9%), followed by similar amounts of *A. fasciculatum* (17.5%) and *U. californica* (16.8%). In spring, goat's diet was dominated by *A. fasciculatum* (36.7%), *E. californicum* (22.5%) and *C. cuneatus* (26.1%). In summer, goats showed major preference for

Q. wislizenii, which together with *Q. durata*, comprised more than 60% of the diet. On the other hand, sheep's diet in fall was dominated by *Q. durata* (55.6%) and grass (27.1%). In spring sheep switched to a diet based mostly on grass (41.4%). In summer, sheep consumed mostly *A. fasciculatum* (41.7%) and *Quercus* spp. (36.3%). Sidahmed et al. (1981a) observed that the preference of browsing Spanish goats was directed primarily toward scrub oak and chamise, while Brennecke and Pittroff, (2003) reported that sheep consumed mostly *A. fasciculatum*, grass and *Q. durata* grazing in chaparral.

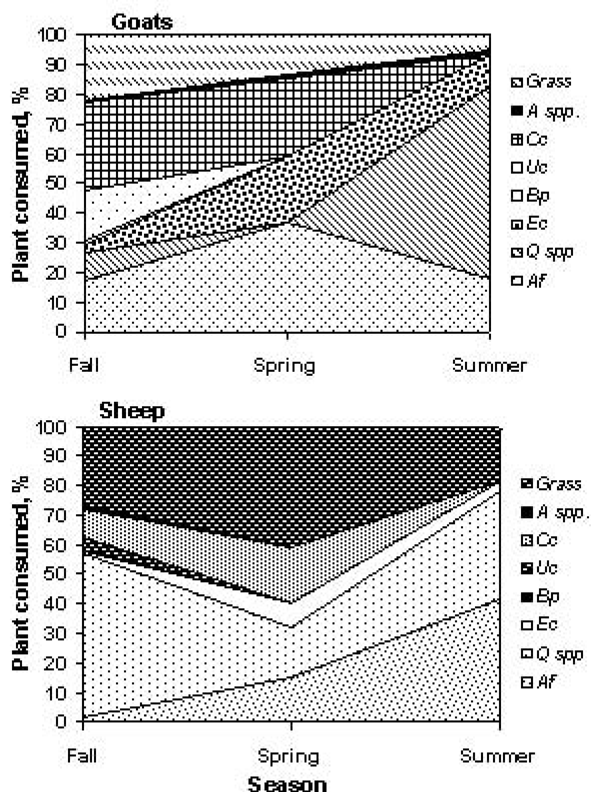

Figure 1. Mean seasonal diets consumed by sheep and goats throughout three seasons. Abbreviations: A spp, *Arctostaphylos* spp.; Cc, *Ceanothus cuneatus*; Uc, *Umbellaria californica*; Bp, *Baccharis pilularis*; Ec, *Eriodictyon californicum*; Q spp., *Quercus* spp., Af, *Adenostoma fasciculatum.*

Intake and Digestibility. Dry matter intake (**DMI**), dry matter digestibility (**DMD**), and nutritional values of diet consumed by goats and sheep grazing in chaparral during each experimental period are listed in Table 1. There was variation between animal species (P<0.01) and among seasons (P<0.001) in the different parameters studied. Interaction between animal species and season was also significant (P<0.001).

In general, low daily intake was recorded for goats and sheep. The DMI values ranged from 30.7 g kg^{-1} BW in fall to 22.4 g kg^{-1} BW in spring for goats, and from 12.7 g kg^{-1} BW in summer to 19.1 g kg^{-1} BW in spring for sheep. During all experimental periods, DMI was higher in goats than in sheep (P<0.01). In addition, there were seasonal differences in the DMD of the diets consumed by goats and sheep. The DMD of the summer diet in goats was higher (P<0.01), than in the fall and spring whereas the DMD of the spring diet in sheep was higher than that of fall and summer (P<0.01). Low DMI was also reported by Sidahmed et al. (1981a) in goats fed with a diet based on oak, chamise and manzanita shrubs. These authors concluded that chaparral species did not meet maintenance energy requirements for goats and sheep causing lost body mass.

Table 1. Dry matter and nutrient intake, digestibility and metabolizable energy of diets consumed by sheep and goats in three seasons in a chaparral community.

Variable	Period			SL[4]	
	F[1]	Sp[2]	Sum[3]	G*S[5]	S[6]
Goats					
DMI (g/kgBW)	30.7	22.4	26.1	*	**
DMI (g/d)	927	1110	1330	*	*
DMD (%)	57.3	53.8	61.5	**	*
CP (g/kgDM)	50	66	68	*	*
NDF (g/kgDM)	461	501	750	**	*
ADF (g/kgDM)	340	385	553	**	*
ME (Mcal/d)	1.3	1.6	1.7	**	*
Sheep					
DMI (g/kgBW)	18.2	19.1	12.7	*	**
DMI (g/d)	813	1213	788	*	*
DMD (%)	46.5	64.1	34	**	*
CP (g/kgDM)	39	69	34	*	*
NDF (g/kgDM)	440	635	478	**	*
ADF (g/kgDM)	311	470	356	**	*
ME (Mcal/d)	1.1	1.9	1.0	**	*

[1]Fall; [2]Spring; [3]Summer; [4]Significance level; [5]Goat vs. Sheep; [6]Seasons.
*p<0.001; **p<0.01
Means of six goats in fall, summer and spring.
Means of five sheep in fall and six sheep in summer and spring.

CP intake by goats was highest in summer (P<0.01), whereas CP intake by sheep was highest in spring (P<0.001). The amount of CP consumed by sheep in spring was almost twice of that consumed in fall and summer. In addition, there was no difference either in CP content of diet consumed by sheep in spring or between the CP content of diets eaten by goats in spring and summer (Table 1). Higher levels of CP in diets selected by goats and sheep in spring may be explained by increased consumption of browse with the highest CP values (*A. fasciculatum*, *E. californicum*, *Q. durata*, and *C. cuneatus*) at that time. Similar results were reported by Ramirez et al. (1991) in goats in a shrubland in northeastern Mexico. Goats consumed considerably higher amounts of fiber in summer than in fall and spring (P<0.001), whereas sheep's fiber intake in spring was 70 and 75% higher than that consumed in fall and summer, respectively (P<0.001). For sheep, these findings could be related with an increment in the consumption of grass species, which comprised 41% of the diet during spring.

Grasses contain much higher NDF levels than do browse leaves or forbs (Holechek et al., 1989). For goats, this could be explained by the presence of browse species with high content of fiber in the diet, such as chamise and oak during summer. Similar results have been reported by Papachristou and Nastis (1992) in goats grazing Mediterranean ecosystems. Higher DMI and DMD of diets consumed by goats in summer compared with that in spring or fall regardless of the high fiber content may be explained by the higher capacity of goats to digest fiber and suggest that goats can digest plants with high cell-wall content better than sheep. Comparative ability to digest ligneous material between ewes and goats has been reported in other studies (Tisserand et al., 1991).

ME intakes for goats and sheep were highest in summer and spring, respectively (P<0.001). There were no differences in ME content of goat's diets between summer and spring. ME intakes for goats and sheep were below their requirements for maintenance (AFRC (Agricultural and Food Research Council), 1998). According to Holloway and Varner, (1985), a diet predominantly composed of brush plants is usually low in energy.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECT OF COBALT SUPPLEMENTATION AND ESTROUS SYNCRONZATION ON BODY WEIGHT GAIN AND CONCEPTION RATES IN YEARLING EWES AND PERIPUBERTAL EWE LAMBS

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ABSTRACT: Conception rates of peripubertal ewe lambs during the fall following their birth and yearling ewes influences flock productivity and economic returns. Our objectives were to evaluate the influence of cobalt (Co) supplementation and estrus synchronization on growth performance and conception rates. Yearling ewes (n = 50) and peripubertal ewe lambs (n = 63) were sorted by breed (Suffolk, Hampshire, Rambouillet, Columbia), allocated to 1of 8 pens (2 pens/breed), and fed control supplement (0.12 kg distillers grain/h/d) or control supplement containing Co (10 mg) for 28 d before and 14 d into the breeding season. All animals received 0.91 kg/d alfalfa hay and 0.91 kg/d grain-based supplement (15% CP, DM basis). On d 1 of the breeding season, estrous cycles of one-half of the ewe lambs from each age group in each pen were synchronized by treatment with GnRH (d 1; 100 µg) and $PGF_2\alpha$ (d 7; 12.5 mg). Fertile rams fitted with marking harnesses were placed with all ewes on d 1 for a 35 d breeding period. Pregnancy status of all animals was confirmed by ultrasound 60 d after ram removal. Supplementation with Co did not influence (P = 0.49) ADG or d to estrus (P = 0.76). Synchronization tended to increase (P = 0.07) the proportion of ewes in estrus during the 10 d interval following $PGF_2\alpha$ administration from 49.4% to 62.0%. However, synchronization did not (P = 0.84) influence overall conception rates. Breed (82.3, 93.8, 75.0, and 90.0% for Columbia, Hampshire, Rambouillet and Suffolk, respectively; P = 0.10) and age (90.1 vs. 80.2% for yearling and peripurberal ewe lambs, respectively; P = 0.09) tended to influence conception rates. Inclusion of supplemental Co in diets of yearling ewes or peripubertal ewe lambs prior to, and early in the breeding season did not influence ADG, d to estrus, or conception rates. Synchronization of estrus with the GnRH and $PGF_2\alpha$ did not influence overall conception rates but tended to increase the proportion of ewes mated by the 10th day of the breeding season to values that would be predicted (62.5%) if all ewes were exhibiting estrous cycles.

Key Words: cobalt, estrus, ewes

Introduction

Ewes that first lamb at one year of age have a greater lifetime production than ewes first lambing at two years of age (Hulet et al., 1969; Botkin et al., 1988). The magnitude of this lifetime difference was estimated at 25% more lambs and 21% more pounds of lamb by Busch and Slyter (1986). The number, however, of ewe lambs expected to reach puberty during the fall breeding season following their birth varies widely (Adam and Robinson, 1994).

The attainment of puberty in most species is influenced by a multitude of factors with the most important factors within breed being age and body weight. Generally, ewe lambs need to be at 50 to 65% of their mature body weight and in good condition before they reach puberty. At the U.S. Sheep Experiment Station anticipated conception rates are 70 to 75% for Rambouillet, 50 to 60% for Targee, and 40 to 45% for Columbia ewe lambs (Glimp, 1989). Goals of the current project were to determine if manipulation of metabolic status with cobalt supplement and induction of ovulation influenced growth performance and conception rates in yearling and peripubertal ewes.

Materials and Methods

Yearling ewes (n = 50) and peripubertal ewe lambs (6-7 months of age; n = 63) were sorted by breed (Suffolk, Hampshire, Rambouillet, Columbia), allocated to 1 of 8 pens (2 pens/breed), and fed control supplement (0.12 kg distillers grain/h/d) or control supplement containing Co (10 mg) for 28 d before and 14 d into the breeding season. All animals received 0.91 kg/d alfalfa hay and 0.91 kg/d grain-based supplement (15% CP, DM basis). All females were weighed prior to and after the cobalt administration period. On d 1 of the breeding season, one-half of the females from each age group in each pen were treated with an ovulatory dose (100 µg) of GnRH. Seven days later, females treated with GnRH received a luteolytic dose (12.5 mg) of $PGF_2\alpha$. Fertile rams fitted with marking harnesses were housed with all ewes from d 1 through 35 of the breeding season. The occurrence of raddle marks were recorded daily and pregnancy status was confirmed by ultrasound 60 d after ram removal.

Conception rate and pregnancy status were analyzed for effects of breed, age, diet, and synchronization using the GENMOD procedure of SAS (SAS Institute Inc., Cary, NC). Effects breed, age, diet, and synchronization on d to estrus and weight gain were tested by analysis of variance using the GLM procedure of SAS.

Results and Discussion

Cobalt is an essential micronutrient that is incorporated into the structure of Vitamin B_{12} by microorganisms in the rumen. Vitamin B_{12} is an important modulator of proprionate formation which is converted to glucose by the liver (NRC, 2007). It was hypothesized that Co supplementation would increase glucose availability resulting in an improved metabolic state manifest by increased weight gain and earlier onset of puberty. However, in the current experiment supplementation with Co did not influence weight gain ($P = 0.49$; Table 1), d to estrus ($P = 0.49$), or conception rates ($P = 0.81$). The possibility that Co supplementation may improve traits of yearling ewes or peripubertal lambs fed less optimum diets remains to be determined.

The estrous synchronization protocol used for this experiment was the "Select Synch" protocol developed for cattle which has the advantage of stimulating ovulation and the onset of estrous cycles in some anestrous animals (Geary et al., 2000). In cattle, 70 to 80% of cyclic cows were in estrus within a 4-d interval following treatment with $PGF_2\alpha$ without any detrimental effects on fertility (Twagiramungu et al., 1995). In contrast, timing of the onset of estrus using the Select Synch protocol in sheep was much less precise. The proportion of ewes in estrus during the 10-d interval following the administration of $PGF_2\alpha$ only tended ($P = 0.07$) to increase from 49.4 to 62.0% and did not differ ($P = 0.36$) among yearling ewes and peripubertal lambs (Table 2). Since approximately 62.5% (i.e. 10 d/16 d estrous cycle) of a group of ewes would be expected to exhibit estrus in a 10-d interval, the Select Synch protocol does not appear to be a precise method of synchronizing estrus in ewes. However, the possibility that estrous cyclic activity was induced earlier in some animals seems apparent (i.e. 49.4 vs. 62.0% of control and synchronized ewes, respectively, were observed in estrus during that 10-d interval). By the end of the 35 d breeding season, however, conception rates did not differ ($P = 0.84$) between control and synchronized animals.

Similar to previous reports, breed (82.3, 93.8, 75.0, and 90% for Columbia, Hampshire, Rambouillet, and Suffolk, respectively; $P = 0.10$) and age (90.1 vs. 80.2% for yearling and peripubertal ewe lambs, respectively; $P = 0.09$) tended to influence conception rates.

In summary, the addition of Co to the diets of yearling ewes or peripubertal ewe lambs prior to, and early in the breeding season did not influence weight gain, d to estrus, or conception rates. Synchronization of estrus with GnRH and $PGF_2\alpha$ did not influence overall conception rates but tended to increase the proportion of ewes mated by the 10th day of the breeding season to values that would be predicted (62.5%) if all ewes were exhibiting estrous cycles.

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Table 1. Weights (kg) of yearling and peripubertal ewes before and after being fed control supplement or control supplement containing Co for 28 d before and 14 d into the breeding season (mean ± SEM)[a]

Ewes	Control supplement	Control supplement + Co
Yearling Ewes, Initial BW	82.3 ± 1.7	84.0 ± 1.4
Yearling Ewes, Final BW	92.3 ± 1.6	94.4 ± 1.4
Peripubertal Ewes, Initial BW	52.0 ± 0.8	58.3 ± 0.9
Peripubertal Ewes, Final BW	51.1 ± 0.7	57.5 ± 0.8

[a]Cobalt did not influence weight gain (*P* = 0.49)

Table 2. Proportion of ewes detected in estrus within 10 d following synchronization with GnRH and $PGF_{2\alpha}$

Ewes	Control ewes (%)	Synchronized ewes (%)
Yearling Ewes	54.2[a]	65.4[a]
Peripubertal Ewes	45.0[a]	59.5[a]
Yearling + Peripubertal ewes	49.4[a]	62.0[b]

[a,b]Means with different superscripts tended (*P* = 0.07) to differ

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECT OF FIELD PEA AND FLAXSEED INCLUSION IN RECEIVING CALF DIETS AND CARRYOVER EFFECT ON FINISHING PERFORMANCE, IMMUNE RESPONSE, CARCASS QUALITY, AND ECONOMICS

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Abstract: One hundred seventy-three medium-frame crossbred steers (initial BW of 293 ± 0.519 kg) were randomly assigned in a 3 year study to evaluate finishing carryover effect when field pea and flaxseed replaced fiber-based ingredients in 50 d receiving calf diets to determine subsequent feedlot performance, immune response, carcass quality, finishing economics, and net return to retained ownership in the cow-calf enterprise. Each year, steers were assigned to one of four pelleted receiving diets: 1) Control (C), 2) 12.5% Flaxseed (FLX), 3) 20.0% Field Pea (PE), and 4) 20.0% Field Pea + 12.5% Flaxseed (PFLX). Pellet NEg was 1.12, 1.26, 1.17, and 1.26 Mcal/kg for C, FLX, PE, and PFLX, respectively. Receiving diet ADG was greater ($P < 0.05$) when FLX occurred in the diet and there was a tendency for improved efficiency ($P = 0.075$) when FLX was present. Feed cost/kg of gain was lowest for FLX and PFLX treatments ($P < 0.05$). Finishing calf receiving weight, harvest weight, DOF, Gain, ADG, ADFI, and G:F did not differ ($P < 0.05$). The effect of receiving diet on carcass measurements did not effect HCW, QG, or USDA % Choice grade ($P > 0.05$). Flaxseed inclusion in the receiving diet was associated with reduced REA ($P = 0.04$), a tendency for greater fat depth ($P = 0.074$), and less favorable YG ($P = 0.083$). Flaxseed has been reported to illicit an immune response that reduces morbidity, increases % IMF, while reducing BF. Replacing fiber-based ingredients with FLX did not reduce either morbidity or medical treatment cost/head ($P = 0.96$). Finishing net return (NR) was effected by yearly fed cattle prices ($P = 0.0001$); however, response due to receiving diet treatment did not differ ($P = 0.943$). Retained ownership NR favored receiving diets treatments that contained field peas. Results suggest that FLX and PFLX are associated with reduced feed cost/kg of gain during the receiving period; however, when retained through finishing, receiving diets formulated with PE and PFLX were associated with the highest finishing net return.

Key words: Beef Cattle, Field Pea, Flaxseed

Introduction

Field peas are a nutrient dense, palatable feedstuff with high rumen degradable protein characteristics that can replace corn and barley in a variety of beef cattle feeding situations (Anderson, 1999; Landblom et al., 2002; Gelvin et al., 2004; Soto-Navarro et al., 2004).

Flaxseed may play a role in enhancing immune resistance reducing morbidity and mortality among calf-feds. Receiving diet research conducted by Drouillard, et al. (2001) compared the value of flaxseed to tallow and suggested that the addition of 10 – 15% flaxseed during the first 5 to 6 weeks after weaning would result in improved feed intake, growth, feed efficiency, and may reduce the incidence of bovine respiratory disease (BRD). Additionally, fat inclusion in the receiving diet from either flaxseed or tallow improved carcass quality grade without a marked increase in subcutaneous fat. Drouillard, et al. (2001) concluded that calves fed flaxseed during the stressful 5-6 week period following weaning illicit stronger immunities and may require less antibiotic therapy.

Previous research with flaxseed has been conducted with corn-based diets. The purpose of this investigation is to evaluate the effect of field pea-flaxseed blends on receiving calf performance, carry-over effect on subsequent finishing performance and carcass merit, immediate and carry-over effect on health status, and feeding economics.

Procedure

One hundred seventy-six steer calves (Angus X Hereford X Gelbvieh) averaging 646 pounds were weaned and randomly assigned to four pelleted receiving diet treatments: 1) fiber-based control (C), 2) fiber-base + 10% flaxseed (FLX), 3) fiber-base + 20% field pea (PE) and 4) fiber-base + 20% field pea and 10% flaxseed (PFLX). Receiving diet supplement nutrient composition and analysis are shown in Table 1. Each treatment consisted of four pen replicates and four steers per pen. Steers were weaned the first week of November and backgrounded an average 50 days at the Dickinson Research Extension Center's feed yard before transfer to a commercial feedyard in Kansas. The pelleted experimental supplements were top-dressed over chopped hay. As daily supplement level was increased, the quantity of chopped hay was reduced until the steers were consuming 9-10 pounds of supplement per day. At the commercial feedyard, the steers were fed to a final harvest end point of 11.43 mm backfat using an electronic cattle management system [Micro Beef Technologies® (ECM)]

To evaluate the effect of flaxseed on health status serum humoral antibody level and BRD incidence were monitored. Three weeks before weaning, calves were vaccinated against economically important bacterial and viral diseases and were administered a booster vaccination at weaning. Blood samples were drawn for serum recovery from the steers 3 weeks before weaning, at weaning, and again 30 days postweaning, and after 60 days in the commercial feedyard. Serum humoral antibody levels for BVD virus types I and II and IBR virus were determined.

In addition, morbidity, mortality, treatment frequency, and treatment cost were recorded.

Receiving, finishing, and carcass data were analyzed using PROC GLM of SAS, percent Choice was analyzed using Chi-square procedures in PROC GENMOD of SAS, and antibody titer data was analyzed using the PROC MIXED procedures of SAS.

Results

Receiving Period:

Three-year performance, efficiency, and economic results for the 50-day receiving-backgrounding period after weaning are shown in Table 2. Since no treatment by year interactions were identified, the data was pooled. Compared to the control diet, supplements that contained flaxseed were 2.09 times higher in fat content and contained 6.2% greater net energy for gain. Average daily feed intake (ADFI) did not differ across treatments ($P = 0.74$). Steers consumed an average 8.4 pounds of the supplement and 9.68 pounds of chopped hay daily. When field pea occurred alone in the supplement, intake did not differ between treatments ($P > 0.10$); however, rate of gain was slower ($P < 0.01$) and feed efficiency was greater ($P < 0.10$) compared to FLX treatments. When flaxseed was included alone in the supplement or as a blend with field pea, inclusion was associated with improved rate of gain ($P< 0.01$) and feed efficiency ($P< 0.10$) when compared to control and field pea test supplements. Economically, compared to control and field pea test supplements, flaxseed and the field pea-flaxseed blended test supplements were associated with the lowest feed cost per pound of gain ($P< 0.01$). Compared to the control, feeding field pea-flaxseed reduced feed cost per unit of gain by 13.1% and compared to field pea alone feed cost per unit of gain was reduced 11.7%.

Finishing Period:

Summaries for finishing animal performance and carcass closeout are shown in Table 3. The ECM system predicted final harvest endpoint with a high degree of accuracy; therefore, harvest weight, days on feed, ADG, ADFI, and feed efficiency did not differ ($P > 0.10$). For carcass, HCW, quality grade, and percent Choice did not differ ($P > 0.10$). When flaxseed occurred in the receiving diet, finishing REA was smaller ($P = 0.044$), fat depth was greater ($P = 0.074$), and YG was negatively impacted, which disagrees with the findings of Drouillard et at. (2001). However, when flaxseed occurred with field pea in the receiving diet, fat depth was similar to other receiving diet treatments. Finishing net return was effected by yearly fluctuations in fed cattle price ($P = 0.0001$); however, response due to receiving treatment did not differ ($P > 0.10$).

Immune Response:

The carryover effect of flaxseed and pea-flaxseed fed during the first 50 days postweaning on antibody titer change and health status during the early finishing period is summarized in Tables 4 and 5. Antibody titer level was low when pre-weaning bacterial and viral vaccinations were administered, increased steadily following the initial and booster vaccinations, but did not differ between dietary treatments for IBR ($P = 0.78$), BVD Type I ($P = 0.11$), and BVD Type II ($P = 0.90$). The incidence of BRD during receiving and finishing was similar for all treatment groups and medical cost did not differ ($P = 0.96$). The results of this study do not agree with that of Drouillard et al. (2002) as in this investigation we were unable to identify stronger immunities and significantly lower medical cost resulting from flaxseed inclusion in the receiving diet 7 weeks before transfer to the commercial feedyard.

Implication

Results suggest that field peas and flaxseed fed during the receiving period improves backgrounding efficiency and reduces feed cost per unit of gain, but during finishing, the carryover effect of flaxseed during the receiving period contributed to increased fat depth and YG was impacted negatively. The data also suggests that when flaxseed and field pea occur together fat depth is not compromised. Similar antibody response, morbidity, and treatment cost during the finishing period suggest that carryover effect resulting from dietary flaxseed inclusion during the 50d receiving period does not appear to enhance finishing immune health status.

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This project was sponsored in part by a USDA/CSREES Cool Season Legume Special Grant #630-01.

Table 1. Receiving diet ingredient composition and nutrient analysis (As Fed).

	Control	12.5% Flax	20% Pea	20% Pea + 12.5% Flax
Flaxseed, %	0.0	12.5	0.0	12.5
Field Pea, %	0.0	0.0	20.0	20.0
Corn, %	15.0	15.0	15.0	10.0
Soybean Hulls, %	21.5	28.803	30.753	34.303
Wheat Midds, %	24.953	11.75	10.0	12.0
Barley Malt Sprouts, %	20.0	15.0	10.0	5.0
Distillers Dried Grain w/ Sol., %	12.25	10.75	8.0	0.0
Other, % [a]	6.297	6.297	6.297	6.297
Analysis:				
Crude Protein, %	15.54	15.54	15.53	15.56
TDN, %	69.08	60.11	70.22	60.58
NDF, %	34.82	33.37	32.93	31.70
NEg, Mcal/kg	0.231	0.258	0.241	0.259

[a]Molasses, 5.0%; Salt, 0.50%; Calcium, 0.55%; Dicalcium Phosphate, 0.10%; TM Premix, 0.075%; Vitamin A & D Premix, 0.025%; Decoquinate Medication, 0.027%; Monensin Sodium, 36.31 gms/kg

Table 2. Three-year effect of receiving diet treatment on 50d weaning transition backgrounding performance.

	Control	12.5% Flax	20% Pea	20% Pea + 12.5% Flax	SE	P-Value
No. Steers	43[a]	43[a]	44a	43[a]		
Initial Wt., kg	292.4	293.3	293.1	293.6	4.34	0.99
50d Final Wt., kg	363.6	371.5	362.9	371.9	4.76	0.38
Gain, kg	71.2[y]	78.2[x]	69.8[y]	78.0[x]	1.99	0.005
ADG, kg	1.42[y]	1.56[x]	1.40[y]	1.56[x]	0.040	0.004
ADFI, kg	8.28	8.34	8.09	8.14	0.183	0.74
Hay/Day, kg	3.88	3.94	3.69	3.74	0.115	0.41
Receiving Suppl./Hd, kg	4.39	4.40	4.39	4.39	0.094	0.99
F:G, kg	5.83	5.34	5.78	5.20	0.090	0.075
Feed Cost/Hd, $	$41.44	$41.20	$39.97	$39.56	0.688	0.17
Feed Cost:kg Gain, $[b]	$0.5820[y]	$0.5269[x]	$0.5726[y]	$0.5072[x]	0.0082	0.012

aOne steer died of bloat.
bMeans in a row with unlike superscripts differ significantly ($P < 0.05$).

Table 3. Three-year effect of receiving treatment on finishing and carcass closeout.

	Control	12.5% Flaxseed	20% Field Pea	20% Field Pea + 12.5% Flaxseed	SE	Trmt.	Year	Trmt x Year
Growth Performance:								
Receiving Wt., kg	356.7	364.5	358.1	366.7	4.36	0.269	0.0001	0.943
Harvest Wt., kg	587.8	582.8	588.3	589.2	6.22	0.898	0.0001	0.481
Days On Feed	147.3	137.0	143.6	141.3	3.91	0.291	0.0001	0.463
Gain, kg	231.1	218.3	230.2	222.5	5.94	0.355	0.0006	0.623
ADFI, kg	8.85	8.87	8.95	8.84	0.155	0.959	0.7790	0.456
ADG, kg	1.568	1.593	1.603	1.575	0.032	0.834	0.0033	0.397
F:G, kg	5.65	5.57	5.59	5.61	0.824	0.609	0.0001	0.888
Carcass Closeout:								
HCW, kg	368.8	366.2	368.9	369.4	4.40	0.955	0.0001	0.284
REA, Sq. Cm.[a]	87.03[xy]	83.23[y]	88.39[x]	87.10[xy]	1.330	0.044	0.0001	0.268
Fat Depth, mm[b]	11.25[x]	13.08[y]	11.79[x]	11.63[x]	0.518	0.074	0.0001	0.063
YG[b]	2.43[x]	2.69[y]	2.39[x]	2.60[y]	0.940	0.083	0.057	0.008
QG	4.70	3.66	3.57	3.77	0.638	0.562	0.124	0.031
Percent Choice, %	60.5	37.2	43.2	44.2		0.112	0.219	0.066
Carcass Value, $	1104.93	1088.67	1106.61	1108.44	18.24	0.862	0.0001	0.015
Calf & Feed Cost, $	1096.03	1086.30	1093.38	1095.39				
Net Return, $	8.90	2.37	13.23	13.05	17.85	0.943	0.0001	0.017

aMeans in a row with unlike superscripts differ ($P < 0.05$).
bMeans in a row with unlike superscripts differ ($P < 0.10$).

Table 5. Effect of dietary field pea and flaxseed replacement on humoral antibody titer values. [a, b]

	Pre-Wean	Weaning	30-Day Post-Weaning	60-Day Finishing
Vaccination	Initial	Booster		
Calf Age/Serum Recovery, days	183	203	233	293
Control				
IBR[c]	4^{d}	22^{e}	13^{f}	11g
BVD Type I[c]	4^{p}	58^{q}	63^{r}	51^{s}
BVD Type II[c]	4^{w}	175^{x}	200^{y}	204^{z}
12.5% Flaxseed				
IBR[c]	4^{d}	18^{e}	13^{f}	7^{g}
BVD Type I[c]	4^{p}	67^{q}	117^{r}	83^{s}
BVD Type II[c]	4^{w}	144^{x}	298^{y}	184^{z}
20% Field Pea				
IBR[c]	4^{d}	19^{e}	11^{f}	8^{g}
BVD Type I[c]	5^{p}	36^{q}	79^{r}	55^{s}
BVD Type II[c]	4^{w}	92^{x}	312^{y}	221^{z}
12.5% Flaxseed + 20% Field Pea				
IBR[c]	4^{d}	17^{e}	10^{f}	11^{g}
BVD Type I[c]	4^{p}	102^{q}	119^{r}	95^{s}
BVD Type II[c]	4^{w}	123^{x}	200^{y}	154^{z}

[a]Treatment and treatment * time interaction means did not differ significantly.
IBR: Treatment (P = 0.78); Treatment * Time (P = 0.89)
BVD Type I: Treatment (P = 0.11); Treatment * Time (P = 0.30)
BVD Type II: Treatment (P = 0.90); Treatment * Time (P = 0.86)
[b]Serum antibody titer value change over time was significant.
IBR: Time (P = < 0.0001)
BVD Type I: Time (P = < 0.0001)
BVD Type II: Time (P = < 0.0001)
[c]Means in a row with unlike superscripts differ significantly (P < 0.0001)

Table 6. The carryover effect of 50-day postweaning receiving dietary treatment on feedlot morbidity and medical cost.

Dietary Treatment	Morbidity, %	Medical Cost/Head[a]
Control	35.4	$26.43
Flaxseed	34.7	$28.70
Field Pea	38.9	$25.83
Field Pea-Flaxseed	36.1	$23.41

[a]Means did not differ: Treatment (P = 0.96); Year (P = 0.08); Treatment * Year (P = 0.20)

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

READABILITY OF THIRTEEN DIFFERENT RFID EAR TAGS BY THREE DIFFERENT MULTI-PANEL READER SYSTEMS FOR USE IN BEEF CATTLE

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ABSTRACT: The objectives of this research were to compare the readability of thirteen different radio frequency identification (RFID) ear tags scanned by three different multi-antenna alley readers (single-lane, dual-lane and multi-animal) using 82 Angus heifers. The readability and flow rates of the heifers were measured through either an 1) Allflex Single-Lane Multi-Panel RFID Reader (ASL; 63.5 cm x 3.05 m), 2) an Allflex Dual-Lane Multi-Panel Reader (ADL; 81.8 cm x 3.06 m) or 3) a Boontech Alley Master Multi-Panel Multi-Animal Alley Reader (BAM; 1.37 m x 9.7 m). Three of the RFID tag brands were half-duplex (HDX) technology: Allflex, Dalton, and Leader while the remaining 10 RFID brands were full-duplex (FDX) technology: Allflex, Animal Profiling Inc. (API), Dalton, Destron1, Destron2, Leader, Verilogic, Y-Tex1, Y-Tex2 and Z-Tag. Observations were measured at different time periods. A total of 5570 observations were collected through the ASL system, 3280 through the ADL system and 3280 through the BAM system. Due to the differing alley dimensions, cattle moved through the three systems at different ($P<0.05$) flow rates (1.3, 2.1 and 8.7 m/sec) for ASL, ADL and BAM, respectively. When the readers were fully tuned and upgraded, readability did not differ ($P>0.05$) for the ASL (99.4%), ADL (99.5%) or BAM (99.7%) systems. Readability of the nine top performing HDX (98.2%) and FDX (96.5%) RFID tags did not differ ($P>0.05$). All flow rates met the National Animal Identification System (NAIS) standards (1 m/sec) set by the USDA. All readers met USDA 95% readability standards when fully tuned and upgraded. Results indicate that it is possible to get read rates that follow standards set by the USDA.
Keywords: Ear Tag, NAIS, Panel Reader, Radio Frequency Identification

Introduction

Currently, the US beef industry has an obsolete system of plastic tags and paper files, coupled with limited man power to deal with a potential disease outbreak and traceback in any logical response time (Ringwall, 2007). Increased requirements by international and domestic markets for source, production practice, and age verification as well as traceback to validate food safety and quality (Blasi et al., 2001; Basarab et al., 2006) demonstrate the need for the development of a more reliable form of cattle identification. Animal Health Australia (2003) recognized that the use of individual animal identification, such as RFID ear tags, could serve as a rapid traceback/trace forward tool and also offer the opportunity for improved surveillance and control of livestock diseases. The ability to trace animal movements and associations will provide a critical tool for animal health professionals in controlling and potentially eradicating diseases such as: BSE, brucellosis, and foot-and-mouth (Basarab et al., 1997; Bailey, 2004).

The key to the National Animal Identification System (NAIS) is rapid multi-animal RFID scanning systems that work automatically and non-invasively at auction markets and slaughter facilities. In addition such a system could facilitate the rapid transfer of valuable management and production information from farm to feedlot, packer, processor and consumer (Basarab et al., 2006). However, the recent pushback from a mandatory program to a voluntary program at the national level (APHIS, 2006) due to lack of interest and funding has not changed the need for a reliable identification system. Before application can occur in production settings, the technology must be tested to determine if scanning RFID tags at the speed of commerce (1m/sec) with 95% readability rates (UDSA, 2007) can be attained.

The objectives of this study were to: 1) compare the readability rates of three commercially available multi-antenna reader systems for their speed, accuracy and reliability in reading both half-duplex and full-duplex RFID tags; and 2) evaluate the readability rates of 13 different electronic ear tags.

Materials and Methods

General

Three experiments were carried out to determine readability and flow rates through three different multi-panel scanning systems. This study design was similar to published research from Lacombe Research Center, Alberta CAN (Basarab et al., 2006). Each experiment consisted of five lots of 15-17 Angus heifers (Exp. 1: 432 kg; 15 mo of age, Exp. 2: 295 kg; 9-10 mo of age, Exp. 3-5: 364 kg; 12-13 mo of age). The average temperature for Exp. 1a was 20.5°C, 18°C for Exp. 1b, 5.2°C for Exp. 2 and 5.3°C for Exp. 3. Each animal was tagged with a prescanned (using a handheld wand reader) RFID tag. The RFID tags were either half-duplex (HDX; Allflex USA, Dalton EU, Leader AU) or full-duplex (FDX; Allflex USA, Digital Angel [Destron] USA, Y-Tex USA, Verilogic USA, Dalton EU, Animal Profiling Inc [API] USA, Leader AU, and Farnam [Z-Tag] USA) ear tag technology. The tags were inserted in the left ear at the orientation suggested by the manufacturer. The test consisted of moving cattle from each tag-type lot through a multi-panel RFID reader system (Allflex Single-Lane [ASL], Allflex Dual-Lane [ADL] or Boontech Alley Master Multi-Animal [BAM; AU]) five

times before commingling all tag-type lots for five more runs through the three readers. This was replicated on two separate days for each experiment (Basarab et al., 2006).

When the multi-panel RFID reader detected the animal's RFID tag, the software on the computer recorded the 15-digit number. If the animals' RFID tag was not detected no number was recorded. The number of positive scans per tag-lot was recorded manually as well as the amount of time taken for each lot to move through the readers (sec/lot).

Statistical Analysis

Data collected were analyzed using the Chi-Square (PROC FREQ) analysis and GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Differences in readability of reader systems and tags were analyzed using LS means. Least squares means were subjected to a means separation test using PDIFF (SAS, 2003). Cattle flow rate through each reader system was subjected to analysis of variance and LS means.

Results and Discussion

The first system tested, Allflex Single-Lane (n=5570; Table 1), had an initial scanning rate of approximately 60% in June, 2006 and improved to 100% by the completion of the evaluation. This was due to redesigning the chute by removing excess metal around the panel readers. Differing results are best demonstrated by Exp. 1a vs. Exp. 1b where read rates were higher after the chute was modified and the scanner was upgraded. The Allflex HDX and Destron1 FDX tags had the highest read rates (100%), while the Verilogic FDX had the lowest read rate (38.5%; $P<0.05$). No differences were measured in the readability of various tags for Exp. 2 or Exp. 3.

The second system evaluated, Allflex Dual-Lane (n=3280; Table 2), measured no differences in read rates for the nine various tags tested. Readability rates were in excess of 98%, and were above the guidelines described by the NAIS (USDA, 2007).

The third system evaluated was the Boontech Alley-Master reader system from Australia (Table 3). Initially (Exp. 2), readability of the five RFID tags was poor (82.8%; $P<0.001$) after the system was installed. Refining the software and repositioning the panels increased ($P<0.001$) read rates (>99%; Exp. 3).

No difference in average readability was measured among the three systems (>99%) when they were upgraded and fully tuned (Table 4). Commingling HDX and FDX tags did not have an affect on readability. Readability of the three HDX tags did not differ (99.7%), while readability of the 10 FDX tags was higher ($P<0.05$) when scanned by the BAM system (99.9%) compared to the ASL (99%) and the ADL (99.3%) reader systems. These data do not include results from the two poorest performing RFID tags (Y-Tex1 and Verilogic). There were differences ($P<0.05$) in readability among the 11 commercially available tags tested (Fig. 1).

Cattle moved through the reader systems at different ($P<0.05$) speeds (Table 4). The BAM system had the fastest flow rate at 8.7 m/sec (31.3 km/hr; SD=9.7) while the ASL system had the slowest flow rate at 1.3 m/sec (7.0 km/hr; SD=0.76). The ADL system was intermediate with an average flow rate of 2.1 m/sec (7.6 km/hr; SD=1.4). Basarab et al. (2006) recorded cattle flow rates of 10.4 km/hr using the ADL system. Results indicate that it is possible to keep the flow of cattle at the required speed (1m/sec) according to the USDA (2007) standards.

Implications

Significant improvements in read rates from the first experiment to the final experiment were measured. All reader systems, once tuned properly with updated technology, had read rates of over 99%, and were able to withstand various environmental temperatures. These reader systems have the capability to work at the speed of commerce. However, reader technology still needs to be enhanced so that the readers function accurately, consistently, and without disrupting the speed of commerce.

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Table 1. The effects of day and tag-type on RFID read rate and cattle flow rate using an Allflex Single-Lane Multi-Panel RFID Reader System.

Main Effects	Cattle Observ.	Scan Rate (%)		Cattle Flow (m/sec)
	5570	81.4		1.3
Tag-Type				
Experiment 1 (June, Sept.)		***1a****	***1b*****	
Allflex HDX	160	99.4^{g}	100.0^{g}	1.12^{i}
Allflex FDX	160	48.8^{bj}	89.3^{ai}	1.17^{h}
Destron1	150	94.7^{bh}	100.0^{ag}	1.06^{j}
Verilogic	150	21.9^{bl}	38.5^{ak}	1.26^{g}
Y-Tex1	150	28.7^{bk}	84.1^{aj}	1.06^{j}
All Tags	780	62.2^{bi}	97.0^{ah}	1.06^{j}
Significance		**<0.001**	**<0.001**	**<0.001**
Experiment 2 (Nov.)				
Allflex HDX	160	100.0		1.31^{hi}
Dalton HDX	170	100.0		1.30^{i}
Dalton FDX	170	100.0		1.30^{i}
Destron2	160	100.0		1.32^{hi}
API	160	100.0		1.37^{g}
All Tags	820	100.0		1.33^{h}
Significance				**<0.001**
Experiment 3 (Feb.)				
Allflex HDX	320	100.0		1.23^{hi}
Leader HDX	340	100.0		1.30^{g}
Leader FDX	340	97.1		1.24^{h}
Y-Tex2	320	99.4		1.22^{ij}
Z-Tag	320	98.1		1.21^{j}
All Tags	820	99.4		1.24^{h}
Significance		**0.091**		**<0.001**

^{ab}Means in same row without common superscript differ (P<0.05).
^{ghijkl}Means in same column without common superscripts differ (P<0.05).
Before (1a*) and after (1b**) unneeded metal was removed and the scanner technology was upgraded.

Table 2. The effects of day and tag-type on RFID read rate and cattle flow rate using an Allflex Dual-Lane Multi-Panel RFID reader system.

Main Effects	Cattle Observ.	Scan Rate (%)	Cattle Flow (m/sec)
	3280	99.5	2.1
Tag-Type			
Experiment 2 (Feb. 9, 22)			
Allflex HDX	160	100.0	1.94^{i}
Dalton HDX	170	100.0	1.99^{g}
Dalton FDX	170	98.8	2.00^{g}
Destron2	160	99.4	1.91^{j}
API	160	98.1	1.99^{g}
All Tags	820	99.4	1.96^{h}
Significance		**0.396**	**0.005**
Experiment 3 (Feb. 26, 27)			
Allflex HDX	160	98.8	1.99^{h}
Leader HDX	170	98.8	1.90^{i}
Leader FDX	170	100.0	2.03^{gh}
Y-Tex2	160	98.8	2.01^{gh}
Z-Tag	160	100.0	2.05^{g}
All Tags	820	99.9	2.00^{h}
Significance		**0.577**	**<0.001**

^{ghij}Means in same column without common superscripts differ (P<0.05).

Table 3. The effects of day and tag-type on RFID read rate and cattle flow rate using a Boontech Alley-Master RFID reader system

Main Effects	Cattle Observ.	Scan Rate (%)	Cattle Flow (m/sec)
	5570	92.4	8.66
Tag-Type			
Experiment 2 (Nov. 21, 22)			
Allflex HDX	160	89.4^{h}	10.1^{g}
Dalton HDX	170	91.2^{h}	9.07^{k}
Dalton FDX	170	98.8^{g}	9.24^{j}
Destron2	160	98.8^{g}	9.90^{h}
API	160	68.8^{j}	9.90^{h}
All Tags	820	80.61^{i}	9.60^{i}
Significance		**<0.001**	**<0.001**
Experiment 3 (March 13, 23)			
Allflex HDX	160	100.0	7.03^{g}
Leader HDX	170	98.8	6.51^{i}
Leader FDX	170	100.0	7.03^{g}
Y-Tex2	160	99.7	6.06^{k}
Z-Tag	160	100.0	6.26^{j}
All Tags	820	99.8	6.60^{h}
Significance		**0.784**	**<0.001**

^{ghijk}Means in same column without common superscripts differ (P<0.05).

Table 4. A summary of the three experiments comparing readability and flow rates of cattle tagged with eleven top performing RFID ear tags while moving through three different optimally tuned, upgraded multi-panel reader systems.

Item		Reader Manufacturer: Allflex Single-Lane	Allflex Dual-Lane	Boontech Alley-Master
Average Readability of all Tags (%)		99.4	99.5	99.7
Readability of Tag Frequencies (%)	**HDX**	100.0^{g}	99.6	99.4
	FDX	99.0^{ah}	99.3^{a}	99.9^{b}
Flow Rate (m/sec)		1.3^{a}	2.1^{b}	8.7^{c}

^{abc}Means in same row without common superscript differ ($P<0.05$).
^{gh}Means in same column without common superscripts differ ($P<0.05$).

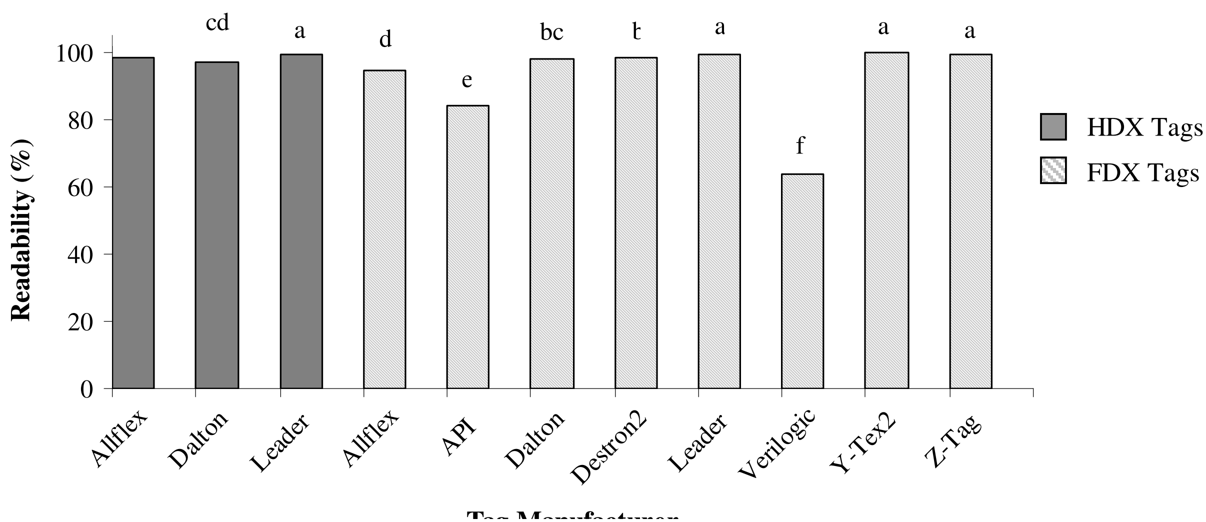

Figure 1. Average read rate of 11 commercially available RFID ear tags scanned by three RFID panel reader systems.

Proceedings, Western Section, American Society of Animal Science

Vol. 58,2007

THE EXTENSION SERVICE AND THE BEEF INDUSTRY: YESTERDAY VS. TODAY

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ABSTRACT: In 1914, the Smith-Lever Act mandated a partnership between agricultural colleges and the USDA to provide for cooperative agricultural extension programs. Agricultural extension work was originally developed to provide applications of research through practical demonstrations. While research is often very specific and narrow in scope, extension is often broader is scope with regard to problem solving. Traditionally, university animal scientists have had many of the same common goals as the beef industry. However, producers sometimes have had the perception that academic arrogance, discipline myopia, uncoordinated research, slow technology transfer, increasing research costs, and counter-productive tenure systems prevent animal scientists from being as responsive as they could be. Extension programs must be available to all, but survival will depend on being both highly useful and well marketed. To illustrate this, a recent study from FL showed that the top three sources of information for beef producers were 1) other producers, 2) county agents, and 3) veterinarians. The sources of information preferred were 1) newsletters, 2) cattle magazines, and 3) extension bulletins. The value of extension is demonstrated by a rancher who said his worst ranch decisions were based on tradition, anecdotal information and the seat of his saddle while the best decisions were based on sound business principles, research-based information and principles of integrated research management. Producers have supported the university through legislative actions. While these traditional funding sources declined or became stagnant, the response has been to seek out gifts, grants, and user fees to supplement traditional funding and to develop additional partnerships. These sources of monies increased 64.4% from 1990 to 1996. Keeping the beef industry from consolidating by preserving old land-grant approaches is illogical. For example, does every state need a specialist in every discipline? Extension has and does make scholarship in the land-grant universities better and more relevant than it would otherwise be because it solves real problems.
Key words: Extension Service, History of CES

The Past (1914-2000)

The Beginning of Cooperative Extension Service (CES). When President Wilson signed the Smith-Lever Act on May 8, 1914, he called it "one of the most significant and far-reaching measures for the education of adults ever adopted by the government". Its purpose was "to aid in diffusing among the people of the US, useful and practical information on subjects related to agriculture and home economics ……" (Rasmussen, 1989). The "cooperative" in the service's title is a reference to its funding, which was and is provided by local, state, and federal sources.

The early directors of the nations' experiment stations realized that they needed to reach farmers if they were to have the continued support and funding from state legislatures. To demonstrate their value to the farmer, many experiment stations issued popular bulletins and leaflets. These reports were a mix of scholarly articles and farm oriented papers. However, most colleges published papers which were to be read by other scholars. While most farm people were literate, many were not comfortable with the printed word and by tradition distrusted "book farming" (Rasmusson, 1989).

Seaman Knapp has often been referred to as the "father" of the Extension Service. During the early 1900's the boll weevil threatened to destroy the cotton industry. The USDA developed a plan to control the weevil, but few farmers followed the recommendations. Knapp, then 70 years old had been a farmer, professor, and president of Iowa Agriculture College. He was convinced that reading pamphlets or observing work on demonstration farms operated at government expense would not cause farmers to change their practices. Knapp decided that the only way to change farming practices was to conduct the research on the farmers own land. His philosophy became "What a man hears, he may doubt; what he sees, he may possibly doubt; but what he does, he cannot doubt". This approach was highly successful and this method of technology transfer is still practiced today. During the 1920's the important issues for county agents included eradicating bovine TB, farm management, transportation, storage and marketing, cooperatives, nutrition, health and welfare.

The original mission of the CES was: "… to aid in diffusing among the people of the United States useful and practical information on subjects relating to agriculture and home economics, and to encourage application of the same …" A more recent mission statement was: "… to enable people to improve their lives and communities through learning partnerships that put knowledge to work." (Ahearn et al, 2003). Why the change? When Cooperative Extension was started in 1914, about 30% of US workers were employed in farming compared to today where approximately one percent of the hired workforce is in farming. Starting in the 1980's, the Extension System shifted from a focus on audience to a focus on issues. The seven base programs emphasized were 1) 4-H and youth development; 2) Agriculture; 3) Community Resources and Economic Development; 4) Family Development and Resource Management; 5) Leadership and Volunteer Development; 6) Natural Resources and Environment Management and 7) Nutrition, Diet and Health (Ahearn et. al, 2003). However, during the 1990's the Extension

System was charged to develop and implement an accountability system based on five issue-oriented goals which were: 1) an agricultural production system that is highly competitive in the global economy; 2) a safe, secure food and fiber system; 3) healthy, well-nourished population; 4) a greater harmony between agriculture and the environment and 5) enhanced economic opportunity and quality of life. The US society is now more diverse because urban populations have continued to increase, yet the demand for affordable food continues. Today land use, obesity prevention, responsible use of pesticides, urban revitalization, non-agriculture commerce, and specific attention to the needs of underserved audiences are among the expanded Extension programs (Bull et al., 2004). These changes in goals and (or) focus have not been without discussion as to the present direction of CES. Some observers believe that Extension has experienced mission creep and should return to a focus on agriculture (Peters, 2004) while others argue that Extension is a "captive" of agriculture interests and should serve a broader national purpose (McDowell, 2001).

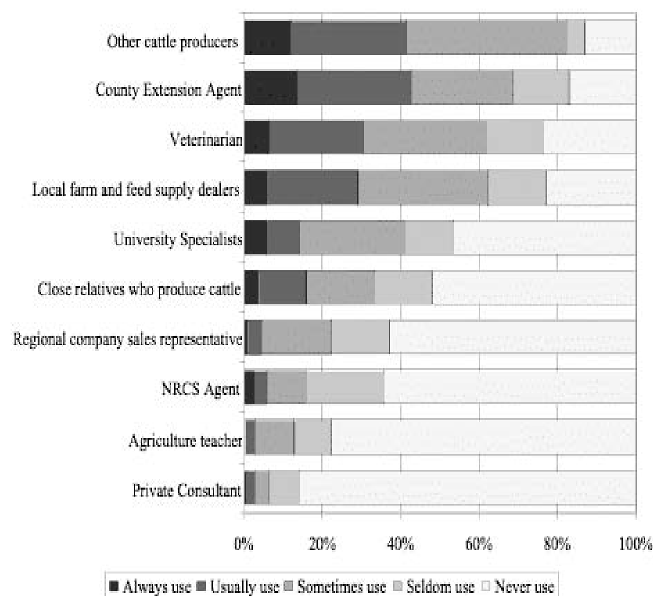
The Present

How has the beef industry changed? The largest 9% of cow/calf producers generate 51% of weanling calves while the largest 2% of feedlot operators produce 85% of finished steers/heifers. The top five packing companies, the top ten supermarket chains, the top ten foodservice distributors and the Top Ten restaurants have market shares of 78%, 55%, 45% and 30%, respectively, of beef or food sales. On average, large farms/ranches have lower production costs and realize higher commodity prices; because large family farms/ranches tend to be more profitable, their share of production is expected to continue to increase. Cow/calf producers reported that the "Top Beef Challenges in the next two to three years were: (a) assuring beef quality and safety, (b) consumer demand and perceptions, (c) improved efficiency and productivity, (d) improved animal productivity, (e) financial management, and (f) staying current with technology (Smith, 2005). Successful operations tend to be growing in capacity, are systems-oriented, maintain high throughput, keep accurate records, use outside consultants, and control production costs. Modern livestock systems have lowered the cost of production by integrating innovative management technologies. But, in order for producers to be successful in the future, access to technology, capital, and timely information will be critical. (Meeker, 1999). Dunn (2003) said that when he managed the family ranch, his worst decisions were based on tradition, anecdotal information and the seat of his saddle. His best decisions were based on sound business principles, research-based information and principles of integrated research management.

Can Extension Compete in Delivering Information and Education? As the value of information increases, sources of that information are changing. Farmers have more choices, are better educated, and farm larger tracts of land than previous generations. Public information sources such as the CES may have dominated in the past, but information from private sources, such as agribusinesses and commercial crop and market advisers, offer strong competition (Boehlje and King, 1998). However, internet technology has created the ability to provide and promote access to expertise and learning opportunities that weren't realistic before. Because of this, the traditional education market is also more easily accessible for competitors—other universities, private education developers, and commercially based education and training organizations.(King and Boehlje, 2000)

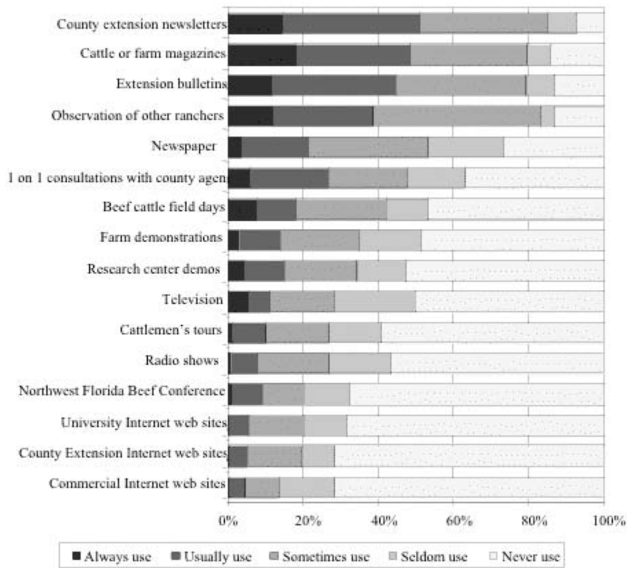
The choice of delivery methods can have an important influence on the impact of Extension programs. The effectiveness of delivering Extension programs can be increased by matching the information sources and channels used by Extension to those preferred by the clientele (Israel, 1991). Figures 1 and 2 show the preferred sources of information (other producers, county agent, veterinarian) and the preferred methods to receive the information (newsletters, magazines, bulletins). Notice where University Internet web sites rank in Fig. 2 compared with newsletters, magazines and bulletins. These data also reemphasize what Knapp observed almost 90 yr ago; ranchers like to get their information from other ranchers.

Figure 1. Preferred Sources of Information by Beef Cattle Producers (Vergot et al., 2005)



Who Pays for Extension? The CES has been a cooperative effort among the federal government, state government and local communities. Although there have been shifts in funding sources, the USDA, CSREES (2001) reported that in 2000, 49 percent of the $1.7 billion budget came from the States, 24% from the Federal Government and 27% from local governments. The University of WY (2002) reported in a strategic plan for CES that in counties where an educator or other position is vacant, this has caused serious problems because the organization is currently using 86 percent of its resources for personnel.

Figure 2. The preferred channels of information for beef cattle producers (Vergot et al., 2005)



Financial support for the organization, from technology to travel and professional development, is inadequate. This system claims to have the lowest starting salaries of any system in the West.

In 2003 more than 85% of Extension programs reported receiving at least 65% or more support funding from state or territorial resources. Of significance was that 20% received more than 80% of program funding from state or territorial resources. Legislative governing bodies clearly are supporting the Extension outreach program as never before and at the same time are demanding more accountability in meeting state and territory priorities at the expense of national goals and initiatives (Payne, 2005). Other western Land Grant Universities are facing similar challenges and as traditional funding sources become stagnant or decline, many Extension organizations are looking for gifts, grants, and fees for services to supplement or replace traditional funding sources. Monies from user fees, grants, and contracts have been the fastest growing source of funds and increased 64.4% from 1990 to 1996. Total Extension funding increased only 17.3% during that same period. Massachusetts' goal for the next decade was to have 50% of Extension funding derived from grants and user fees (Jackson and Johnson, 1999).

After 25 years as a University Professor, I would not have survived the promotion/tenure requirements had it not been for external grants received from the feed and pharmaceutical companies as well as commodity organizations. But, what are the implications of accepting external funds? First, the non-university partners have demands for high quality products, and you must meet or exceed their expectations if you take the money. Second, does accepting these grant monies fit with the mission of the University and require future financial commitments?

Opinions on the Land Grant Institution's Covenant with the Producer. Animal scientists have many common objectives with livestock producers. Their work in research, teaching, and extension is critical for continued progress. However Meeker (1999) expressed the opinion that producers sometimes have the perception that academic arrogance, discipline myopia, uncoordinated research, slow technology transfer, increasing research costs, and counter-productive tenure systems prevent animal scientists from being as relevant and responsive as they could be. The problem of discipline myopia—the inability of animal scientists to cooperate, understand, or even communicate beyond the boundaries of their traditional discipline—is perceived as a problem. Technology transfer is considered to be too slow, caused by basic researchers not communicating with extension personnel and resulting in agents who are not "up-to-date". Academic arrogance, the tendency of Ph.D.s to "talk down" to people and the tendency of some university faculty to consider their position in society far above that of industry workers with equal education, hinders communication and cooperation within the livestock industry. The tenure system is considered to be antiquated. Young faculty are seen as spending too much time and effort attending to the details of achieving tenure, and these activities are often seen by livestock industries as irrelevant. Faculty with tenure are often viewed as unduly protected from the realities of the changing global economy and recalcitrant to react to the forces that dictate a faster response in other sectors of society (Meeker, 1999; Vanderwort, 2005)

Was moving away from communicating with producers intentional? In my opinion it was not, but current circumstances have created changes at universities that cause me concern. McDowell, (2002) believes current behavior is partly caused by 1) state legislatures not providing the amounts necessary to sustain research, 2) much of the funding for research is via competitive federal grants, 3) results paid for by corporations have value in the private sector if they can capture control of the findings; and 4) universities are in a mad scramble for funds from the NSF, the NIH, and private corporations. "Publish or perish" is a passé concept and has been replaced by "patent or perish" for many faculty. An example is the reported selling of a whole department's research output to a single corporation like the University of California-Berkley did with Navartis.

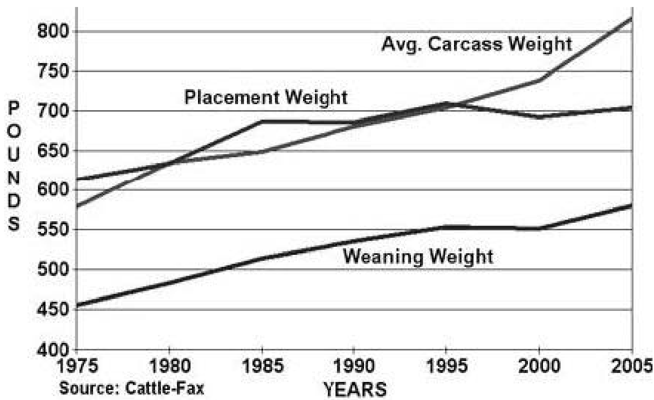
The Future

Challenges for the Beef Producer. From 1986 until 2007, approximately 237,000 beef producers left the industry (Trends, 2007). However, the total number of cows is just 2.3% smaller than 1986 suggesting that more cows are in fewer hands and the size of operations are increasing. If you study the trends (Fig. 3), it appears that the beef industry will continue to produce calves with heavier weaning weights, heavier carcass weights and to a lesser degree placement weights of feeder cattle.

The 2005 National Beef Quality Audit indicated that while the percentage of choice quality cattle improved, the percentage of yield grade 4 cattle likewise became poorer. Was this decline in yield grade a result of feedlot management and/or genetic selection? Smith (2006) presented several beef industry goals to be achieved by 2010. Among the goals were: 1) to clarify beef market

signals that encourage production of cattle, carcasses and cuts that conform to industry targets; 2) to move expeditiously toward source and age verification to build supply lines of cattle to fit domestic and export markets; 3) to minimize production of excess fat, 4) strive for

Figure 3. Changes in weaning, placement and carcass weights, 1975-2005



uniformity/consistency in cattle production; 5) consider tenderness in genetic and management decisions and 6) foster communication among sectors of the beef supply chain. Purcell (2002) recommended that for the beef industry to remain healthy it must 1) further improve production efficiency and keep production costs down; 2) continue to invest in new product and new market development; 3) continue to develop a more open perspective on the value of exports; and 4) continue to price cattle on an individual carcass merit basis.

Challenges for Beef Cattle Extension. Extension is a process where the credibility of the agent/specialist giving the advice is an important factor in the weighting that ranchers assign to that advice. Credibility is developed over time through provision of nonbiased, practical, useful answers that assist producers in the day-to-day farm operations (Vanclay, 2004). We have recognized that we can't often provide one-on-one advice so we work to provide multiple methods of delivering extension information such as fact sheets, field days, ranch demonstrations, TV, radio, and evening seminars. I have believed in partnering with feed and pharmaceutical companies, commodity representatives, beef committees and the local banks to provide educational seminars.

Factors such as advances in internet technology, changes in the numbers and types of clientele, increased operating costs, and reduced funding require significant changes in resource allocations, organizational structure, and the way Extension conducts its future business (Washington and Fowler, 2005).

In summary "… players who feel challenged and scared may be the ultimate winners. Those who don't yet feel challenged and are not scared don't have a chance." (King and Boehlje, 2000). It is my belief that being an extension specialist maybe the best possible occupation that a young graduate could ever have.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

ASSESSING THE INDUSTRY'S NEEDS: A PRODUCTION PRACTICES SURVEY OF COW-CALF PRODUCERS IN NORTHEASTERN OREGON

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ABSTRACT: Upon arriving in Baker County as the new Agriculture Extension Agent for Oregon State University in the fall of 2005 I mailed out a cow-calf production survey to livestock producers in Baker and Union Counties. This 27 question survey was modified from an earlier 22 question survey that Dr. Dave Bohnert et al. utilized in 2004. Objectives of this survey were 1) to better understand current cow-calf production practices and 2) to enhance Extension Livestock educational efforts in Northeastern Oregon. A total of 415 surveys were mailed out to livestock producers in Northeastern Oregon. A total of 103 surveys were returned and are included in this evaluation (Baker=72; Union=31). Ninety-two percent of the respondents stated they were a commercial cow/calf operation. Herd sizes varied widely with 13, 24, 29, 21, and 13% of respondents listing 0-50, 51-200, 200-400, 400-1000, and greater than 1000 head respectfully. Fifty-eight percent of respondents always cull open cows. Of the 42% (43 respondents) that do not cull all open cows, the top 3 reasons for keeping an open cow were young or proven (33%), production history (17%), and if the cow lost her calf through no fault of her own (11%). The most frequent culling rate for cows was 10-15% (53%), while 55% of survey respondents reported annual mature cow death loss to be less 0.5%, with 0.5-1% accounting for 34% or producers. Seventy-six percent of producers raised their own replacement heifers, with 63% rating their heifer development program as excellent and 37% stating theirs needs improvement. When asked to describe their heifer replacement rate, 57% of respondents stated they replace a constant percent every year, with 18% stating they replace at the same rate as they cull, only keeping enough to maintain cow numbers. The most common annual cow cost was $251-$300 (26%), with $301-$350 and $201-$250 accounting for 24 and 16% respectfully. Results of this survey will be utilized to better understand current cow-calf production practices, and to assist in addressing and developing future Extension educational efforts in Northeastern Oregon.

Key words: Northeastern Oregon, Cow-Calf, Production Survey

Introduction

The task of assessing livestock producers' educational needs for an Extension Agent is a very important task but is also quite difficult and time consuming. Upon arriving in Baker County Oregon as the new Extension Ag Agent for Baker and Union Counties, I wanted to survey the local cow/calf producers to assess their current production practices, and educational background in order to better provide for their educational needs relating to livestock production. I felt that an understanding of current production practices by cow-calf producers was necessary to develop effective Extension programs aimed at improving the economic efficiency of beef production in the Northeastern Oregon.

I modified a previous 22 question survey utilized by Bohnert et al., (2005) into a more comprehensive 27 question survey developed to obtain information on current cow-calf management practices. The results of this survey will allow Oregon State University Extension personnel to better understand beef production practices in Northeastern Oregon and develop Extension beef programs that address current educational needs and/or deficiencies in production practices.

Materials and Methods

The production practices survey was modified and sent to cow-calf producers in Baker and Union Counties in Northeastern Oregon. The survey posed 27 questions related to cow/calf management, female replacement programs, bull management, and annual cow-herd economics. The survey was mailed to 415 individuals who were either members of the Oregon Cattleman's Association or were on the local County livestock mailing lists. The mailings included a self addressed form that allowed the survey respondents to mail the completed surveys back in complete anonymity.

As of January 1, 2007, the cutoff date for this report, 103 surveys had been returned (Baker 72; Union 31). Information from each response was entered into SPSS 13.0, 2004 (SPSS Inc., Chicago, Illinois) database to facilitate data summary and analysis.

Survey Results

General Respondent Information

Although the survey results varied between counties the survey results from both counties are combined for this report. We received 72 and 31 surveys back from Baker and Union counties respectfully. Though often different from Bohnert et al. survey results, our results were striking similar in many ways.

The average herd size varied widely, with the most common herd being 301-400 head which was reported by 15.7% of respondents (Table 1). The least common herd size, 51-100, 401-600 and 601-1,000 head, was noted by 10.5% of respondents.

We listed seven categories for type of beef operation, and respondents were asked to check all that applied. Many respondents listed multiple operation types, indicating the diverse nature of beef production operations in Northeastern Oregon. Categories that we provided on the survey, with the proportion of respondents listing each in parenthesis, were: registered seed stock producer (13%), commercial producer (87%), outside year round operator (7.9%), irrigated/improved pasture operator (63%), common allotment range operation (11%), desert range operator (8%).

The two most frequently listed calf weaning weights were 251-272 kg, and over 272 kg which were selected by 37 and 33% of cow-calf producers respectfully (Table # 2). The next two most common weaning weights were 227-250 kg and less than 226 kg by 19 and 11% of producers, respectively.

Culling Practices

The most common cow culling rate was 11-15% which was noted 53% of the time followed by 0-10% which was listed by 32% of respondents. The remaining two culling rates, 16-20%, and varies depending on cattle cycle, were selected by 11, and 3% of producers, respectively (Table 3).

The proportion of respondents that always cull open cows, no exceptions, was 58% (60 of 103 respondents). The reasons given by the remaining 42% of respondents for not always culling open cows are listed in Table 4. It should be pointed out that many of the survey respondents listed multiple reasons for not culling open cows; therefore, the percentages listed in Table 4 were calculated as the number of responses listed for each category divided by the 43 respondents that claimed to not always cull open cows. The majority of responses (33%) listed a young cow as the primary reason to not cull. The next two most frequently stated reasons for not culling an open cow were past performance (17%) and if it was "not the cows fault" that she was open (11%). The remaining reasons for not culling an open cow were listed less than 10% of the time.

Surprisingly when asked if they always cull dry cows that lost a calf but are currently pregnant, 74% of producers responded no with only 24% responding yes with 2% not responding to this question. The top 3 reason given for not always culling dry but pregnant cows are; not the cows fault of her own (34%), young cow (8%), and past performance (5%).

Cow Replacement Programs

The response for annual mature cow death loss was 55, 34, 8, and 3% of survey respondents stating that annual death loss was 0-0.5, 0.5-1.0, 1.0-1.5, and > 1.5%, respectively (Table 5).

Approximately 53% of cow-calf producers stated that they replace a constant percentage of their cow herd each year. This was followed by 18% stating that they replace the same amount as are culled and 11% retaining more cows when cow prices are low. Other considerations listed for determining the annual cow replacement rate included selling more cows when cash was short (5%), keeping more cows when cow prices are high (4%), 8% didn't provide any response (Data not shown).

When asked where they get their replacement heifers, 76% of respondents stated they raise and develop their own. The most frequently listed replacement strategy beyond simply raising their own was a combination of raising their own and purchasing bred heifers (21%), with very few producers choosing to purchase bred cows (3%) (Data not shown).

Heifer replacement programs were rated as excellent by 63% of respondents while 34% felt that they could use improvement. Three percent of cow-calf producers didn't provide a response in relation to their heifer development program.

Annual Cow Cost/Economics

The reported annual cow cost for survey respondents is listed in Table 6. The most common response (26%) was $251-$300 per year. However, there was a wide range in reported cow cost with 65% of all cow-calf producers listing costs between $200 and $350. Almost 19% of producers either didn't know or didn't provide an estimate of their annual cow cost.

Bull Management

Most survey respondents (34%) purchase their bulls from bull sales, followed by private treaty (26%) and only slightly fewer (21%) purchase bulls from a combination of private treaty and bull sale. Therefore, 81% of the survey respondents purchase their bull battery from either a bull sale or by private treaty. Only 3% of respondents listed the local auction barn as the location they purchase bulls and only 10% of producers stated they do a combination of raising their own bulls and purchasing from bull sale. Three percent of survey respondents did not state how they purchased their bulls (Data not shown).

Yearling bulls were preferred for purchase by 47% of cow-calf producers followed by 2-yr olds at 16%. Approximately 8% of respondents purchase both yearlings and 2-yr olds. Interestingly, 29% of producers chose not to respond. Table 7 provides a breakdown of the price that survey respondents pay for bulls.

The responses to our questions regarding annual testing of bulls are provided in Table 8. Briefly, 37% of respondents conduct semen tests only, while 18% have complete breeding soundness exams performed. Only 13% of cow-calf producers test for trichomoniasis and 24% conduct no pre-breeding exams of there bulls at all. Eight percent of respondents provided no data for this question.

The average age that most cow-calf producers dispose of their bulls was 5-yr (37%). The next most frequent disposal age was 6-yr or older, which was selected by 29% of producers. This was followed by 4 (18%) and 3-yr old bulls (5%). Four percent of respondents didn't list a bull disposal age. The remaining respondents selected multiple ages (data not shown).

The most frequently listed cow:bull ratio was 20-25:1, which was noted by 42% of cow-calf producers. The next most common ratios were 15-20:1 (34%) and greater than 25:1 (18%). Approximately 5% of respondents didn't provide a cow:bull ratio (Data not shown).

Cow Reproduction

Spring calving was listed by 79% of producers and fall calving was selected by only 3% of respondents. Interestingly, 18% stated that they had both spring and fall calving cows.

Length of breeding season information is provided in Table 9. Approximately 87% of the cow-calf producers use a breeding period of 90 days or less. Consequently, 13% of respondents listed a breeding season greater than 90 d.

Pregnancy rate is one of the most important performance variables for a cow herd; however, 21% of respondents stated they do not routinely verify pregnancy of their cows. Of the survey respondents who routinely preg-check their cows 79% said their herd's pregnancy rate was greater than 91%, with 13% saying their rate was 86-90%. The remaining 8% reported between 71 and 85% pregnancy rate (Table 10).

Feed Resources

When asked how many tons of hay they feed per cow each year 52% of cow/calf producers reported feeding 2-2.5 ton per year, with 27% reporting 2.5-3 ton per year. Three percent of cow/calf producers reported feeding over 3 ton per year, with 5% reporting feeding 0.5-1, 1-1.5, and 1.5-2 tons each. Approximately 4% of respondents didn't respond to this question. (Table 11.)

Eighty-four percent of cow/calf producers surveyed reported that they raise the majority of their winter feed, with 13% reporting they purchase the majority and only 3% reporting a 50/50 mix of raising/purchasing. (Data not shown).

When asked if they routinely collect and analyze their hay/forage for quality only 32% reported yes, with the vast majority 66% stating that they do not routinely analyze their feed sources for quality. Two percent failed to provide an answer to this question.

When asked if they utilize the forage analysis information when formulating winter rations only 52% of the producers who conducted forage analysis stated they used this information, with the other 48% reporting no to this question.

Calf Marketing Strategies

When asked how they market their calves 26% of cow/calf producers reported selling calves at the local auction yard, followed by 24% retaining ownership through slaughter, 19% selling private treaty, 13% selling through video sales or internet auctions, and the remaining 18% stating they do a combination of all the above. (Table # 12) When asked if they currently participate in a retained ownership / niche marketing program 29% stated yes with the remaining 71% stating no.

Conclusions

Though not totally inclusive I believe the results of this survey will be extremely valuable to the Extension Livestock programming efforts in Northeastern Oregon. To date I have utilized the results of this cow/calf producer survey when working with and talking to local cow/calf producers, local media and members of the allied beef industry. I have presented the results to the local cattlemen/livestock producer groups and will continue to utilize the results of this survey in designing, developing and conducting future livestock education efforts in Northeastern Oregon. Livestock management deficiencies such as not verifying pregnancy of cows, not testing forages for quality, and not conducting breeding soundness exams on bulls are examples of areas that I believe future educational efforts could have substantial positive economic impacts for local cow/calf producers.

Literature Cited

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Table 1. Average herd size of survey respondents[a]

Herd Size, (hd)	Percentage
0-50	13.2
51-100	10.5
101-200	13.2
201-300	13.2
301-400	15.7
401-600	10.5
601-1000	10.5
>1000	13.2

[a] 103 total respondents

Table 2. Average weaning weights[a]

Weaning Weight (kg)	Percentage
< 226	11
227 - 250	19
251 – 272	37
> 272	33

[a] 103 total respondents

Table 3. Annual culling rate[a]

Culling rate (%)	Percentage
0-10	31.6
11-15	52.6
16-20	10.5
Varies Depending on Cycle	2.6

[a] 103 total respondents

Table 4. Reasons for keeping open cows[a]

Reason	Percentage
Young or proven	33.3
Past performance	16.7
Sentimental	5.6
Not cows fault	11.1
Rebreed and sell	6.7
Economics	6.8
Genetic base	8.6
No selection on survey	11.2

[a] 43 respondents; multiple selections by most respondents

Table 5. Annual mature cow death loss[a]

Death Loss (%)	Percentage
0 – 0.5	55.3
0.5 – 1	34.2
1 – 1.5	7.9
> 1.5	2.6

[a] 103 total respondents

Table 6. Annual cow cost of survey respondents[a]

Cow cost, ($)	Percentage
201-250	15.8
251-300	26.3
301-350	23.7
351-400	7.9
> 400	7.9
No selection on survey	18.4

[a] 85 respondents

Table 7. Price paid for bulls by survey respondents[a]

Purchase price, ($)	Percentage
1,001-1,300	10.5
1,301-1,900	10.5
1,901-2,200	15.8
2,201-2,500	5.3
2,501-3,000	23.7
> 3,000	28.9
No selection on survey	5.3

[a] 98 total respondents

Table 8. Bull testing practices of survey respondents[a]

Type of test	Percentage
Trichomoniasis	2.6
Semen	36.8
Trichomoniasis + semen	10.5
Breeding soundness exam	18.4
No testing	23.7
No selection on survey	7.9

[a] 95 total respondents

Table 9. Length of breeding season listed by survey respondents[a]

Breeding season, (d)	Percentage
≤ 45	10.5
45-60	52.6
60-90	23.7
90-120	10.5
>120	2.6

[a] 103 total respondents

Table 10. Tons of hay per cow per year listed by survey respondents[a]

Tons per year	Percentage
0.5 - 1	5.3
1 – 1.5	5.3
1.5 – 2	5.3
2 – 2.5	50.0
2.5 – 3	26.3
> 3	2.6

[a] 103 total respondents

Table 11. Cow reproduction rates[a]

Pregnancy Rate (%)	Percentage
71-85	8
86-90	13
> 91	79

[a] 103 respondents

Table 12. Calf marketing strategies[a]

Route of Marketing	Percentage
Local auction yard	26
Retain ownership - slaughter	24
Private treaty	19
Video/Internet auction	13
Combination of above	18

[a] 103 total respondents

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

THE FUTURE OF INFORMATION DISSEMINATION TO THE BEEF CATTLE INDUSTRY

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ABSTRACT: Agriculture and the lives of people who make their living on the land are embedded in visceral experiences – a world of place, consequences, tough decisions, ever-present change, and always uncertainty. But change is not unique to farms and ranches. The forces of change brought on by technological innovation, market forces, public policy decisions, and the whims of politicians and consumers are also significantly impacting those who serve agriculture. The very infrastructure that stimulated the explosion of agricultural productivity by U.S. farmers and ranchers is undergoing substantial retooling, restructuring and in some cases - dismantlement. In February of 2005, our team was drafted to evaluate the future of the information system for the beef industry. Our mission was not to provide a solution but rather to scout the landscape. We have endeavored to that end. The heart of our report is that the traditional information infrastructure is in crisis. The front lines are manned by talented and highly motivated professionals. However, our current institutions responsible for information discovery and delivery are struggling. Our belief is that the status quo is incapable of delivering desirable outcomes and that significant, if not radical, improvement must be initiated to avoid losing our role as a world leader in the beef industry.
Key words: beef, dissemination, extension, future, information systems, Land Grant University

Introduction

The very infrastructure that stimulated the explosion of agricultural productivity by U.S. farmers and ranchers is undergoing substantial retooling, restructuring and in some cases - dismantlement. In February of 2005, our team was drafted by the Production Research Committee of NCBA to evaluate the future of the information system for the beef industry. Our mission was not to provide a solution but rather to scout the landscape. The heart of our report is that the traditional information infrastructure is in crisis. While the front lines are manned by talented and highly motivated professionals. Our current institutions responsible for information discovery and delivery are struggling. Our belief is that the status quo is incapable of delivering desirable outcomes and that significant, if not radical, improvement must be initiated to avoid losing our role as a world leader in the beef industry.

The challenge is to deal with the following questions:

- Is the status quo of beef cattle research information dissemination capable of meeting future needs?
- Is the status quo sustainable?
- Which future do we wish to create in regards to research and development for the beef industry?
- Who will pay?
- Who are the users?
- Who is responsible for providing beef information dissemination service (private, public, joint)?

The process of dealing with these questions has lead us to the following conclusions: failure to invest in the intellectual assets of the beef industry will contribute to reduced food security for U.S. consumers, heightened conversion of agricultural lands to development, and fewer opportunities for people to work in the beef industry. A profitable and sustainable beef industry is knowledge dependent. However, the traditional infrastructure of discovery and information dissemination is in decline. Participants in the industry require unbiased, multi-disciplinary information that is relatively easy to access and that helps people make better choices. Creating a national beef cattle information dissemination effort through a partnership between the industry, the land grant university system, and the key research agencies of the USDA offers the best opportunity to arrest the declining state of the beef production information infrastructure. Furthermore, such a system recognizes the value of interaction between producers, specialists, and information providers and endeavors to facilitate a "knowledge based" community.

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Historic Perspectives

Early in the American experience, the architects of the republic understood the consequences of illiteracy, ignorance, and holding knowledge as the birthright of the aristocracy. Enabled by the world altering vision that an educated citizenry was the best assurance of democracy, the land grant system of universities, USDA, and a host of knowledge-based institutions were created. Certainly the primary motivation to invest in agricultural intellectual capital was to assure domestic tranquility. However, the needs of agriculture and rural communities were a critical consideration in the national investment in food production research and information systems. To ensure farmer access, the creation of the information infrastructure for agriculture was accompanied by the notion of providing such service for minimal cost to the user.

As a result, the agricultural community in the U.S. has become dependant, at least in part, on information sources that are largely public in nature and inexpensive to access. The power of a Goggle search engine on the World Wide Web has created a faster and more convenient route to gaining access to multiple pieces of information. However, these benefits do not change the fact that the information comes piecemeal. Without a supporting systems-based approach to interpreting and applying the information, ease of access to information fails to produce lasting value.

The argument can be made that information delivery in the beef industry is being transformed into a more proprietary system where the private arena is more central and the cost of access to information is more expensive either as the result of pricing changes, contractual agreements, conditions of market entry, or aligning information with specific goods and services in the marketplace. As the industry consolidates, the needs of large and small firms diverge. As competition heightens larger business entities (farms, processors, distributors, etc) tend to hold the advantage in using information to adopt appropriate technologies and practices. Furthermore, supply chains tend to hold information as an economic and competitive advantage thus heightening the trend towards a more proprietary system.

Information Systems

Perhaps the greatest challenge is to agree on the purpose and mission of a beef industry information system. The needs are diverse and various players in the business may find their needs in competition with one another. Some information needs are unique to individual segments or even enterprises while in other instances the need is more collective or community based. Nonetheless, defining the target is critical to building the correct infrastructure with the capability of delivering true value. Which of the following are important topics that the industry wants to have addressed?

- Profitability, people, pasture, partnerships, and product
- Ecosystem management
- Meeting consumer needs
- Transferring businesses across generations
- Building human opportunity by creating new jobs
- Enhancing national security by assuring some degree of food supply self-sufficiency
- Improving financial controls
- Capturing higher returns for adding value
- Enhancing risk management
- Dealing with governmental regulation
- Increase learning and the exchange of knowledge by providing accessible, meaningful information at a reasonable price
- Improving business relationships
- Capture business growth opportunities – domestic and international

We need information that assists us in the interpretation of the world around us and puts societal signals into a context upon which we can develop proactive responses to these social forces that are best dealt with from a collective as opposed to enterprise approach. External forces put pressure on industry to better understand and respond to consolidation, food safety, animal welfare, and environment.

Information is also a valuable tool to generate better decisions within the enterprise. When considering the within enterprise realm, the focus of information should be on those critical control points where control can be exerted and where outcomes have significant impact on the goals of the business. The dimensions of this information are bounded by financial, biological, climatic, geographic, and human needs. Thus, "the one size fits all" approach is not only inappropriate but also unproductive. Nonetheless, information about the following control points is critical to the day-to-day operation of a beef cattle business:

- Data management as a decision tool.
- Genetics and reproductive efficiency
- Biosecurity/Animal Health
- Financial
- Human Resources
- Forage, nutritional, and environmental resources
- Creating beef products that meet varied customer needs

The world outside the beef enterprise must also be monitored and interpreted. Understanding these external pressures help to frame the strategic elements of the management plan. The general categories of external forces that must be measured include the following:

- Marketplace – domestic and foreign
- Macro trends
- Changes to the infrastructure of the industry

- Social issues
- Governmental policy and regulation
- Technological changes

The challenge in the future will be the integration of information from a variety of sources and disciplines into effective decision tools. Historically, this process was left to individual managers and their advisors. The scientific community has typically been more focused on discovery at increasingly more specialized levels. But there is a trend today for multi-disciplinary scientific discovery and interpretation. This trend facilitates building a community that will engage specialists as well as innovative ranchers.

If we assume that it is a kind of collective intelligence to be utilized by our grandchildren then changes are required in the approach to discovery, dissemination, and utilization of information and knowledge. The communities of geography and profession have been our traditional focus. However, our sense of community will be broadened in the future by the dramatic forces of change in information and communication technology. Change is occurring in both agricultural and scientific communities.

The need for access to reviewed, unbiased data will continue to be important but rather than depending on the slow process of peer review and the release of experimental results through traditional journal formats, a new process needs to emerge that shortens the time between project completion and release of new information. Any new structure will have to deal with a more efficient means of getting unbiased data from the world of discovery to the field of application. For example, the ability to access search engines that rank or classify the "quality" of the information from a particular source would enhance our ability to better filter the breadth of information to meet defined goals. It will also be increasingly important to help users of information to more effectively filter new ideas, technologies, and protocols

It is reasonable to assume that future producers will desire to move beyond the limits of their communities of place and profession and to engage a broader community through a variety of virtual tools and applications.

The notion that what people learn in school will carry them through the rest of their life is gone. Today, constant and rapid changes in communication technologies offer ubiquitous access to vast amounts of information, making it a struggle to keep up with the pace and volume of information in our lives. For example, the innovations in many scientific fields makes it difficult to keep up with the literature in one area of expertise and nearly impossible for an individual to keep pace in several technical fields.

The Users

Before we try to investigate how future beef producing communities will access data, information, knowledge and wisdom, it might be useful to attempt to define today's beef producing communities.

1) Professional cattleman – information driven – brand friendly
2) Professional cattleman –tradition bound/resistant to change – commodity focused
3) Professional farmer – cattle as a by-product of land ownership (lots of variation in terms of passion for the cattle enterprise)
4) Professional in another industry – cattle as a secondary income
5) Recreational cattle producer – life style operator – income not an issue
6) Lifestyle cattle producer – margin operator
7) Cause driven landowner (Nature Conservancy, Ducks Unlimited, etc)
8) Service provider (educators, veterinarians, sales, consultants, bankers, etc)

Tables 1 and 2 provide a summary of our estimation of the demand for general categories of information, the desired system of delivery, the structure of the information, and the motivation of end user categories to gain access. In the development of a new model of information dissemination, the architects will have to determine whether to serve all or part of these end user categories based on an assessment of each group's longevity, willingness to invest, and level of demand for the service.

Traditional sources and distributors of information include the following - land grant universities, the extension service and the Agricultural Experiment Stations, USDA mission area of Research, Education, and Economics, general circulation agricultural publications, food animal veterinarians, state and national cattlemen's organizations (NCBA, breed associations, etc.), and various forms of electronic media.

Threats to the continuation of these information sources include but are not limited to the following:

- Federal and state budget cuts have been significant; as such we should expect dramatic changes in extension, experiment stations, USDA and Colleges of Agriculture.
- The nation's veterinary colleges are placing less emphasis on the food animal industries. The industry should expect to have fewer independent food animal practitioners.
- Consolidation is changing the private sources of information as well. Less animal health, nutrition, and other livestock related companies are likely to exist in the future.
- Consolidation is bound to reduce the number of state cattlemen's organizations and breed associations.

Information Providers

Who do producers trust to provide them information relative to their beef cattle enterprises? The 1997 NAHMS Beef Cow-calf Management Survey provides a national perspective as to the answer. The veterinarian has the highest level of credibility with producers as a

trusted information source and as Table 3 illustrates, this advantage is not closely challenged. Of grave concern to university and extension information providers is that one-third of producers do not see them as important or even somewhat important sources of information. Surprisingly, producer associations also receive low marks for credibility as information sources – a fact that should be of concern to national and state cattlemen's organizations.

Why are extension/university and producer organizations given such low levels of trust as sources of information? In the case of producer associations, it is likely that state and national groups have not made provision of information a high enough priority, haven't been able to successfully form alliances with information providers such as extension/university, or have not successfully interpreted and met producer needs and demands. It may also be the result of the R&D system's failure to provide sufficient ownership to users. This disconnect is likely the culprit behind the low levels of credibility.

In the case of extension/university receiving low marks, there are a number of hypotheses as to the reason for the credibility gap with producers. These include:

- Too much focus on discipline-based, linear approaches to problem solving.
- Specialists have been too eager to promote specific technologies or protocols as stand alone solutions. A lack of awareness on the part of the land grant system of the social, environmental, and economic consequences of technological change has eroded credibility. This dilemma has been heightened by the long-standing, nearly singular focus on increasing productivity.
- Shift in resources away from applied agricultural programs by the land grant university system.
- Federal and state funding for extension, experiment stations, and agricultural colleges is declining. Indirect cost recovery has taken on increasing levels of importance in the public research arena. Unfortunately agricultural research, particularly those projects funded by industry and check-off dollars, has not been competitive with NIH type research for generation of indirect cost monies.
- The services have traditionally been offered free of charge or at under-valued prices thus leading producers to view the offering as low value. Note that the highest credibility comes from information sources (veterinarian) where the producer is making direct payment to the provider.
- Local knowledge and farmer led innovations have been ignored or trivialized.
- Communication tends to be unilateral, i.e. the specialist provides information to producer directly or through the regional and county network but the loop is severed or disjointed such that the producers and specialists rarely engage in a meaningful relationship. Industry feedback is limited; a contribution of ideas and engagement in conversations from individual farmers/ranchers is either non-existent or very limited. Discovery focused agencies are poor communicators of results or have not been sufficiently directed to do so.
- Poor coordination by individual states, universities and agencies has resulted in information/results from multiple sources not being linked to improve convenience and value to end-users.
- Peer review has created a long time gap between project completion, reporting in journals, and dissemination to producers.
- Industry has failed to significantly invest in research and information dissemination activities.

The Future

We believe that one of the following three scenarios will emerge.

Scenario One: Status Quo

If the industry fails to respond to the multitude of signals that the current information discovery and delivery infrastructure is failing, the following outcomes are likely:

- Shrinking resources for the land grant university system will lead to the rapid regionalization of Colleges of Agriculture resulting in a handful of programs that emphasize beef cattle. Even today, the list would not exceed 15.
- Shrinking federal resources for agricultural research will lead to the closure of large ARS research centers as well as the almost total shift of the research focus to issues impacting consumers directly with little concern given to the needs of the beef supply chain.
- Individual supply chain entities will begin to develop research and information sharing that is completely proprietary in nature. Access will be totally limited to participants in the supply chain.
- Product and service suppliers will capture the information delivery system and producers will be left with a system that focuses on the desire of these entities to sell products. Unbiased interpretation of data will fall by the wayside.
- The industry will become dependent on existing published information.
- International competitors (South America, China, Australia, and Canada) will gain a competitive advantage as they take over the research and development leadership for a global beef industry.

Scenario Two: The Quick Death of the Current System

While unlikely that the existing publicly held information infrastructure (USDA, land grant system) would be discontinued suddenly, just such a scenario

occurred in New Zealand. The New Zealand case saw the substitution of previously publicly held entities with private sector ventures. As such market forces as opposed to the traditions and needs of the old regime drove the creation of contemporary services.
New Zealand is home to a healthy farm consulting environments because the information and data system moved into free enterprise.

Scenario Three: Build a better mousetrap
The rate of technological advancement is so rapid that almost as fast as these possibilities are read they could become obsolete. Nonetheless, if the beef industry wishes to build a better mousetrap it must make a concentrated effort to monitor innovations in information dissemination technologies, predict trends and envision systems that will meet its needs in the future.

- Computer systems, reference systems, satellite services, video, audio, instant communication and other technologies will most likely be assembled into central units with mobile peripherals.
- Conversation centers should run the gamut from forums much like today's discussion groups and web blogs with audiences as small as one-on-one to worldwide interactions. Sponsorship and ownership of conversation centers will be both privatized and public.
- What we know as the web and Internet will probably be reorganized into stratums. Reference resources will be easier accessed. Regulation will increase.
- Decision support and software will be bolstered with artificial intelligence advancements. The majority of systems management support and information accumulation to knowledge bases will probably be privatized in agriculture unless current trends change.
- Software development will advance the ability of decision makers to integrate complex information.
- Integration of technologies will provide economic opportunities.

One question to consider will be how much of future technologies will be state sponsored and how much will be proprietary systems? Beef producers in the future will continue to juggle a variety of information needs from simple facts to diverse cause and effects in multiple areas that deal with products, economics, environment, and social ramifications. Virtual communities will expand as a result of broad access to computer systems. As a result people will have access to and contribute to a far broader range of effective information creation and transfer. However, quantifying that effective information will present new challenges.

The Challenge: Redefining Organization Culture

The transition from industrial-age to information age organizations will involve creating a new organizational culture. Organizational culture is that collection of values, norms, rituals, shared beliefs, and metaphors that define what's important in the organization. The information-age organization has an integrative culture based on integrative thinking.

Integrative thinking that actively embraces change is more likely in companies whose cultures and structures are also integrative, encouraging the treatment of problems as "wholes, " and considering the wider implications of actions. Such organizations reduce rancorous conflict and isolation between organizational units; create mechanisms for exchange of information and new ideas across organizational boundaries; ensure that multiple perspectives will be taken into account in decisions; and provide coherence and direction to the whole organization.

The industrial-age organization is comparable to model toy kits that come as a set of discrete parts with instructions on how to put those parts together one correct way. The information-age organization is similar to the new toy transformers that come with linkages between a number of different parts that can be put together to carry out one purpose (an airplane), but with a few transformations of the parts can be reassembled to look like something else (a robot) with a new shape and a new purpose.

The challenge for information providers isn't only to create an information-age organizational culture, but also to communicate the appropriateness and necessity of such a new culture to traditional constituencies and funding sources that may be more comfortable with the old industrial-age way of doing things-even as they make demands for information age relevance, effectiveness, and leadership.

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Table 1. Demand for fact/principle information and integrative decision support information by end user category

End user	Demand for fact and principle based information	Demand for integrative decision support information
Professional – info - brand	Moderate	High
Professional - tradition	Moderate	Low
Professional farmer	Moderate	Low
Other industry professional	High	Moderate
Recreational cattle producer	Low to moderate	Low
Lifestyle cattle producer	Low	Low
Cause driven	Low	High (seek nontraditional sources)
Service provider	High	Moderate

Table 2. Estimated preferred delivery system, information structure, and motivation to obtain information by end user category

End User	Desired information delivery system	Desired structure of information	Motivation to access information
Professional – Info-brand	Multiple, high interactivity with range of people	Systems, complexity is okay on broad fronts	Grow business
Professional - tradition	Traditional, Interactive with other producers	Recipe, bullet point, input for enterprise decision making, complexity okay with regards to cattle production	Improved efficiency/ease of operation
Professional farmer	Instant access	Answer questions, broad	Bottom line of enterprise
Other industry professional	Multiple, focus on instant access	Answer questions, broad with focus on convenience and simplicity	Annual profitability
Recreational cattle producer	Instant access, face to face	Answer questions, broad with a focus on ease of operation	Improve the life experience
Lifestyle cattle producer	Conversation	Production	Improve production
Cause driven	Multiple	Systems, focus on ecological impact	Enhance the resource in line with owner goals
Service provider	Multiple	Answer questions, supplement existing knowledge service, anticipate change	Serve clients, sell more goods and services to clients

Table 3. Percent of cow-calf managers ranking information sources by level of importance

Source	Percent ranking as: Not important	Percent ranking as: Somewhat important	Percent ranking as: Very Important
Extension/univ/vo-ag	32.4	43.5	24.1
Veterinarians	8.2	31.0	60.8
Beef popular press	30.7	53.9	15.4
Producer associations	58.0	32.2	9.8
Other producers	30.4	46.9	22.7
Salespersons	41.7	42.3	16.0
Consultants	77.5	16.1	6.4
Radio, TV, etc	55.5	36.5	8.0

Source: USDA: APHIS: VS, Part 1: Reference of 1997 Beef Cow-calf Management Practices. June, 1997.

Rank (very important) – vets, extension, other producers, sales, beef popular press, producer associations, radio/TV, and consultants

Rank (not important) – consultants, producer associations, radio/TV, sales, extension, popular press, other producers, veterinarians

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

TEACHING CATTLE PRODUCERS TO MANAGE RISK – THE BEEHIVE MASTER BEEF MANAGER PROGRAM

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ABSTRACT: Sustainable cattle production is vital to the economy of rural Utah and the Intermountain West. Cattle producers face many different types of risk, though they often accept risk as part of production agriculture. Production risk, however, often carries with it impacts which affect other types of risk exposure. The Beehive Master Beef Manager Program (BMBM) was developed as one way to educate cattle producers about risk, beef quality assurance (BQA) and best management practices (BMP). However, the primary objective of the program is to assist producers to identify perceived risks within their operation, and then teach them principles to aid them as they develop strategies to manage these risks. The BMBM program originally began as a Beef Check-off funded Pilot Project and is now offered in five geographical areas of Utah. Since production agriculture is multi-faceted and numerous interrelationships exist between various production enterprises and the risk which accompanies those enterprises, BMBM takes a holistic approach to managing risk. Cattle producers have been taught about the various types of risk using the Right Risk computer simulation software. They have then been asked to determine which aspects of risk have the highest priority. The results have been compiled for each teaching location and priorities for future educational programming have been established to meet the producer-identified needs. Some of these needs are similar across locations, but differences also exist. One of the strengths of this program is that the producers establish the educational priorities to meet their needs. The *Cow-Calf Management Guide & Cattle Producer's Library* has served as the primary resource for the sessions held to date, coupled with supplemental materials for topics not covered adequately in the *Guide.* Classes taught included introduction to risk management, cattle marketing options, heifer selection, understanding Expected Progeny Differences, mineral nutrition, weaning strategies to minimize stress, fall cattle management strategies, herd disease management and making management decisions to manage market, human, institutional and production risk. Self-assessed understanding of topics covered in the class sessions was determined through the use of pre- and post-workshop evaluations. Two-sample t-tests assuming unequal variances were conducted for each topic in each locale where that topic was taught. With only one exception, statistically significant increases in understanding occurred for a majority of topics at all locales ($P<0.05$). Funding for the program was provided by Western Center for Risk Management Education.

Introduction

We live in a world of uncertainty. Many cattle producers recognize there are some things which are not within their ability to manage in their operations. However, they often overlook some areas which, if managed, could improve their profitability and optimize the productivity of their operation.

Kay and Edwards in their text entitled *Farm Management* state that, "Many agricultural decisions have outcomes that take place months or years after the initial decision is made." (Kay and Edwards, 1994) As a result of this interval there can be substantial risk in

many different aspects of any given agricultural enterprise.

Utah State University Extension and industry partners have developed an educational program which assists cattle producers to identify perceived risks in their operations and teaches them skills which help them to mitigate or manage those risk factors. The objectives of the Beehive Master Beef Manager program are to teach cattle producers:

1. The different types of risk,
2. To identify and prioritize risk factors in their operation and,
3. To implement Best Management Practices (BMP) to manage their risk.

Materials and Methods

In 2004, a committee was organized with the idea of developing a curriculum and series of workshops for cattle producers to help them implement Best Management Practices as a way to manage risk in their operations. Initially the committee was comprised of university Extension personnel and state cattle industry leaders.

Funding to conduct a pilot project was secured in 2005 through a grant from the National Cattlemen's Beef Association Beef Quality Assurance program and initial workshops were held during the fall and winter of 2005-2006 in Richfield (Sevier County) and Tooele (Tooele County), Utah.

The reference text for the workshops is the *Cow-Calf Management Guide and Cattlemen's Library*, a text consisting of more than 900 fact sheets covering 14 different subject areas developed by Extension beef specialists from the 13 western states.

During the initial meetings, producers were asked informally to prioritize their educational needs. These were then compiled and the first-year curriculum was developed. Two workshops were taught in Tooele and three workshops were taught in Richfield during the first year. Workshop topics for year 1 included weaning strategies and preconditioning, farm financial records and marketing options for cow-calf producers.

Following year 1 workshops, the teaching team, with the help of producers who had attended the workshops, decided the producers needed more in-depth knowledge of risk and how it impacts their operations before establishing educational priorities. It was also determined that pre- and post-workshop evaluations would be the most effective way to measure workshop impact and increases in understanding.

In year 2, funding for the program was secured through a grant from USDA/CSREES through the Western Center for Risk Management Education. The program was also expanded from the two original sites to three additional sites in Roosevelt (Duchesne & Uintah Counties), Randolph (Rich County) and Beaver (Beaver County), Utah.

The Right Risk computer simulation software was used to introduce cattle producers to the impact of risk and the subsequent benefits of risk management using one of several ranch simulations as an example. Following the Right Risk exercise, attendees were then asked to complete a survey where they prioritized their specific educational needs within six broad categories and prioritize which categories were their highest priorities, with 1 being their highest priority and 5 being their lowest.

The survey instrument used to establish site priorities was patterned after a matrix used in a web-based risk management education program developed by Oklahoma State University Cooperative Extension. (See Table. 1) (OSU, 2006)

Survey results were then compiled by site and the category with the lowest composite ranking was assigned the highest priority. Likewise, the topic within that category with the lowest ranking was similarly assigned the highest priority. The curriculum was then established to address these site-specific educational priorities.

Three to four workshops were held per teaching site in year two.

Pre- and Post-workshop evaluations were conducted for each workshop. These evaluations determined self-assessed understanding by each participant before the workshop and at the workshops conclusion. The evaluations were analyzed using a two-sample t-test assuming unequal variances.

Results and Discussion

The Beehive Master Beef Manager Program teaches cattle producers about the various types of risk that can have impacts on their operations, how to prioritize their perceived risk and demonstrates how various best management practices can help them manage their risk factors.

The greatest strength of the program is that the participants establish the curriculum based upon the risk priorities which they identify, rather than a curriculum established by a committee of educators. While there are similarities between teaching sites relative to curriculum content, the priorities established by the producers at each site relative to the order in which they are presented vary greatly.

Over the two-year history since the inception of the program, more than 70 cattle producers managing more than 27,500 head have participated at five regional teaching sites. A total of 17 workshops have been conducted at the five sites.

Workshop topics have included introduction to risk, Right Risk decision-making software, fall cattle management and weaning strategies, preconditioning calves, ranch financial records, cattle production records, managing disease in calves and adult cattle, mineral nutrition, selecting heifers, understanding and using EPDs in bull selection and marketing opportunities for calves and culls.

The t-test analyses conducted on pre- and post-workshop self-tests showed that, with only one exception, significant learning occurred across the majority of topics covered ($P<0.05$). The exception where this did not occur was an introductory workshop where fall cattle management and weaning strategies were included. This suggests that many participants in the audience already understood the concepts presented and/or had implemented the suggested BMPs, and thus did not experience significant learning relative to those topics. However, relative to the discussion on the different types of risk, significant learning did occur among this group ($P<0.05$).

Conclusion

The Beehive Master Beef Manager Program has proven to be an effective avenue through which information relative to risk management and best management practices can be conveyed to cattle producers. The success of the program has been due, in large part, to the buy-in which the producers have as a result establishing the curriculum priorities for their teaching location. We will continue to use it as an effective way to disseminate timely information to our cattle producers and hope to expand the program in the future to encompass more teaching sites throughout the state.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECTS OF SEASON, MANAGEMENT, AND DIET ON PREVALENCE OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* IN DAIRY CATTLE[1]

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ABSTRACT: Shiga toxin-producing (**STEC**) caused numerous outbreaks of human illnesses worldwide. Because dairy cattle are STEC reservoirs, contamination of their products continues to be a human health risk. Several STEC outbreaks were traced to beef from culled dairy cows, water, or produce contaminated with cattle feces. To identify on-farm factors that decrease STEC prevalence, four dairy farms (averaging 712 cows) in California were used. A total of 1,268 fecal samples were collected from heifers (n = 261) and cows in first (n = 424), second (n = 298), or later (n = 285) lactations over one year (approximately 80 samples per farm per season). Across seasons, STEC were recovered in all farms at similar (P > 0.05) rates (averaging 1.8%). Prevalence rates of STEC were not different (P > 0.05) between cows and heifers (averaging 2.0%) and were not affected (P > 0.05) by lactation, days in milk (1 to 60, 61 to 150, or $ 151 d), or season (averaging 1.6, 1.6, and 1.7%, respectively). The STEC isolates belonged to 16 serotypes (O15:H! [nonmotile], O116:H!, O125:H20, O127:H19, O128:H20, O136:H10, O136:H12, O136:H19, O136:HUT [untypeable H antigen], O157:H7, O157:H!, O166:H6, OX13 [a new O antigen]:H19, OX13:H20, OUT [untypeable O antigen]:H7, and OUT:H!). Of these serotypes, three (O157:H7, O157:H!, and OUT:H!) caused hemolytic uremic syndrome (**HUS**), two (O15:H! and OUT:H7) caused other human illnesses, and eight (O125:H20, O127:H19, O128:H20, O136:H10, O136:H19, O166:H6, OX13:H19, and OX13:H20) have not been reported previously in cattle. Prevalence of STEC was not affected (P > 0.05) by farm factors such as manure handling, frequency of cleaning the feed bunk, water source, and pen size, type, or density. However, dietary ingredients appeared to affect STEC prevalence. For example, higher (P < 0.05) prevalence rates were associated with feeding yeast cultures (2.0 vs 0.6%) and with total or partial replacement of soybean meal with cottonseed meal in the protein supplement (3.8 vs 0.7%). Thus, decreasing fecal shedding of STEC by dairy cattle appears possible by dietary manipulation.

Key words: Food Safety, *Escherichia coli*, Dairy Cattle

Introduction

Consumer's concerns with safety of beef and dairy products have increased in recent years due to increased number of human illness outbreaks caused by STEC. These illnesses (Griffin and Tauxe, 1991) range from mild diarrhea to the life-threatening HUS. Other symptoms include bloody diarrhea, severe hemorrhagic colitis, and thrombotic thrombocytopenic purpura that is characterized by central nervous system abnormalities (Paton and Paton, 2000). These illnesses were caused by more than 100 STEC serotypes (WHO, 1998). These include *E. coli* O26:H11, O26:H!, O91:H10, O91:H21, O103:H2, O103:H!, O111:H2, O111:H8, O111:H!, and O157:H7. Pathogenic *E. coli* strains not only produce toxins but can have other virulence factors that may increase severity of human illnesses (Paton and Paton, 2000).

Dairy cattle are considered reservoirs of O157:H7 (Besser and Hancock, 1994; Mechie et al., 1997) and non-O157:H7 (Rahn et al., 1997; Kobayashi et al., 2001) STEC. Several STEC isolates were detected in raw milk (Sandhu et al., 1996; Chiueh et al., 2002), cheeses (Pradel et al., 2000; 2001), and ground beef from dairy cattle (Doyle, 1991). Also, STEC outbreaks were attributed to consumption of raw milk (Allerberger et al., 2001; Lahti et al., 2002), cheeses (Deschênes et al., 1996), yogurt (Morgan et al., 1993), and dairy beef (Ostroff et al., 1990). Due to the rising concerns with safety of foods of dairy origin, more efforts have been devoted to develop and implement pre-harvest (on-farm management practices) and post-harvest (milk processing and meat packing) control measures to decrease the risk of STEC contamination of dairy products. This study was designed to determine effects of season on STEC prevalence in California dairy cattle. Another objective was to identify on-farm factors (management practices and dietary ingredients) that could be manipulated to decrease STEC

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[1]The author would like to acknowledge the support of the Integrated Research, Education, and Extension Competitive Grants Program—National Integrated Food Safety Initiative (grant No. 2001-51110-11463) of the USDA Cooperative State Research Education, and Extension Service. Some of the VTEC-Screen kits used in this study were kindly provided by Denka Seiken Co., Ltd. (Tokyo, Japan).

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prevalence.

Materials and Methods

Cattle Population

Owners of dairy farms were solicited for voluntary participation in this study through lists of producers compiled by Veterinary Medical Officers of the USDA and Farm Advisors employed by the University of California Cooperative Extension. Four dairy farms (averaging 712 Holstein cows and heifers per herd) were enrolled in the study and were located in the southern San Joaquin Valley in California. In these farms, fecal samples were collected from 1,007 cows and 261 heifers over one year.

Fecal Sampling

Each dairy farm was visited once in each season for fecal sampling. From each farm, approximately 80 fecal samples were collected from heifers and cows at different stages of lactation. These samples were collected from the rectum of approximately 20 cows or heifers in each of four different pens with each pen containing animals at similar stage of lactation. A minimum of 5 g fresh feces were placed in a sterile snap-seal plastic cup and shipped on ice to our laboratory for analysis # 24 h post-sampling. A standardized questionnaire was administered to the dairy farm owner or manager to collect data related to the cattle and their feeding and management practices.

Enrichment and Initial Selection of STEC Isolates

Initial selection of *E. coli* isolates was conducted by adding 1 g feces from each animal to 25 mL enrichment medium (brain heart infusion [**BHI**]; Hardy Diagnostics, Santa Maria, CA), mixing vigorously, and bringing the total volume to 50 mL with the enrichment medium containing cefixime (Sigma, St. Louis, MO) at 50 µg/L, novobiocin (Sigma) at 20 mg/L, potassium tellurite (Sigma) at 2.5 mg/L, and vancomycin (Sigma) at 40 mg/L. The diluted feces (1:50) were immediately incubated at 37°C for 12 h with continuous shaking (120 rpm) to allow for antibiotic selection and toxin induction. At the end of incubation, the enriched samples were serially diluted to $10^{!7}$ in BHI medium, plated in duplicate onto sorbitol-MacConkey (**SMAC**) agar (Hardy Diagnostics), and incubated at 37ºC for 18 h. At the end of this incubation, sorbitol-fermenting (pink colonies) and non-fermenting (white colonies) bacteria on SMAC plates were subcultured on 4-methylumbelliferyl-$-D-glucuronide (**MUG**) MacConkey (**MMUG**; Hardy Diagnostics) agar grid plates. Ten (sorbitol positive or negative) or less (when unavailable) colonies from each category were randomly selected and transferred to the MMUG plates. The MMUG plates were incubated at 37ºC for 18 h and observed on a UV light box (Fotodyne, New Berlin, WI). Results were recorded for MUG positive or MUG negative (blue fluorescence or no fluorescence under UV light, respectively). When available, two colonies from each of the potential four biochemical categories (sorbitol positive/MUG positive, sorbitol positive/MUG negative, sorbitol negative/MUG positive, and sorbitol negative/MUG negative) were selected at random. These potential *E. coli* isolates were transferred from the MMUG plates to 5-mL tubes containing 2 mL of tryptic soy broth (**TSB**; Hardy Diagnostics) and incubated at 37ºC for 6 h with continuous shaking. At the end of incubation, the culture was diluted with equal volume (2 mL) of sterile glycerol, mixed well, and stored at ! 80ºC. At that time, the same isolates from the MMUG plates were subjected to biochemical testing for *E. coli*.

Screening for Potential STEC Isolates

Enriched fecal samples were screened for STEC by using the VTEC (verotoxin-producing *E. coli*)-Screen kit (Denka Seiken Co., Ltd., Tokyo, Japan). A total of 5 mL enriched feces and 100 µL polymyxin solution (Denka Seiken) were incubated at 37°C for 30 min with continuous shaking to ensure optimal extraction of Shiga toxins from the bacterial periplasmic space. The mixture was centrifuged at 900 × *g* for 20 min and the supernatant was removed to test for Shiga toxins in 96-well V-bottom microtiter plates (Costar, Corning, NY). In these plates, the culture supernatant (25 µL) was mixed with equal volume of supplied diluent (phosphate buffered saline and 0.08% sodium azide). An equal volume of latex particles sensitized with rabbit polyclonal anti-Shiga toxin 1 (**Stx1**) and anti-Shiga toxin 2 (**Stx2**) immunoglobulin G antibody was mixed in the appropriate wells. The plates were mixed, covered, incubated at room temperature, and examined for latex agglutination after 18 h. The positive and negative control toxins supplied with the kit were tested with each assay. A positive result was recorded when agglutination in the sample well was two levels above the control well.

Identification of STEC Isolates

The isolates that were selected based on sorbitol fermentation and $-glucuronidase activity (maximum eight for each sample) were tested for *E. coli* biochemically by using the API 20E Identification System (bioMérieux Vitek, Inc., Hazelwood, MO). Only isolates confirmed as *E. coli* were stored at ! 80ºC for further testing. *E. coli* isolates were grown in 5 mL BHI at 37ºC for 12 h with continuous shaking. At the end of incubation, the cultures were subjected to the same agglutination assay (VTEC-Screen kit) described above to identify and preserve *E. coli* isolates that are STEC.

Serotyping of STEC Isolates

The STEC isolates were initially serotyped in our laboratory for the O and H antigens by the slide agglutination method using rabbit antisera kits (Denka Seiken) containing 51 O antisera (O1, O6, O8, O9, O15, O18, O20, O25, O26, O27, O28ac, O29, O44, O55, O63, O74, O78, O86, O91, O103, O111, O112ac, O114, O115, O119, O121, O124, O125, O126, O127, O128, O136, O142, O143, O144, O145, O146, O148, O151, O152, O153, O157, O158, O159, O161, O164, O165, O166, O167, O168, and O169) and 22 H antisera (H2, H4, H5, H6, H7, H9, H10, H11, H12, H16, H18, H19, H20, H21, H27, H28, H34, H40, H41, H42, H45, and H51). The STEC isolates that could not be typed for the

H antigen were subjected to a motility test to assess presence or absence of bacterial flagella. The test medium (Remel, Lenexa, KS) contained tetrazolium dye to aid in visualization of bacterial motility. The STEC isolates were grown on TSB agar containing 5% defibrinated sheep blood at 37ºC for 18 h. Using a sterile needle, a colony of each STEC isolate was picked to stab a motility test medium tube two-thirds of the way down to the bottom without touching the wall of the tube. The medium tubes were incubated at room temperature and were examined for motility after 24 and 48 h. The nonmotile STEC isolates were considered H$^{!}$. The isolates that could not be typed by the above mentioned O and H antisera were sent to the Gastroenteric Disease Center at the Pennsylvania State University (University Park, PA) for serotyping.

Control Cultures

The antibiotics used during fecal enrichment and initial selection were tested on sterilized cattle feces that were inoculated with cultures containing four different *E. coli* O157:H7 isolates. These isolates were ATCC 43890 (producing only Stx1), ATCC 43889 (producing only Stx2), ATCC 43895 (producing Stx1 and Stx2), and ATCC 43888 (not producing either toxin). The *E. coli* O157:H7 isolates were grown in TSB at 37°C for 6 h with continuous shaking before mixing with the sterilized feces after reaching room temperature. The sterilized/inoculated feces were treated exactly the same as the test fecal samples and, therefore, were used as controls. The results showed no detrimental effects of the antibiotic combination used on growth and recovery of the *E. coli* O157:H7 isolates. Recovery of these isolates was accomplished by using the same isolation methods described above. Determination of specific toxin production was achieved by using the VTEC-Reversed Passive Latex Agglutination (Denka Seiken).

Verotoxicity of the STEC Isolates

The STEC isolates were grown in 5 mL TSB at 37ºC for 18 h with continuous shaking, the cultures were centrifuged (3,000 × *g* for 10 min), and the supernatants were filtered twice through 0.22 µm sterile syringe filters (ISC BioExpress, Kaysville, UT). The sterile supernatants were used for determination of toxicity of STEC isolates to Vero (African green-monkey kidney) cells (Konowalchuk et al., 1977; Smith and Scotland, 1993) in duplicate. Negative controls consisting of Eagle minimal essential medium (Mediatech, Inc., Herndon, VA), TSB medium, and non-STEC O157:H7 (ATCC 43888) were analyzed with each set of 96-well flat-bottom microtiter plates (Costar). In addition, each set of plates contained a positive control panel of supernatants from three *E. coli* O157:H7 (ATCC 43890, ATCC 43889, and ATCC 43895). The STEC-positive and STEC-negative isolates were determined by absence or presence of a confluent monolayer, respectively. Results were recorded after 24 and 48 h of incubation.

Statistical Analysis

A significant difference in the odds of STEC for factors with two levels was determined using an exact likelihood ratio test and computation of the exact 95% confidence interval for the odds ratio by using LogXact 4 Software for Windows (Mehta and Patel, 1999; 2000). The *P*-value was set at < 0.05 for statistical significance.

Results and Discussion

Across seasons, STEC were recovered in all farms at similar ($P > 0.05$) rates (averaging 1.8%). Prevalence rates of STEC were not different ($P > 0.05$) between cows and heifers (averaging 2.0%) and were not affected ($P > 0.05$) by lactation, days in milk (1 to 60, 61 to 150, or $ 151 d), or season (averaging 1.6, 1.6, and 1.7%, respectively). The STEC isolates belonged to 16 serotypes (O15:H$^{!}$, O116:H$^{!}$, O125:H20, O127:H19, O128:H20, O136:H10, O136:H12, O136:H19, O136:HUT, O157:H7, O157:H$^{!}$, O166:H6, OX13:H19, OX13:H20, OUT:H7, and OUT:H$^{!}$). Of these serotypes, three (O157:H7, O157:H$^{!}$, and OUT:H$^{!}$) caused HUS (WHO, 1998; Anonymous, 2001, Blanco et al., 2003), two (O15:H$^{!}$ and OUT:H7) caused other human illnesses (WHO, 1998; Anonymous, 2001, Blanco et al., 2003), and eight (O125:H20, O127:H19, O128:H20, O136:H10, O136:H19, O166:H6, OX13:H19, and OX13:H20) have not been reported previously in cattle. Prevalence of STEC was not affected ($P > 0.05$) by farm factors such as manure handling, frequency of cleaning the feed bunk, water source, and pen size, type, or density. However, dietary ingredients appeared to affect STEC prevalence. For example, higher ($P < 0.05$) prevalence rates were associated with feeding yeast cultures (2.0 vs 0.6%) and with total or partial replacement of soybean meal with cottonseed meal in the protein supplement (3.8 vs 0.7%). Thus, decreasing fecal shedding of STEC by dairy cattle appears possible by dietary manipulation.

Implications

Fecal testing of 1,268 dairy cattle in four California farms over one year revealed prevalence of STEC isolates belonging to 16 serotypes at 1.8%. Because all STEC isolates were toxic to Vero cells, three serotypes are known to cause HUS, and two others are known to cause various human illnesses suggest the seriousness of the STEC problem. Thus, it is critically important to test dairy cattle for STEC before entering the food chain. The results also suggest that decreasing fecal shedding of STEC by dairy cattle is possible through dietary manipulation.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

RELATIONSHIP BETWEEN RESIDUAL FEED INTAKE AND MEAT QUALITY IN STEER PROGENY OF DIVERGENT INTRAMUSCULAR FAT EPD ANGUS BULLS[1]

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ABSTRACT: The relationship between feed efficiency [residual feed intake (RFI)] and meat quality has not been well characterized in beef cattle selected for varying levels of intramuscular fat. Daily DMI was recorded for 35 steers (initial weight, 305 ± 27.7 kg), which were the progeny of 4 Angus sires with low, moderate, or high EPDs for percent intramuscular fat. Steers were fed for an 84-d post-weaning growth period and weighed bi-weekly, followed by a 90-d finishing period prior to harvest. Complete carcass data and strip loins were collected from each carcass. Strip loins were cut into steaks, aged for 14 d, frozen, and later analyzed for palatability by a trained sensory panel. An RFI value was calculated for each steer, and used to classify steers into 3 groups: efficient (< -0.5 SD; n = 9; RFI = -0.60 kg/d), marginal (± 0.5 SD; n = 18; RFI = -0.01 kg/d), and inefficient (> 0.5 SD; n = 8; RFI = 0.65 kg/d). There were no differences among RFI group means for ADG, initial or final BW, fat thickness, yield grade, REA, percent lipid, HCW or cooking loss. Inefficient steers had greater DMI than marginal ($P < 0.05$) and efficient ($P < 0.01$) steers, and marginal steers tended ($P = 0.07$) to have greater DMI than efficient steers. Feed conversion ratio (FCR) was greater ($P < 0.01$) for inefficient vs. marginal and efficient steers. There was a correlation between RFI and DMI (r = 0.61; $P < 0.001$), FCR (r = 0.64; $P < 0.001$), marbling (r = 0.41; $P < 0.05$), quality grade (r = 0.40; $P < 0.05$), juiciness (r = 0.48; $P < 0.01$), flavor (r = 0.47; $P < 0.01$), and Warner-Bratzler shear force (r = -0.33; $P = 0.05$). However, RFI was not correlated with ADG, yield grade, REA, HCW, percent lipid, cooking loss, or sensory panel tenderness. In summary, data from this small number of observations suggest that feed efficiency of Angus steer progeny of sires divergent for intramuscular fat EPD may be related to several meat quality factors that influence the value and palatability of beef.

Key words: Beef Cattle, Meat Quality, Residual Feed Intake

Introduction

Marbling plays an important role in beef palatability, which is essential for consumer satisfaction; however, the beef industry has been unable to meet the quality demands of the consuming public (NCBA, 2006). Fortunately, expected progeny differences (**EPDs**) are a genetic tool available for producers to effectively select for specific traits, including marbling and percent intramuscular fat (Gwartney et al., 1996).

It has been shown that increased marbling and reduced cost of production can be simultaneously used in a selection strategy (Exton et al., 2004), based on the use of Angus bulls superior for both marbling and residual feed intake (**RFI**) in Australia. However, the long-term effects on these and other production and product quality traits that may result from their simultaneous selection are not known.

An animal's RFI value is the difference between its actual feed intake (measured individually) and its predicted feed intake (predicted based on BW and ADG). A considerable amount of animal-to-animal variation exists for the difference in actual and predicted feed intakes in beef cattle (Herd et al., 2003). Therefore, since feed costs comprise the largest cost on most beef cow/calf operations, efforts to reduce feed costs without negatively affecting growth, reproduction, carcass, or meat quality characteristics would be beneficial.

The RFI trait offers a genetic selection method to improve beef cattle efficiency without also increasing growth rate and mature size (Johnson et al., 2003). Selection of parents for low RFI (considered to be efficient) resulted in progeny that consumed less feed as yearlings but weighed the same at harvest as offspring from high RFI parents (Richardson et al., 2001). Preliminary evidence suggests that selection for RFI probably does not negatively affect cow mature weight or carcass quality of progeny, but can offer an advantage in selection for reduced cow maintenance requirements (Johnson et al., 2003).

Residual feed intake has been reported to be moderately heritable, based on estimates in growing beef cattle that range from 0.16 to 0.43 (Herd et al., 2003). Due to the correlation between post-weaning RFI and ADFI, selection for efficiency using the RFI trait could potentially improve feed efficiency in cattle through reduced feed intake (Herd et al., 2003). A direct genetic prediction for RFI is not yet available in the U.S., due to the lack of cost-effective methods to characterize large numbers of cattle for RFI. However, it is likely that the beef seedstock industry will develop such an EPD in the near future. In the interim, efforts to determine the relationship between RFI and carcass and end-product characteristics are needed.

Therefore, the objective of this research was to determine the relationship between RFI and meat quality

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

and palatability in a population of steers sired by Angus bulls with low, moderate, or high intramuscular fat EPDs.

Materials and Methods

All procedures involving animals were approved by the University of Idaho Animal Care and Use Committee prior to the initiation of the project. Thirty-five Angus-sired steer calves (progeny of 4 Angus sires with low, marginal, or high intramuscular fat EPDs) from the University of Idaho Nancy M. Cummings Research, Extension, and Education Center in Carmen, ID were weaned, sire-verified via DNA parentage analysis (MMI Genomics Inc., Davis, CA), and transported to the University of Idaho Monson Research Feedlot in Moscow, ID. Two weeks after arrival, calves were stratified by sire and BW and randomly assigned to 1 of 8 pens (4 to 5 calves/pen) equipped with individual Calan gate feeders (American Calan, Northwood, NH) for the collection of ADFI.

After training to the Calan gates for a 2-week period, an 84-d RFI evaluation was initiated. Calves (initial weight, 305 ± 27.7 kg) were provided ad libitum access to a growing-phase ration (Table 1) twice daily (50% per feeding), and orts were removed and recorded daily. During the 84-d period, steers were weighed every 14 d at approximately 0800 h, prior to the morning feeding.

At the conclusion of the RFI evaluation period, calves were fed 3 step-up rations during a 21-d period prior to receiving the finishing diet (Table 1). Steers were evaluated for ultrasound backfat thickness (**UFT**) with a Classic 200 real-time ultrasound unit (Pie Medical Equipment Co., Maastrict, The Netherlands) equipped with an 18-cm, 3.5-MHz linear array transducer by a certified ultrasound technician on d 70 and just prior to harvest. All steers were harvested on d 174 at a commercial abattoir based on an average UFT of 1.0 cm.

Table 1. Ingredient composition and proximate analysis of diets fed during the growing and finishing phases[a]

Ingredient	Growing ration	Finishing ration
Corn silage, %	26.61	-
Barley, %	24.62	36.00
Rolled corn, %	24.30	47.50
Alfalfa hay, %	23.67	8.00
Grass hay, %	-	6.00
Soybean meal, %	-	1.60
Urea, %	-	0.60
Trace mineralized salt, %	0.54	0.30
Limestone, %	0.26	-
Chemical composition:		
DM, %	65.02	84.05
CP, %	10.30	12.72
ADF, %	20.97	8.71
NE_m, Mcal/kg	1.74	1.96
NE_g, Mcal/kg	0.99	1.24
Crude fat, %	2.00	2.88

[a]DM basis

After chilling at 2°C for 24 hr, complete carcass data was collected on each carcass, including: HCW, REA, backfat thickness, KPH fat, calculated yield grade, USDA yield grade, marbling score, and USDA quality grade. In addition, one complete boneless strip loin (Institutional Meat Purchase Specification 180; NAMP, 1992) was removed from each carcass during fabrication, vacuum-packaged, boxed, and transported on ice to the University of Idaho Meats Laboratory. Strip loins were cut into 2.54-cm steaks, individually vacuum-packaged, and aged at 2°C for 14 d prior to storage at –20°C for later analysis.

Steaks were thawed and broiled on DeLonghi Alfredo grills (model BG-16, DeLonghi America Inc., Carlstadt, NJ) to an internal temperature of 71°C (AMSA, 1995) as monitored by thermocouples and a Digi-Sense scanning thermocouple thermometer (Cole Parmer, Niles, IL). After cooling to 20°C, six 1.27-cm cores were removed parallel to the muscle fiber and peak Warner-Bratzler shear force values were recorded.

A trained 10-person sensory panel also evaluated steaks for tenderness, juiciness, flavor, and off-flavor. After broiling as described, steaks were cut into 2.54- × 1.27- × 1.27-cm cubes and served warm to each panelist. Panelists evaluated 7 samples per session (2 sessions/d) for tenderness (1 = extremely tough, 10 = extremely tender), juiciness (1 = dry, 10 = juicy), flavor (1 = bland, 10 = intense), and off-flavor (1 = none detectable, 10 = pronounced) using a 10-point scale with anchored end-points (AMSA, 1995).

Data Analyses. Residual feed intake was defined as the difference between actual DMI and DMI predicted by a regression model. To calculate an RFI value for each steer, DMI was predicted by linear regression, via PROC REG (SAS Inst., Inc., Cary, NC), of DMI on mid-test $BW^{0.75}$ (**MBW**), ADG (Koch et al., 1963), and d 70 UFT (Basarab et al., 2003). The calculated RFI values were used to individually classify steers into 3 RFI groups: efficient (< -0.5 SD), marginal (± 0.5 SD), and inefficient (> 0.5 SD). Performance (growing phase and carcass characteristics) and end-product measurements were evaluated across RFI groups using a mixed linear model (PROC MIXED), and means were partitioned using the PDIFF option. Correlations were calculated using the PROC CORR procedure of SAS.

Results and Discussion

Means, SD, and minimum and maximum values for performance traits of all steers during the 84-d RFI evaluation phase are reported in Table 2. As described above, steers were classified into 3 RFI groups: efficient (n = 9; RFI = -0.60 kg/d), marginal (n = 18; RFI = -0.01 kg/d), and inefficient (n = 8; RFI = 0.65 kg/d). Means for the 3 groups are included in Table 3.

There were no differences among RFI groups for initial BW, ADG, final (d 84) BW, or d 70 UFT. Similarly, HCW, REA, adjusted fat thickness, KPH, calculated yield grade, percent lipid, and percent cooking loss were similar across all RFI groups.

During the RFI evaluation phase, inefficient steers had greater DMI than marginal ($P < 0.05$) and efficient ($P <$

0.01) steers, and marginal steers tended ($P = 0.07$) to have greater DMI than efficient steers. Likewise, values for both feed conversion ratio (**FCR**) and G:F were greater ($P < 0.01$) for inefficient vs. marginal and efficient steers, but not different between marginal and efficient steers. Since steers were classified into groups based on RFI value, all groups differed ($P < 0.001$) from each other for RFI.

Several carcass traits were different among the RFI groups. Inefficient steers had greater marbling scores than efficient ($P = 0.02$) and marginal ($P = 0.03$) steers. However, efficient and marginal steers did not differ ($P = 0.59$) for marbling score. Interestingly, USDA quality grade was greater ($P = 0.02$) for inefficient vs. efficient steers, and not different between marginal and inefficient ($P = 0.15$) or efficient ($P = 0.79$) steers.

The mean Warner-Bratzler shear force value for efficient steers was greater ($P = 0.05$) than that of inefficient steers, and tended ($P = 0.09$) to be greater than marginal steers. Relative to traits evaluated by a trained sensory panel, tenderness was not different between inefficient and either efficient or marginal steers, although marginal steers tended ($P = 0.07$) to have greater tenderness scores than efficient steers. Juiciness scores were greater ($P < 0.01$) for inefficient than efficient steers, and tended ($P = 0.10$) to be greater for marginal vs. efficient steers. Sensory panel flavor scores were greater ($P < 0.01$) for inefficient steers compared to efficient steers, and tended ($P = 0.06$) to be greater for inefficient vs. marginal steers. Flavor scores were not different between the efficient and marginal groups. Off-flavor scores tended ($P = 0.06$) to be greater for marginal vs. inefficient steers, but were not different between efficient and the inefficient or marginal groups.

Partial correlations and *P*-values of RFI and FCR with other performance, carcass, and end-product traits are reported in Table 4. Relative to growing phase performance, RFI was highly correlated with DMI and FCR (positively), and G:F (negatively). In contrast, RFI was not correlated with ADG (however, FCR was negatively correlated with ADG), initial or final (d 84) BW, or d 70 UFT. For carcass and end-product characteristics, RFI was not correlated with HCW, REA, adjusted fat thickness, KPH, calculated yield grade, percent lipid, cooking loss, or sensory panel tenderness. However, RFI was positively correlated with marbling score, USDA quality grade, and sensory panel juiciness and flavor. There was also a negative correlation between RFI and Warner-Bratzler shear force and sensory panel off-flavor score.

The strong relationships observed among RFI and DMI, FCR, and G:F (and lack of relationship between RFI and ADG) in the current experiment are consistent with previous research (Herd and Bishop, 2000; Arthur et al., 2001; Basarab et al., 2003; Baker et al., 2006). However, results for RFI relative to carcass characteristics differ from some previous research.

Relationships reported in the literature among RFI and ultrasound and(or) carcass measurements are inconsistent. Baker et al. (2006) observed no differences across RFI groups for HCW, REA, backfat, KPH, yield grade, marbling score, or USDA quality grade (RFI grouping method was identical to the current experiment). Arthur et al. (2001) reported a low ($r = 0.14$) correlation between RFI and fat thickness, and Basarab and coworkers (2003) reported a tendency ($P = 0.07$) for a correlation ($r = 0.15$) between RFI and carcass marbling in low (inefficient) RFI steers. Herd and Bishop (2000) observed a negative correlation ($r = –0.43$) between RFI and carcass lean content, while Crews et al. (2003) reported correlations between RFI and fat thickness ($r = 0.45$) and marbling score ($r = 0.17$). In contrast, no relationship among RFI and intramuscular fat measured by ultrasound was reported by Carstens et al. (2002). It should be noted that in the present study, steers were the progeny of only 4 Angus sires. Thus, it is not known whether or not there is a sire effect influencing the variables measured.

Additionally, few experiments have evaluated end-product quality (shear force, palatability, etc.) in cattle that have been characterized for RFI. Baker et al. (2006) reported no differences among RFI groups for Warner-Bratzler shear force, in contrast to the current experiment. It should be noted that all of the steaks had average shear force values of 4.0 kg or lower, and therefore were considered acceptable (Huffman et al., 1996). No difference among RFI groups for sensory panel tenderness or juiciness scores was also reported by Baker et al. (2006). However, the authors did report a tendency ($P = 0.10$) for lower juiciness scores in efficient vs. marginal and inefficient steers. Additionally, it was reported that off-flavor scores were lower for efficient vs. inefficient steers.

In conclusion, data from this small number of observations suggest that feed efficiency of Angus steer progeny of sires divergent for intramuscular fat EPDs may be related to several meat quality factors that influence the value and palatability of beef. Additional research which incorporates complete end-product evaluation (Warner-Bratzler shear force and sensory panel analysis) of steaks from steers evaluated for RFI is needed.

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Table 2. Mean, SD, and range of performance traits of steers evaluated for residual feed intake (RFI)

Trait	Mean	SD	Minimum	Maximum
Initial BW (d 0), kg	305.4	27.68	249.0	351.0
ADG, kg	1.3	0.15	1.0	1.7
DMI, kg/d	9.6	0.84	7.6	11.4
RFI, kg/d	0.0	0.52	-1.34	1.20
FCR (DMI:ADG)[a]	7.3	0.65	6.4	9.2
G:F (ADG:DMI)	0.14	0.012	0.11	0.16
Final BW (d 84), kg	416.2	33.26	356.0	486.0
d 70 UFT[b], cm	0.66	0.121	0.46	0.91

[a]FCR = feed conversion ration.
[b]UFT = ultrasound fat thickness.

Table 3. Least squares means for performance traits and carcass characteristics of steers classified into "efficient" (> 0.5 SD above mean; n = 9), "marginal" (± 0.5 SD from mean; n = 18), and "inefficient" (< 0.5 SD below mean; n = 8) groups based on residual feed intake (RFI) values

Trait	RFI Group			SEM	*P*-value
	Efficient	Marginal	Inefficient		
n =	9	18	8	-	-
Initial BW (d 0), kg	309	301	310	8.7	0.682
ADG, kg	1.29	1.34	1.30	0.047	0.678
DMI, kg/d	9.01[e]	9.58[e]	10.24[f]	0.228	0.007
RFI, kg/d	-0.604[e]	0.011[f]	0.654[g]	0.0827	0.001
FCR (DMI:ADG)[a]	6.97[e]	7.21[e]	7.92[f]	0.174	0.004
G:F (ADG:DMI)	0.144[e]	0.140[e]	0.127[f]	0.0031	0.005
Final BW (d 84), kg	418	414	419	10.5	0.927
d 70 UFT[a], cm	0.64	0.69	0.61	0.037	0.257
HCW, kg	325.3	319.4	325.3	7.39	0.767
REA, cm^2	79.1	79.2	78.6	1.83	0.977
Adjusted fat thickness, cm	1.17	1.29	1.17	0.078	0.433
KPH fat, %	2.9	2.9	2.7	0.12	0.373
Calculated yield grade	3.05	3.09	3.04	0.105	0.904
Marbling score[b]	573.3[e]	598.9[e]	711.3[f]	35.80	0.043
USDA quality grade[c]	1.8[e]	1.8[e]	2.4[f]	0.15	0.029
Lipid, %	4.8	5.3	6.1	0.54	0.313
Cooking loss, %	21.4	22.3	21.3	0.88	0.655
Warner-Bratzler shear force, kg	3.2[e]	2.8[ef]	2.7[f]	0.16	0.107
Sensory panel tenderness[d]	6.7	7.4	7.3	0.27	0.178
Sensory panel juiciness[d]	5.5[e]	6.0[ef]	6.5[f]	0.24	0.029
Sensory panel flavor[d]	5.4[e]	5.8[ef]	6.4[f]	0.22	0.025
Sensory panel off-flavor[d]	1.4	1.7	1.2	0.19	0.155

[a]FCR = feed conversion ration; UFT = ultrasound fat thickness.
[b]Marbling scores are coded as 400 = Slight, 500 = Small, 600 = Modest, 700 = Moderate, 800 = Slightly Abundant;
[c]USDA quality grades are coded as 1 = Select, 2 = Choice, and 3 = Prime.
[d]Tenderness, juiciness, flavor, and off-flavor like/dislike: 1 = dislike extremely, 9 = like extremely.
[e,f,g]Means in the same row without common superscripts are different ($P < 0.05$).

Table 4. Partial correlations (*P*-values) of residual feed intake (RFI) and feed conversion ration (FCR; DMI:ADG) with other performance traits and carcass characteristics in steers evaluated for RFI

Trait	RFI	FCR
Initial BW (d 0), kg	0.03 (0.87)	0.22 (0.20)
ADG, kg	0.00 (1.00)	-0.64 (0.0001)
DMI, kg/d	0.61 (0.0001)	0.13 (0.44)
RFI, kg/d	-	0.64 (0.0001)
FCR (DMI:ADG)[a]	0.64 (0.0001)	-
G:F (ADG:DMI)	-0.63 (0.0001)	-0.99 (0.0001)
Final BW (d 84), kg	0.02 (0.92)	-0.06 (0.72)
d 70 UFT[a], cm	0.00 (1.00)	-0.28 (0.10)
HCW, kg	-0.07 (0.68)	-0.07 (0.71)
REA, cm^2	-0.04 (0.84)	-0.29 (0.09)
Adjusted fat thickness, cm	0.02 (0.91)	-0.28 (0.11)
KPH fat, %	-0.26 (0.14)	-0.06 (0.74)
Calculated yield grade	-0.06 (0.75)	-0.01 (0.95)
Marbling score	0.41 (0.01)	0.25 (0.14)
USDA quality grade	0.40 (0.02)	0.27 (0.11)
Lipid, %	0.24 (0.16)	0.10 (0.58)
Cooking loss, %	-0.02 (0.90)	0.10 (0.57)
Warner-Bratzler shear force, kg	-0.33 (0.05)	-0.24 (0.17)
Sensory panel tenderness	0.20 (0.24)	0.25 (0.15)
Sensory panel juiciness	0.48 (0.004)	0.28 (0.10)
Sensory panel flavor	0.47 (0.004)	0.42 (0.01)
Sensory panel off-flavor	-0.34 (0.05)	-0.37 (0.03)

[a]FCR = feed conversion ration; UFT = ultrasound fat thickness.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

SERUM THYROXINE, TRIIODOTHYRONINE AND IGF-1 CORRELATE WITH POSTWEANING GROWTH AND CARCASS TRAITS IN LAMBS

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ABSTRACT: Forty two lambs (90 ± 22 d and 24 ± 2.2 kg) were used to examine relationships between serum concentrations of triiodothyronine (T_3), thyroxine (T_4) and IGF-1 during the postweaning period with body weight, ADG, feed conversion and carcass traits. Lambs came from Suffolk (SF), Charollais (CH), Blackbelly (BB), Pelibuey (PB), and Katahdin (KH) rams and BB and PB ewes. Lambs were housed in individual stalls and fed *ad libitum* with a diet of alfalfa hay and concentrate. Served feed and orts were weighed and recorded daily and lambs weighed every 14 d and bled every 28 d for the first 3 samplings and then every 14 d until slaughter (SF and CH = 45 - 50 kg, KH = 40 - 45 kg, BB and PB = 35 - 40 kg). Correlation coefficients between body weight and hormone concentrations were high (P< 0.05) for all sire breed groups (r = 0.78 to 0.99). Canonical correlation (Rc) analyses were used to determinate the relationship between groups of variables: **WF**, ADG and feed conversion; **LFC**, weight of liver, kidney and pelvic fat and testis, rib eye area and fat thickness; **C**, weight of carcass cuts (front and rear legs, and loin) as a percentage of chilled carcass weight; **CC**, percentage of lean, fat and bone in cuts mentioned before; and **HC**, serum hormone concentrations measured at slaughter and at different classes of rates of body weight to slaughter weight: R1, 27 - 58 %; R2, 58.1 - 70 %; R3, 70.1 - 76 %; and R4, 76.1 – 95 %. Variables to be included in the LFC group were selected by principal component analysis. Canonical correlations were important between WF and HC at R4 (Rc = 0.61; P < 0.05); LFC and HC at slaughter (Rc = 0.51; P = 0.31) and at R1 (Rc = 0.78; P = 0.07); C and HC at slaughter (Rc = 0.40; P = 0.86); and between CC and HC at slaughter (Rc = 0.72; P = 0.10) and at R3 (Rc = 0.88; P < 0.05). Serum concentrations of IGF-1, T_3 and T_4 in lambs do relate to postweaning growth and carcass traits, when measured near or at slaughter.

Key words: T_3, T_4, IGF-1, Growth, Lambs

Introduction

Triiodothyronine (T_3) and thyroxine (T_4) hormones along with IGF-1, are important factors in normal growth and normal development of animals. Preweaning concentrations of T_3 and T_4 appear to be related to growth patterns in lambs (Storer et al., 2005), and a deficiency in early life has been related to a delay on growth (Garcia et al., 2005). It is known that a positive association exists between the thyroid hormones and weaning weight (Garcia et al., 2005) and the combination of serum thyroid hormones and IGF-1 is a tool for examining onset of puberty in ewe lambs (Storer et al., 2005). The knowledge of the relation in hormone changes during the preweaning period with growth could be a valuable tool for the early selection of lambs with faster growth (McBee et al., 2006). In addition, it would be interesting to know the circulating levels of these hormones from weaning to slaughter and how they relate with growth and body composition. Therefore, the objective of the present study was to examine the relationships of serum concentrations of T_3, T_4 and IGF-1 during the postweaning period with body weight, ADG, feed conversion and carcass traits.

Materials and Methods

Animals. Forty two weaned male lambs with an average age and weight of 90 ± 22 d and 24 ± 2.2 kg, respectively, sired by 2 Suffolk, 2 Charollais (CH), 2 Blackbelly (BB), 2 Pelibuey (PB) and 2 Katahdin (KH) rams with 16 BB and 16 PB ewes were used. Numbers of lambs per sire breed were 10, 10, 10, 7 and 5, respectively. Lambs received a vitamin A injection and were treated for internal and external parasites and then were housed in individual stalls. Previous a 15-d adaptation period with a 50:50 forage:concentrate diet, lambs received a finishing diet fed *ad libitum* consisting of alfalfa hay (20.83 %), dry rolled corn (57.58 %), cotton seed meal (11.8 %), corn gluten meal (4.96 %), cane molasses (3.57 %), salt (.44 %), mineral and vitamin premix (.44 %), and calcium carbonate (.37 %). Amounts of feed and orts were recorded daily and lambs weighed every 14 d with a 12 h feed withdrawal, until they reached the slaughter weight of 45 - 50 kg (SF and CH), 40 - 45 kg (KH) and 35 - 40 kg (BB and PB).

Blood Collection. Blood samples were collected every 28 d and after the third collection every 14 d by jugular venipuncture. Lambs were fasted 12 h before bleeding and bled in the same order and at the same time every day. All samples were collected into 10-mL vacuum serum separator tubes which were allowed to stand at room temperature for 30 min before centrifugation at 4° C for 15 min at 1200 x g. Serum was then transferred to plastic vials and stored frozen until analyzed.

Hormone Analysis. Serum concentration of IGF-1 was quantified by RIA as described by Berrie et al. (1999), and T_3 and T_4 were quantified by RIA using components of commercial kits (Coat-A-Count; Diagnostic Products Corp. Inc., Los Angeles, CA), as described by Wells et al. (2003) and Richards et al. (1999), respectively. Within and between assay coefficient of variation (CV) for IGF-1, T_3 and T_4 was 18.1 %, 3.6 % and 8.49 % or less, respectively.

Canonical correlation (Rc) analyses were used to determinate the relationship between groups of variables: WF, ADG and feed conversion; LFC, weight of liver, kidney and pelvic fat and testis with skin, rib eye area and fat thickness; C, weight of carcass cuts (front and rear legs, and loin) as a percentage of chilled carcass weight; CC, percentage of lean, fat and bone in cuts mentioned before; and HC, serum hormone concentrations measured at slaughter and at different classes of rates of body weight to slaughter weight: R1, 27 - 58 %; R2, 58.1 - 70 %; R3, 70.1 - 76 %; and R4, 76.1 - 95 %. Variables to be included in the LFC group were selected by a principal component analysis.

Results and Discussion

Pearson correlation coefficients between body weight and serum hormone concentrations were high for all sire breed groups ($r = 0.78$ to 0.99; $P < 0.05$). Serum hormone concentrations during the feeding period showed a quadratic trend, similar to growth.

Values obtained for the first and second principal components of the carcass traits with a cumulative explained variance of 50 % are shown in Table 1. Figure 1 shows that lambs could be divided in two groups according to the biological group, lambs from hair breed sires and wool breed sires.

The canonical correlations were important between WF and HC at R4, with Rc = 0.61 ($P < 0.05$), being greater the contribution of IGF-1 and T_3 in HC and feed conversion in WF (Table 2); between LFC and HC at slaughter with Rc = 0.51 ($P = 0.31$) and at R1, with Rc = 0.78 ($P = 0.07$), suggesting a greater relation of the components of this group of variables at the beginning of the feeding trial (Table 3); between C and HC at slaughter with Rc = 0.40 ($P = 0.86$) more importantly IGF-1 and T_3, with most of the components of the group of variables, except rear leg (Table 2); and between CC and HC at slaughter with Rc = 0.72 ($P = 0.10$) and at R3 with Rc = 0.88 ($P < 0.05$), suggesting that the relation of cuts composition with hormone concentrations was higher as the lambs were heavier or got closed to slaughter weight (Table 4). The relation between hormone concentrations with growth and body composition in male lambs in this study are similar to those found by Storer et al. (2005) with Rambouillet ewe lambs when they analyzed growth traits, pubertal response, and serum concentrations of T_3, T_4, GH and IGF-1. They concluded that serum concentrations of T_3 and T_4 are related to growth patterns and this relation could be extended to puberty, which is achieved during the postweaning period.

Very high canonical correlation coefficients were obtained (Rc = 0.69 to 0.98; $P < 0.05$) between LFC and WF, C and CC, suggesting that the carcass traits selected through a principal component analysis have a relation with the efficiency of the lamb to gain weight during the postweaning period and with the body composition at slaughter.

Implications

Serum concentrations of IGF-1, T_3 and T_4 in lambs are related to postweaning growth and carcass traits, when measured near or at slaughter weight.

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Table 1. Principal component analysis for carcass traits

Variable	Principal Component 1 (PC1)	Principal Component 2 (PC2)
Liver (kg)	-0.230	0.206
Empty guts (kg)	-0.193	0.431
Kidney and pelvis fat (kg)	0.014	0.463
Hot carcass (kg)	-0.348	0.021
Carcass yield (%)	0.037	-0.323
Testis w/skin (kg)	-0.089	0.268
Fat thickness (cm)	-0.145	0.029
Rib eye area (cm2)	-0.304	-0.080
Front leg, lean (kg)	-0.286	-0.210
Front leg, fat (kg)	-0.207	-0.029
Rear leg, lean (kg)	-0.293	-0.218
Rear leg, fat (kg)	-0.217	0.115
Loin, lean (kg)	-0.313	-0.072
Loin, fat (kg)	-0.127	0.322
Longissimus dorsi (kg)	-0.265	-0.237
Neck (kg)	-0.037	0.130
Shoulder (kg)	-0.209	-0.144
Loin (kg)	-0.240	-0.110
Rear leg (kg)	-0.263	0.006
Thorax (kg)	-0.229	0.231
Eigenvalue as proportion	0.381	0.119

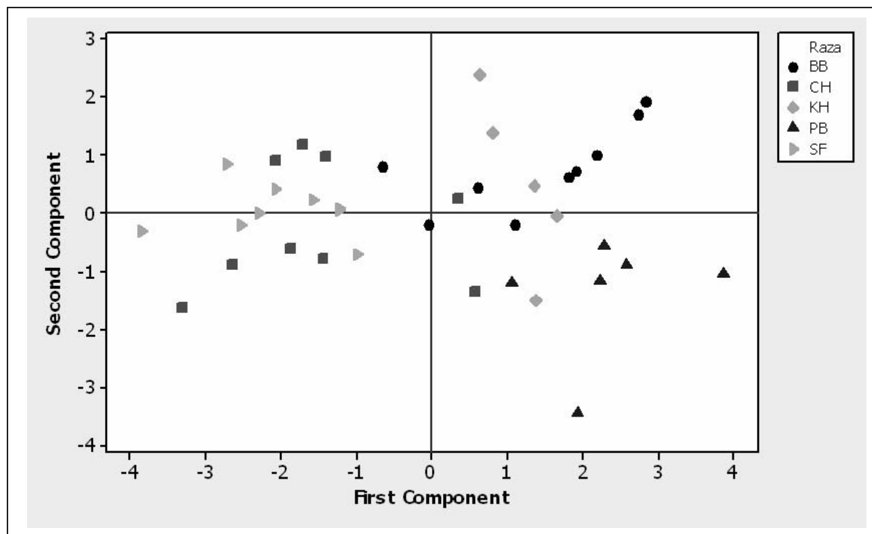

Figure 1. First and second principal components values for carcass traits (see Table 1) by sire breed group (BB = Blackbelly, CH = Charollais, KH = Katahdin, PB = Pelibuey y SF = Suffolk)

Table 2. Standarized canonical coefficients for the first canonical variables and canonical correlations for WF (ADG and feed conversion) and HC (serum concentrations of T_3, T_4 and IGF-1 when lambs reached 76.1 to 95 % (R4) of their slaughter body weight); and for C (weight of carcass cuts such as front and rear legs, and loin, as percentages of chilled carcass weight) and HC (serum concentrations of T_3, T_4 and IGF-1 at slaughter)

R4				Slaughter			
WF		HC		C		HC	
Avg. Daily Gain	-0.15	IGF-1	0.60	Front leg	0.56	IGF-1	0.70
Feed Conversion	-1.13	T_3	0.69	Rear leg	0.07	T_3	0.68
		T_4	-0.16	Loin	0.50	T_4	0.17
				Longissimus dorsi	-0.84		
Canonical correlation = 0.61 (P < 0.05)				Canonical correlation = 0.40 (P = 0.86)			
Eigenvalue as proportion = 0.97				Eigenvalue as proportion = 0.94			

Table 3. Standarized canonical coefficients for the first canonical variables and canonical correlations for LFC (liver weight, kidney and pelvic fat weight and carcass traits such as rib eye area and fat thickness) and HC (serum concentrations of T_3, T_4 and IGF-1 at slaughter and when lambs reached 27 to 51 % (R1) of their slaughter body weight)

Slaughter				R1			
LFC		HC		LFC		HC	
Liver	-0.66	IGF-1	-0.66	Liver	-0.08	IGF-1	0.67
Kidney and pelvic fat	0.69	T_3	0.01	Kidney and pelvic fat	0.25	T_3	-1.43
Testis w/skin	-0.42	T_4	0.66	Testis w/skin	0.56	T_4	1.21
Rib eye area	0.64			Rib eye area	0.34		
Fat thickness	0.34			Fat thickness	0.62		
Canonical correlation = 0.51 (P = 0.31)				Canonical correlation = 0.78 (P = 0.07)			
Eigenvalue as proportion = 0.67				Eigenvalue as proportion = 0.80			

Table 4. Standarized canonical coefficients for the first canonical variables and canonical correlations for CC (lean, fat and bone percentages in cuts mentioned in table 2) and HC (serum concentrations of T_3, T_4 and IGF-1 at slaughter and when lambs reached 70.1 to 76 % (R3) of their slaughter body weight)

Slaughter				R4			
CC		HC		CC		HC	
FL, Lean	-0.18	IGF-1	0.001	FL, Lean	-0.83	IGF-1	0.19
FL, Fat	-0.11	T_3	0.11	FL, Fat	0.10	T_3	-0.36
FL, Bone	-0.44	T_4	0.08	FL, Bone	-0.51	T_4	1.18
RL, Lean	-0.09			RL, Lean	0.08		
RL, Fat	0.09			RL, Fat	0.05		
RL, Bone	0.01			RL, Bone	0.90		
L, Lean	0.04			L, Lean	0.32		
L, Fat	0.02			L, Fat	0.002		
L, Bone	-0.01			L, Bone	-0.58		
Canonical correlation = 0.72 (P = 0.10)				Canonical correlation = 0.89 (P = 0.05)			
Eigenvalue as proportion = 0.72				Eigenvalue as proportion = 0.74			

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

FEEDING BARLEY BETA-GLUCAN ENHANCES IMMUNE RESPONSE IN MICE[1]

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ABSTRACT: We conducted 3 research trials using mice to investigate the effects of feeding barley beta-glucans on immune response after infection with influenza virus. In Trials 1 and 2, 75-80 mice were fed diets of high beta-glucan barley (HBG; 7.6% beta-glucan), low beta-glucan barley (LBG; 3.8% beta-glucan), corn (0.4% beta-glucan), or a commercial mouse chow (STD, Trial 2 only; 0.5% beta-glucan). In Trial 3, 20 mice were fed diets of Geraldine barley (3.11% beta-glucan), Geraldine barley plus purified barley beta-glucan (BBG; 5.3% beta-glucan), corn (0.10% beta-glucan), or corn plus purified barley beta-glucan (CBG; 6.1% beta-glucan). After 10 d diet adaptation, mice from each treatment were infected with influenza virus (d 0; 50 µL/mouse). Weight, intake, and immune response were measured on d -10, -6, 0, 3, 5, 7, 10, 12 and/or 15. In Trial 1, mice fed HBG had greater (P = 0.001) BW than mice fed LBG and corn on d 5 and 10. In Trial 2, BW by mice fed HBG and LBG was greater (P = 0.04) on d 0 than BW by mice fed STD. Mouse BW did not differ (P = 0.43) between treatments in Trial 3. In Trials 1 and 2, DMI did not differ ($P > 0.10$) between diets, averaging 10.0 and 10.2% BW, respectively. Mice fed HBG had greater (P = 0.001) serum and lung IgG on d 7 and less serum and lung IgG on 10 d compared to mice fed corn and LBG. Mice fed LBG had greater (P = 0.001) lung TNFα than corn-fed mice on d 5, and mice fed corn and LBG had less lung TNFα than mice fed HBG on d 7. Lung albumin was greater (P = 0.002) in mice fed HBG and corn than mice fed LBG on d 7, and least for HBG-fed mice, intermediate for LBG-fed mice, and greatest for corn-fed mice on d 10. Viral recovery was less (P = 0.04) in mice fed LBG than mice fed corn and HBG on d 7. On d 10 in Trial 3, mice fed BBG tended (P = 0.11) to have greater lung lactate dehydrogenase than mice fed barley, and mice fed CBG tended (P = 0.10) to have greater serum IgA compared to mice fed corn. Beta-glucan intake ranged from 0.02 to 0.8 g/kg BW in corn-based diets and from 0.7 to 11.6 g/kg BW in barley-based diets. The immune system of mice can be stimulated by feeding barley-based diets.

Key Words: Barley, Intake, Mice

[1] The authors wish to thank Ann Harmsen, Soo Han, and Katie Shampeny for their technical assistance in the lab and Tammy Marcotte and her colleagues at the MSU Animal Resource Center for their animal care expertise on this project.

Introduction

Beta-glucans are naturally occurring forms of carbohydrate found in yeast, fungi, oats, and barley that have been shown to stimulate the mammalian immune system (Williams, 1997). Research has shown that cells of the immune system have specific receptors that recognize beta-glucan (Williams, 1997), including beta-glucans from barley (Czop and Austen, 1985). Oral administration of purified beta-glucans (Yun et al., 1997; Davis et al., 2004) including beta-glucans from barley (Delaney et al., 2003) has stimulated the immune system. Sealey et al. (2006) reported that fish fed diets based on barley varieties containing 5.2 or 8.2% beta-glucan had improved survivability compared to fish fed wheat-based diets (~1% beta-glucan). The objective of our research was to determine if immune modulating activities could be conferred to mice through consumption of barley-based diets high in beta-glucan.

Materials and Methods

Trial 1. Seventy-five C57BL/6 mice were fed diets of high beta-glucan barley (HBG; 9.4% beta-glucan in grain), low beta-glucan barley (LBG; 4.7% beta-glucan in grain), or corn (<0.5% beta-glucan in grain). Mice were housed 5/cage with 25 mice/treatment. Diets were balanced for CP, fat, lysine, methionine, vitamins, and minerals according to mouse nutrient requirements (NRC, 1995). Diet composition data are presented in Table 1. Weight and intake data were collected on d -10, -4, 0, 3, 5, 7, and 10. After a 10 d diet adaptation period, mice from each treatment were infected with PR8 influenza virus (day 0; 50 µL/mouse). Five mice (1 cage) from each treatment were sacrificed on d 5, 7, and 10. Serum samples were collected and analyzed by ELISA for influenza-specific IgG and IgA levels. Bronchoalveolar lavage (BAL) samples were also collected and analyzed for influenza-specific IgG and IgA by ELISA; TNFα, IFNγ, IL-2, IL-4, and IL-5 by using a Cytokine Bead Array kit (BD Biosciences, San Diego, CA), serum albumin by colorimetric assay (Sigma Diagnostics, St. Louis, MO), viral recovery as described in Wiley et al. (2001), and cell content by cell differential count. Body weight and intake data were analyzed using the PROC MIXED procedure of SAS (Littell et al., 1998). Immune data were analyzed using the GLM procedure of SAS with treatment, day, and their interaction in the model. Means were separated using LSMEANS when $P < 0.05$.

Trial 2. Eighty C57BL/6 mice were fed diets based on HBG, LBG, corn, and a commercial mouse chow (STD; 20 mice/diet; 5 mice/cage) as previously described in Trail 1. The same experimental protocol was followed as in Trial 1, except only body weight and intake were monitored. Data were analyzed using the PROC MIXED procedure of SAS (Littell et al., 1998). Means were separated using LSMEANS when $P < 0.05$.

Trial 3. Twenty C57BL/6 mice were fed diets of Geraldine barley (BAR; 3.11% beta-glucan), Geraldine barley plus a purified beta-glucan from barley (65% BG) that was used to raise the beta-glucan content of the diet (BBG; 5.3% beta-glucan), corn (0.10% beta-glucan) or corn plus purified barley beta-glucan (CBG; 6.1% beta-glucan). The same timeline and experimental procedures were followed as in Trial 1 and 2; however, all mice were sacrificed on d 10 and samples were collected for analysis of IgG, IgA, and albumin as previously described and lactate dehydrogenase (CytoTox 96 kit, Promega, Madison, WI). Body weights were analyzed using the PROC MIXED procedure of SAS (Littell et al., 1998). Immunity data were analyzed using PROC GLM. Contrasts were also performed in order to compare supplemented vs nonsupplemented diets. Means were separated using LSMEANS when $P < 0.05$.

Results

Trial 1. After 10 d of diet adaptation, mice fed HBG had greater ($P = 0.001$) BW than mice fed corn, with mice fed LBG being intermediate and similar to other treatments (Table 2). Mice fed HBG weighed more ($P = 0.001$) than mice fed LBG and corn on d 5 and 10 post-infection. Mice fed HBG and LBG weighed more ($P = 0.001$) than corn-fed mice on d 7. Ten days after infection with influenza, mice fed barley diets had BW similar ($P > 0.05$) to those prior to infection, while mice fed corn had not recovered their BW to pre-infection levels.

Dry matter intake was similar ($P = 0.10$) between treatments averaging 10.0% BW. Beta-glucan intake (g/kg BW) differed between treatments ($P = 0.001$) and ranged from 0.1 to 0.5 for corn-fed mice, 3.9 to 10.1 for HBG-fed mice, and 1.5 to 5.1 for LBG-fed mice.

Numbers of macrophages, monocytes, lymphocytes, neutrophils, eosinophils, and levels of IL-5 in BAL did not differ ($P > 0.17$) between treatments (data not presented).

Mice fed LBG had lower ($P < 0.05$) IL-4 and IL-2 in BAL than mice fed corn or HBG (2.4 vs avg 3.4 pg/mL for IL-4 and 1.8 vs avg 2.3 pg/mL for IL-2). Level of TNFα in BAL was greater ($P = 0.001$) in LBG-fed than corn-fed mice on d 5, but was greater in mice fed HBG than corn and LBG on d 7 (Table 3). On d 7, BAL IFNγ was greatest ($P = 0.002$) in mice fed HBG, intermediate in mice fed corn, and lowest in mice fed LBG. Levels of IgG and IgA in BAL and serum peaked on d 7 for HBG and d 10 for corn and LBG. This resulted in greater ($P = 0.001$) antibody levels in HBG-fed mice on d 7 and lower levels in HBG-fed mice on d 10 compared to other treatments. Viral recovery was lower ($P = 0.04$) in mice fed LBG than corn or HBG on d 7. Albumin in BAL of mice fed HBG and corn was greater ($P = 0.002$) than in mice fed LBG on d 7, but on d 10 was lowest ($P = 0.002$) in mice fed HBG, intermediate in mice fed LBG, and highest in mice fed corn.

Trial 2. Body weight differed ($P = 0.04$) between treatments on d -6, 0, and 15 (Table 4). Mice fed HBG had greater ($P = 0.05$) BW on d 0 than mice fed all other diets. Mice fed HBG and LBG recovered BW sooner after infection (d 12) than mice fed corn and STD (d 15). By d 15, only mice fed LBG had surpassed their d 0 pre-infection BW. Dry matter intake did not differ ($P = 0.78$) between treatments averaging 10.2% BW. Beta-glucan intake (g BG/kg BW) ranged from 0.1 to 0.6 by corn-fed mice, 0.1 to 0.8 by STD-fed mice, 0.7 to 5.8 by LBG-fed mice, and 1.8 to 11.6 by HBG-fed mice.

Trial 3. Mouse BW differed ($P = 0.001$) between days being highest on d 0 (21.7 g) and lowest on d 7 and 10 (avg 17.7 g). Mouse BW did not differ between treatments ($P > 0.43$) or contrasts ($P > 0.45$). A high SE (0.43) was observed in this trial which could be due to yeast cell contamination in lung samples and may have influenced results. Mice consuming BBG tended ($P = 0.11$) to have higher LDH values than mice consuming barley (3.88 vs 2.30 unit/mL); while mice consuming CBG tended ($P = 0.10$) to have higher IgA levels in serum than mice consuming corn (0.24 vs 0.14 absorbance at 405 nm). Dry matter intake (% BW) by mice was 10.4 for barley, 8.1 for BBG, 8.6 for corn, and 6.8 for CBG. Beta-glucan intake (g BG/kg BW) by mice ranged from 0.02 to 0.13 for corn, 1.1 to 6.9 for CBG; 2.2 to 4.4 for barley, and 1.5 to 7.0 for BBG.

Discussion

Body weight is a clinical sign of sickness and immunity response was in agreement with BW response in Trial 1. Weight results were slightly different between Trials 1 and Trial 2. This could be due to differences in initial BW or viral batch used to infect mice. It should be noted that the HBG barley was a waxy starch variety which may have had more energy than the normal starch variety used in the LBG diet (Miller et al., 1994). This would explain differences in BW prior to infection and also may have influenced the ability of mice to fight infection.

Macrophages have the beta-glucan receptor, but this did not result in greater proliferation of macrophages in the current study. In contrast to our data, Estrada et al. (1997) reported that intraperitoneal injection of oat beta-glucan increased numbers of macrophages, but not neutrophils and lymphoctyes in mice. Similar to our data, Estrada et al. (1997) also reported that oat BG stimulated production of IL-2, IL-4, and IFNγ in cultured spleen cells and caused a small increase in TNFα by macrophages. Oat beta-glucan also increased the number of cells secreting IL-4 and IFNγ (Yun et al., 1997) and immunoglobulins in serum.

Our results suggest that antibodies and inflammatory mediators response to a pathogen occurred 3 d earlier in mice consuming barley diets. Antibodies fight viruses outside the cell and are most effective at preventing or lessening a viral invasion, while TNFα is a mediator of inflammation that is produced by macrophages and is essential for an animal's resistance to infection. The peak in TNFα on d 5 in LBG-fed mice could help explain lower viral counts in LBG-fed mice on d 7. Interferon-γ is also

produced by macrophages and is one of the most potent antiviral cytokines. While barley-based diets did not increase the number of macrophages, it does appear that macrophage activity was stimulated.

Lower albumin levels in LBG mice on d 7 may correspond to less damage in the lungs of these mice while lower albumin levels in HBG fed mice on d 10 may have indicated faster repair. Lactate dehydrogenase is a cytoplasmic enzyme found in BAL only after cell death and higher levels would correspond to increased cell damage. Albumin and LDH results between trials are in general agreement. Barley diets in Trial 3 were formulated with barley containing "normal" rather than high levels of BG. The barley diet may have already had an optimum level of BG for immune system stimulation; however, corn-fed mice may have benefited from supplemental BG. There may be a threshold level for beta-glucan or there may be other components in the barley that affect immune status.

Other researchers have stimulated the immune system of mice with oral beta-glucan doses as low as 0.10 to 0.22 g BG/kg BW (Yun et al., 1997; Davis et al., 2004). Interestingly, even mice fed corn or a commercial mouse chow consumed amounts of BG similar to these doses.

Implications

Mice fed barley-based diets had improved immune response and weight recovery after influenza challenge. Adequate doses of beta-glucan can be achieved by consuming 80% barley diets without additional beta-glucan supplementation.

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Table 1. Composition of diets fed to mice in Trials 1, 2, and 3

	Trial 1 and 2[1]				Trial 3[2]			
Item	HBG	LBG	Corn	Standard	Barley control	Barley+BG	Corn control	Corn+BG
DM, %	90.9	91.0	90.0	89.8	95.4	95.1	94.8	94.7
CP, %	18.6	17.9	16.3	24.3	16.2	16.4	16.3	16.2
ADF, %	-	-	-	-	4.00	3.82	1.79	1.84
Starch, %	49	55	59	35.0	47.6	55.7	44.3	48.8
Beta-glucan, %	7.6	3.8	0.4	0.5	3.11	5.30	0.10	6.07
Fat, %	6.8	6.7	7.6	4.8	6.41	5.73	6.66	5.70
GE, Mcal/kg	4.74	4.72	-	4.53	4.38	4.45	4.55	4.53

[1] Diets formulated with 80% of diet being high beta-glucan barley (9.4% beta-glucan; HBG), 80% of diet being low beta-glucan barley (4.7% beta-glucan; LBG), or 75% Corn. Standard was a commercially available mouse chow.
[2] Diet formulated with 80 or 76 % of diet being Geraldine barley (3.64% beta-glucan) for Barley control and Barley+BG, respectively, and 78 or 67% of diet being corn for Corn control and Corn+BG, respectively.

Table 2. Body weights and intake by mice fed diets based on high beta-glucan barley (HBG), low beta-glucan barley (LBG), or corn in Trial 1

		Day								*P*-values		
Item	Trt	-10	-4	0	3	5	7	10	SE	Trt	Day	Trt*Day
BW, g	Corn	16.1^{b}	17.6^{c}	17.9^{dA}	17.3^{cd}	15.8^{bA}	14.1^{aA}	13.6^{aA}	0.27	0.002	0.001	0.001
	HBG	16.3^{a}	18.2^{c}	18.7^{dB}	18.1^{bc}	17.4^{bB}	16.0^{aB}	16.5^{bcC}				
	LBG	16.2^{b}	17.7^{c}	18.2^{dAB}	17.8^{cd}	16.3^{bA}	15.4^{aB}	15.7^{abB}				
DMI, % BW		-	13.1^{e}	13.1^{e}	11.2^{d}	7.4^{b}	3.9^{a}	9.1^{c}	0.46	0.10	0.001	0.16
BGI, g/kg BW	Corn	-	0.5^{A}	0.5^{A}	0.4^{A}	0.2^{A}	0.1^{A}	0.3^{A}	0.456	0.001	0.001	0.001
	HBG	-	9.8^{deC}	10.1^{eC}	8.8^{cdC}	7.0^{bC}	3.9^{aC}	7.7^{bcC}				
	LBG	-	5.1^{dB}	4.8^{cdB}	4.1^{cdB}	2.5^{bB}	1.5^{aB}	3.7^{bcB}				

[abcd] Within a row, means without a common superscript letter differ ($P < 0.05$).
[ABC] Within a column and item, means without a common superscript letter differ ($P < 0.05$).

Table 3. Immune response following influenza infection in mice fed diets based on high beta-glucan barley (HBG,) low beta-glucan barley (LBG), or corn in Trial 1

Item	Treatment	d 5	d 7	d 10	SE	*P*-value (Trt*Day)
Bronchoalveloar lavage						
Viral recovery, $\log_{10}$ plaque forming units	Corn	5.99^{b}	5.22^{bB}	0.04^{a}	0.399	0.04
	HBG	6.48^{c}	5.12^{bB}	0.04^{a}		
	LBG	6.39^{c}	2.94^{bA}	0.04^{a}		
TNFα, pg/mL	Corn	109.0^{aA}	217.3^{bA}	86.7^{a}	25.83	0.001
	HBG	169.9^{bAB}	311.5^{cB}	31.3^{a}		
	LBG	211.3^{bB}	176.0^{bA}	51.5^{a}		
IFNγ, pg/mL	Corn	65.4^{a}	1298.1^{bB}	12.2^{a}	113.34	0.002
	HBG	112.3^{a}	1952.3^{bC}	3.0^{a}		
	LBG	69.5^{a}	826.1^{bA}	5.8^{a}		
Albumin, mg/mL	Corn	0.52^{a}	1.66^{bB}	1.92^{bC}	0.158	0.002
	HBG	0.57^{a}	1.72^{bB}	0.86^{aA}		
	LBG	0.65^{a}	1.04^{abA}	1.30^{bB}		
IgA, absorbance at 405 nm	Corn	0.008^{a}	0.031^{aA}	0.779^{bB}	0.1507	0.001
	HBG	0.004^{a}	1.124^{bB}	0.077^{aA}		
	LBG	0^{a}	0.110^{aA}	0.866^{bB}		
IgG, absorbance at 405 nm	Corn	0.004^{a}	1.260^{bB}	1.877^{cB}	0.1138	0.001
	HBG	0.004^{a}	1.880^{cC}	0.953^{bA}		
	LBG	0^{a}	0.846^{bA}	1.594^{cB}		
Serum IgG, absorbance at 405 nm	Corn	0.037^{a}	1.071^{bA}	2.145^{cB}	0.0995	0.001
	HBG	0.047^{a}	2.082^{cB}	0.937^{bA}		
	LBG	0^{a}	0.835^{bA}	2.020^{cB}		

[abc] Within a row, means without a common superscript letter differ ($P < 0.05$).
[ABC] Within a column and item, means without a common superscript letter differ ($P < 0.05$).

Table 4. Body weights and intake by mice fed diets based on high beta-glucan barley (HBG), low beta-glucan barley (LBG), corn, or a commercial mouse chow (STD) in Trial 2

		Day										*P*-values
	Trt	-10	-6	0	3	5	7	10	12	15	SE	Trt*day
BW, g	Corn	14.8^{c}	14.9^{cAB}	15.5^{dA}	14.7^{c}	13.9^{b}	12.1^{a}	12.4^{a}	13.4^{b}	14.9^{cAB}	0.17	0.04
	HBG	14.9^{b}	15.5^{cB}	16.6^{dB}	15.4^{c}	14.5^{b}	12.6^{a}	13.0^{a}	14.5^{b}	15.9^{cB}		
	LBG	14.9^{c}	14.7^{cA}	15.7^{dA}	14.9^{c}	14.4^{c}	12.7^{a}	13.5^{b}	14.8^{c}	16.2^{dB}		
	STD	14.8^{c}	14.6^{cA}	15.4^{dA}	14.8^{c}	14.0^{bc}	12.1^{a}	12.2^{a}	13.4^{b}	14.3^{cA}		
DMI, % BW		-	16.0^{e}	12.9^{d}	11.1^{c}	8.0^{b}	2.2^{a}	8.3^{b}	11.6^{c}	11.9^{c}	0.78	0.88^{z}
BGI,g/kgBW	Corn	-	0.6^{A}	0.5^{A}	0.4^{A}	0.2^{A}	0.1^{A}	0.3^{A}	0.4^{A}	0.4^{A}	0.46	0.001
	HBG	-	11.6^{fC}	9.7^{eC}	8.7^{dC}	5.3^{bC}	1.8^{aB}	6.7^{cC}	8.5^{dC}	9.3^{eC}		
	LBG	-	5.8^{B}	4.4^{B}	4.0^{B}	4.0^{B}	0.7^{A}	3.2^{B}	4.3^{B}	4.7^{B}		
	STD	-	0.8^{A}	0.6^{A}	0.6^{A}	0.4^{A}	0.1^{A}	0.4^{A}	0.6^{A}	0.5^{A}		

[abcd] Within a row, means without a common superscript letter differ ($P < 0.05$).
[AB] Within a column and item, means without a common superscript letter differ ($P < 0.05$).
[z] Treatment effect ($P = 0.24$), Day effect ($P = 0.001$).

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

COMMERCIAL PHYTASES (*Aspergillus níger)* IN BROILER DIETS

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ABSTRACT: To evaluate the effect of added commercial phytases (*Aspergillus niger*) to improve phytic phosphorus utilization in broilers fed soy-corn mash basal diet, 480 one day old chicks of the commercial Cobb x Cobb hybrid were used in a factorial arrangement 3x4 with four replications of 10 birds for each treatment. Diets contained four levels of phytases (0, 300, 400 and 500 U/kg), with increasing total phosphorus (Pt) levels (0.45, 0.55 and 0.65%) by adding a commercial dicalcium phosphate. Diets with 24% crude protein, 3100 Kcal ME/kg and constant level of Ca (1%) were fed *ad libitum* to birds. Body weight, tibia bone ash and Pt retention were measured at 4 weeks of age. Chicks body weight increased (P<0.05) along with total phosphorus levels, with values of 1173, 1325 and 1379g, respectively. There was a tendency (P=0.09) to greater body weight with phytase additions with values of 1104, 1137, 1232 and 1222 g, respectively. Feed intake was greater (P<0.05) with 0.65% Pt diets, with no differences between lower levels (0.55 and 0.45% Pt), with values of 1865, 1768 and 1514 g, respectively. Phytase additions showed a tendency to increase feed intake at all Pt levels, resulting in no significant differences. Feed conversion was better (P<0.05) at 0.45% Pt level (1.29), with no differences between higher Pt levels (1.34 and 1.35). Bone ash content (%) increased (P<0.05) with phosphorus levels, with values of 38.65, 40.83 and 43.00% and with phytase additions, with values of 37.17 and 40.11, 41.87 and 42.26 and, 42.66 and 43.76 respectively for 0 and 500U/kg enzyme levels, for each increase of Pt in the diets. Bone P was 14.18, 16.37 and 17.63% P, respectively for dietary Pt levels. Total P net retention (%) was greater (P<0.05) for 0.65% Pt level, followed by 0.55 and 0.45%, with mean values of 69, 65 and 61, respectively. Total phosphorus excretion decreased with phytase addition, being more evident with 0.65% dietary Pt. It is concluded that phytase supplementation increased body gain and feed intake only at low dietary phosphorus level (0.45%), while the enzyme was more effective on bone mineralization at all phosphorus levels. In addition within each dietary Pt levels, the exogenous phytase increased Pt retention.

Key words: Phosphorus, Phytase, Broiler

Introduction

Cereal and oil grains phytates represent a potential phosphorus sources in poultry and swine feeding. However phytates are poorly utilized by these species, because of limited phytase enzyme secretion in the digestive tract of non ruminant animals. To improve P bioavailability, synthetic microbial phytases can be used to hydrolyze phytic phosphorus in poultry and swine diets. In this regard, phytic phosphorus is highly available in ruminants because of microorganisms with phytase activity that are present in the pre-digestive compartments of these species (Clark *et al*., 1986; Morse *et al.*, 1992)

Therefore, exogenous phytases are important in poultry and swine diets to improve phytic phosphorus hydrolysis, with the production of inorganic orthophosphates, phosphoric esters and myoinositol. This allows that a greater fraction of phytic phosphorus can be transformed into a usable form of the element. In addition, less inorganic phosphates are used in diet formulations, reducing the risk of environmental contamination by fecal phosphorus excretion. Reduction of the negative metabolic effects of phytic acid is an additional advantage related to the use of exogenous phytases in the diet (Denbow *et al*., 1995).

Synthetic phytases are phosphomonoesterases (Lasztity and Lasztity, 1988; Harland and Morris, 1995) present in microorganisms like fungi (*Saccharomyces cerevisae, Aspergillus spp)* and bacteria (*Bacillus subtilis, Pseudomonas spp).*

Abundant scientific and practical information suggest that exogenous phytase addition in poultry diets produces significant increases in phytic phosphorus utilization (Zhang *et al*., 2000; Beltrán *et al*., 2000; Bedford, 2000; Ahmad *et al.*, 2000; Carlos and Edwads, 1998; Denbow *et al*., 1998) with a consequent effect on animal performance.

Therefore, this paper summarizes research on the effect of exogenous phytases and increasing levels of inorganic phosphorus in broiler diets, on productive responses, bone mineralization and phosphorus retention in this specie.

Materials and Methods

The effect of commercial phytases on phytic phosphorus utilization was determined in broilers by measuring growth, body weight, feed intake, feed conversion, phosphorus retention and bone mineralization.

A total of 480 one day old chicks of the commercial hybrid Cobb x Cobb were used in a factorial arrangement 3x4 with four replications of 10 birds for each treatment. Diets contained four levels of phytases of *Aspergillus niger* (0, 300, 400 and 500 U/kg), with increasing total phosphorus (Pt) levels (0.45, 0.55 and 0.65%) by adding a commercial dicalcium phosphate. Diets, as soy-corn mash, with 24% crude protein, 3100

Kcal ME/kg and constant level of Ca (1%), trace minerals and vitamins were fed (Table 1)to broilers up to 4 weeks of age.

Table 1. Composition of broiler diets with different phosphorus and phytase levels

Ingredients	Total phosphorus (%) 0.45	0.55	0.65
Corn	48.6	48.2	47.8
Soy 48%	40.6	40.7	40.7
Salt	0.5	0.5	0.5
Ca carbonate	1.9	1.5	1.1
Methionin	0.27	0.27	0.27
Vegetal oil	7.2	7.4	7.5
Vit/min[1]	0.5	0.5	0.5
Phosphate	0.47	1.03	1.6
ME[2], kcal/kg	3100	3100	3100
CP[3], %	24	24	24
Ca, %	1	1	1
P total, %	0.45	0.55	0.65
Pd, %[4]	0.24	0.35	0.45
Phytase (U/kg)[5]	0	0	0
	300	300	300
	400	400	400
	500	500	500

[1]Vitamin and minerals (per kg feed): vitamin A, 4000UI; vitamin D, 200 UI, riboflavin, 3mg; phantotenic acid, 5mg: niacin, 20 mg; cholina, 450 mg; vitamin B12, 10 µg; vitamin E, 2 mg; Mn, 65 mg; Cu, 8 mg; Zn, 50 mg; Fe, 25 mg; Mg, 500 mg.
[2]ME: Metabolizable Energy (estimated)
[3]CP: Crude protein (N X 6.25).
[4]Bioavailability (NRC, 1994)
[5]U/kg: Phytase unit

Birds were kept in metallic batteries with water provision and feed *ad libitum*. Weekly, individual body weight and feed intake were measured.

At 4 week of age, after total body weights were measured, six birds were selected by body weight mean of each treatment and sacrificed for extraction of both tibias, to determine bone ash and phosphorus content by conventional methods (AOAC, 1984).

Additionally, phosphorus apparent net retention was determined using a balance technique, with 10 birds per treatment, during three consecutive days, measuring feed intake and cloacae excretions. These were stored at 0° C (Calvert *et al*, 1978). The following equation was used to calculate phosphorus apparent net retention (PANR):

$$PANR\ (\%) = \frac{\text{Ingested P - excreted P}}{\text{Ingested P}} \times 100$$

Data were analyzed for variance (ANAVAR), using a statistical model corresponding to a factorial arrangement and the F test was applied to verify the significance of the mean squares of the variation sources (P<0.05). Averages were compared by Tukey method. Models include main effects of phosphorus and phytase levels and phosphorus x phytase interaction. Analysis was made using the SAS statistical program. (1996).

Results and Discussion

Broiler body weights, at 4 week of age, increased (P<0.05) with total phosphorus levels (Pt) with values of 1173, 1325 and 1379g, respectively, for 0.45, 0.55, 0.65% Pt. A tendency (P=0.09) of greater body weight with phytase additions was registered. Diets with 0.45% Pt had values of 1104, 1137, 1232 and 1222, respectively for the same order of phytase additions (Table 2). An interaction (P<0.05) between total phosphorus and phytase levels was registered, with greater response to the enzyme addition at lower Pt level in the diet.

Table 2. Body weight, feed intake and feed conversion of broilers fed with increasing phosphorus and phytase levels

Treatment: Total phosphorus (%)	Phytases (U/kg)	Variables: Body weight (g/bird)	Fee intake (g/bird)
0.45	0	1104 ± 55.5^{b}	1370 ± 189^{a}
	300	1137 ± 72.3^{b}	1520 ± 143^{a}
	400	1232 ± 30.9^{a}	1611 ± 37^{a}
	500	1222 ± 33.1^{a}	1556 ± 41^{a}
Average		**1173.7 ± 47.9^{A}**	**1514 ± 102.5^{C}**
0.55	0	1289 ± 68.48^{a}	1766 ± 116^{a}
	300	1329 ± 21.83^{a}	1749 ± 91^{a}
	400	1335 ± 60.34^{a}	1744 ± 80^{a}
	500	1346 ± 32.54^{a}	1812 ± 39^{a}
Average		**1325 ± 45.80^{B}**	**1768 ± 81.5^{B}**
0.65	0	1357 ± 26.51^{a}	1834 ± 15^{a}
	300	1362 ± 22.36^{a}	1856 ± 59^{a}
	400	1394 ± 22.36^{a}	1878 ± 44^{a}
	500	1403 ± 41.64^{a}	1893 ± 61^{a}
Average		**1379 ± 28.22^{C}**	**1865 ± 44.75^{A}**

A,B Differences (P<0.05) between phosphorus levels independent of phytase levels
a,b Differences (P<0.05) between phytase levels for each phosphorus level
[1] Feed conversion: Feed intake kg/kg body weight
± Standard Deviation (n= 10)

Feed intake (g/bird chick) was higher (P<0.05) with 0.65% Pt, followed by levels of 0.55 and 0.45% Pt, with values of 1514, 1768 and 1865, respectively. Addition of exogenous phytases showed a tendency (P>0.05) to increase feed intake.

A better feed conversion was registered (P<0.05) at 0.45% Pt level (1.29), with no differences between higher Pt levels (1.34 and 1.35). On the other hand, at all phosphorus levels there was no significant response due to exogenous phytase addition.

Higher body weights are related to the effect of phytase incorporation in the diets due to a greater release of P from the phytate molecule (Qian *et al.*, 1996; Sebastián *et al.*, 1996). In addition, phytases improve inositol utilization (Simons *et al.*, 1990); and starch digestibility (Knuckles and Betschart, 1987), and promote greater protein utilization (Farell *et al.*, 1993).

Nevertheless, inorganic phosphorus in the diet decreases phytase activity, when the amount of P is adequate to animal requirements (Power and Khon, 1993).

The finding that phytase addition at high dietary phosphorus levels had no affect on feed conversion agrees with data reported by Kornegay *et al*. (1996), who evaluated broiler feed diets with 0.27, 0.36 and 0.45% available phosphorus with or without phytase addition. On the other hand, Sebastián et *al*., (1998) indicated that there is no improvement of feed conversion because the increase of body growth by phytase additions is also associated with an increase of feed intake.

Bone ash content (%) increased (P<0.05) with phosphorus levels, with values of 38.65, 40.83 and 43.00% and with phytase additions, with values of 37.17 and 40.11, 41.87 and 42.26 and, 42.66 and 43.76 respectively for 0 and 500U/kg enzyme levels, for each level of dietary phosphorus (Table 3). The increase of bone ash due to enzyme addition was more evident at 0.45% P level and diminished with the increment of the element in the diets. Diets with 0.65% Pt and with 500U/kg phytase were significant different (P <0.05) in relation to lower enzyme levels (Kornegay *et al*., 1996).

Table 3. Bone ash and phosphorus content of broilers fed diets with increasing phosphorus and phytase levels

Treatment		Variables	
Total Phosphorus (%)	Phytase (U/kg)	Ash %	Phosphorus %
0.45	0	37.17±1.51[c]	13.90±0.10[a]
	300	38.81±1.84[b]	13.63±0.86[a]
	400	38.50±1.15[cb]	14.61±1.01[a]
	500	40.11±1.61[a]	14.59±0.58[a]
Average		**38.65±1.53[C]**	**14.18±0.64[C]**
0.55	0	41.87±1.55[b]	16.10±0.65[a]
	300	41.19±1.22[c]	16.19±0.57[a]
	400	42.00±1.75[ab]	16.83±0.64[a]
	500	42.26±0.96[a]	16.34±1.031[a]
Average		**41.83±1.37[B]**	**16.37±0.72[B]**
0.65	0	42.66±1.05[b]	17.34±0.15[a]
	300	42.99±0.95[ab]	17.54±0.35[a]
	400	42.60±1.05[b]	17.66±0.49[a]
	500	43.76±0.95[a]	17.96±0.29[a]
Average		**43.00±1.00[A]**	**17.63±0.32[A]**

A,B Differences (P<0.05) between phosphorus levels independent of phytase levels
a,b Differences (P<0.05) between phytase levels for each phosphorus level
± Standard deviation (n= 10)

Bone phosphorus showed similar pattern of bone ash with values of 14.18, 16.37 and 17.63 % P, for 0.45, 0.55 and 0.65% Pt, respectively, independently of phytase levels. Increase phytase level in diets, for each total phosphorus level, had no effect on P content of bone.

Total P net retention (%) was greater (P<0.05) for 0.65% Pt level, followed by 0.55 and 0.45%, with mean values of 69, 65 and 61, respectively for each P level. Total phosphorus excretion decreased with phytase addition, being more evident with 0.65% dietary Pt (Table 4). These values were 57; 77; 67 and 75%, respectively for 0, 300, 400 and 500U/kg of phytase.

Table 4. Phosphorus total net retention in broilers fed diets with ncreasing phosphorus and phytase levels.

Treatment	P Intake (g/d)	P excreted (g/d)	ANR Pt[2] (%)
0.45-0	7.15	3.00[a]	58[b]
0.45-300	6.88	2.96[a]	57[b]
0.45-400	7.69	2.85[a]	63[a]
0.45-500	7.91	2.80[a]	65[a]
Average	**7.41**	**2.90**	**60.7[C]**
0.55-0	11.90	5.10[a]	57[b]
0.55-300	11.53	4.49[ab]	61[b]
0.55-400	12.13	3.59[b]	70[a]
0.55-500	12.21	3.36[b]	72[a]
Average	**11.94**	**4.14**	**65[B]**
0.65-0	14.58	6.20[a]	57[c]
0.65-300	12.98	3.04[b]	77[a]
0.65-400	12.86	4.20[b]	67[b]
0.65-500	14.80	3.67[c]	75[a]
Average	**13.81**	**4.28**	**69[A]**

[1]Ten chicks per treatment
a,b,c Average in the same column with different letters are different (P<0.05)
A,B,C Average in the same column with different letters are different (P<0.05)
[2]ANR: apparent net retention = $\frac{\text{P intake} - \text{P excreted}}{\text{P intake}} \times 100$

Total phosphorus excretion decreased with phytase incorporation, independently of phosphorus levels (Table 4), being this more evident with 0.65% Pt diet.

Similar results were reported by Yi *et al*. (1996[ab]) using a corn-soy diet with 350 U/kg phytase. On the other hand, in this study, phytase addition to the basal diet with 0.45% Pt, reduced excreted phosphorus from 18 to 38%. Yi *et al*. (1996[a]) found reduction values between 42 and 47%.

The results of this research that indicate that Phosphorus net retention was influenced by inclusion of phytases in the diet agree with the findings of other authors (Denbow *et al*., 1998; Sebastián *et al*, 1997).

Regression equations between the different criteria and levels of total phosphorus and phytase presented high R^2 values, that were better described by linear equations (P<0.05) for feed intake, weight gain, bone ash and phosphorus retention and excretion. This indicates that phosphorus and phytase addition, when globally considered, improved animal performance and phytates bioavailability, and showed a reduction of phosphorus excretion with each dietary phosphorus level.

Kornegay *et al*. (1996) suggest that when adequate phytase levels in diet (500-759 U/kg) are included and phosphorus level is 0.1 % unit below the recommended values (NRC, 1994), the excretion of phosphorus is reduced from 31.8 to 35.7%, in comparison with phosphorus excretion with no exogenous phytase additions.

Implications

It is concluded that synthetic phytase supplementation in soy-corn broiler diets increases body gain and feed intake only at low dietary phosphorus level (0.45%), while the enzyme was more effective on bone mineralization at all phosphorus levels. In addition, within each dietary Pt levels, the exogenous phytase increases Pt retention.

The convenience of phytase use will depend on biotechnological advances and on cost/benefit relation. Environmental considerations may have an important role in this feeding system.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

PROTEIN SUPPLEMENTATION OF RUMINANTS CONSUMING LOW-QUALITY COOL- OR WARM-SEASON FORAGE: DIFFERENCES IN INTAKE AND DIGESTIBILITY

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ABSTRACT: Four steers (252 ± 8 kg BW; Exp. 1) and four wethers (38 ± 1 kg BW; Exp. 2) were used in two 2 × 2 factorial design experiments to determine the influence of protein supplementation of low-quality cool- (C3; bluegrass straw) and warm-season (C4; tall grass-prairie hay) forage (6.3 and 5.7% CP, respectively) on intake and nutrient digestion. Steers and wethers were allotted to 4 × 4 Latin squares with 20-d periods. Animals were provided forage at 120% of the previous 5 d average intake. Soybean meal (SBM; 52% CP) was used as the CP supplement. In Exp. 1, feed and digesta were collected on d 14 through 18 for estimation of nutrient digestibility and ruminal fluid was sampled on d 20. In Exp. 2, feed, feces, and urine were collected on d 16 to 20 for calculation of N balance. Contrasts were: 1) supp. vs unsupp.; 2) C3 vs C4; 3) supp. × forage type. A supp. × forage type interaction ($P < 0.01$) was noted for forage and total DMI in Exp. 1, with supplementation increasing intake of C4 and C3 forage by 47 and 7%, respectively. DM digestibility responded similarly with a supp. × forage type interaction ($P = 0.05$; supp. increased digestibility 12% with C4 and 9% with C3 forage). Also, supp. × forage type interactions were noted for ruminal liquid retention time ($P = 0.02$; supp. decreased retention time from 15.3 to 11.7 h with C4 and from 9.7 to 9.1 h with C3 forage) and particulate passage rate ($P = 0.02$; supp. increased particulate passage 46% with C4 and 10% with C3 forage). As in Exp. 1, a supp. × forage type interaction ($P = 0.01$; supp. increased digestibility 18% with C4 and 7% with C3 forage) was observed with DM digestibility in Exp. 2. In contrast, only supplementation effects were noted for N balance ($P = 0.002$) and N digestibility ($P < 0.001$), which increased with supplementation. These data suggest that intake and digestion of low-quality C3 and C4 forages by ruminants are not similar and, more importantly, the physiological response of ruminants differs with protein supplementation of C3 versus C4 forages.

Keywords: Cattle, Metabolism, Sheep

Introduction

Forages represent the predominant class of feed within most ruminant livestock operations. Due to differences in plant variety, stage of maturity, and management practices, forages vary significantly with respect to quality parameters such as DM digestibility, CP, and palatability. In addition, many ruminants, especially in the Intermountain West, consume low-quality forages (<7% CP) for extended periods during the annual production cycle (Turner and DelCurto, 1992). In an effort to meet the nutritional needs of these animals, supplemental CP is often provided because it has been shown to increase forage OM intake (Lintzenich et al., 1995), forage DMD (DelCurto, 1990), and animal performance (Bodine et al., 2001).

The forage types available to ruminants can be broadly grouped into cool-season (**C3**) and warm-season (**C4**). Physiological and biochemical differences distinguish C3 (first organic product during carbon fixation is three-carbon 3-phosphoglycerate) from C4 (first organic product is the four-carbon oxaloacetate) grasses (Lambers et al., 1998). It is generally considered that C3 grasses have a higher nutritional quality than C4 grasses (Barbehenn et al., 2004), which has been attributed to higher levels of nonstructural carbohydrates, protein, and water and lower levels of fiber (Wilson et al., 1983; Barbehenn and Bernays, 1992).

Despite agronomic research evaluating physiological differences between C4 and C3 grasses and the nutritional evaluation of low-quality forage CP supplementation, information on the comparative utilization of low-quality C3 vs. C4 grasses by ruminants is limited. Therefore, the objective of this experiment was to compare intake, digestibility, and N balance of ruminants offered low-quality C4 (tall grass-prairie hay) and C3 (bluegrass straw) grasses with and without protein supplementation.

Materials and Methods

All experimental procedures used in this study were approved by the Oregon State University Institutional Animal Care and Use Committee (ACUP# 3372).

Experiment 1: Influence of CP Supplementation of C3 versus C4 Forage on Intake, Digestibility, and Ruminal Fermentation by Steers

Four ruminally cannulated Angus x Hereford steers (252 ± 8 kg BW) were used in a 4 × 4 Latin square design and housed in individual pens (2 x 4 m) within an enclosed barn with continuous lighting. Steers were provided continuous access to fresh water and low-quality C3 (bluegrass straw) or C4 (tall grass-prairie hay) forage (6.3 and 5.7% CP, respectively; Table 1). Forage was provided daily (0700) at 120% of the average intake for the previous 5 d, with feed refusals from the previous day determined before feeding. A trace mineralized salt mix

was provided daily. In addition, an intramuscular injection of vitamins A, D, and E was administered to each steer at the onset of the trial to safeguard against deficiency. Treatments were arranged in a 2 × 2 factorial design (two forage types with or without supplemental protein). Soybean meal (**SBM**) was placed directly into the rumen via the ruminal cannula for supplemented treatments. The amount of CP supplied by SBM was 0.09% of BW/d. The supplemented treatments were formulated to provide 100% of the estimated DIP requirement assuming a microbial efficiency of 11%.

Experimental periods were 20 d, with intake measured beginning d 14 and concluding d 18. On d 15, treatment effects on ruminal DM, indigestible ADF (**IADF**), and fluid contents were determined by manually removing the contents from each steer's reticulo-rumen 4 h after feeding. The total ruminal contents were weighed, mixed by hand, and sub-sampled in triplicate (approximately 400 g). The remaining ruminal contents were immediately replaced into the animal. Ruminal samples were weighed; dried in a forced-air oven (55°C; 96 h); reweighed for DM; ground to pass a 1-mm screen in a Wiley mill; and composited within period and steer.

Samples of forages, SBM, and orts were collected on d 14 through 18 and dried at 55°C for 48 h. Total fecal collection was conducted on d 16 to 20. Steers were fitted with harnesses and fecal bags on d 16 (0700). Bags were emptied once daily, feces manually mixed, and a 2.5% sub-sample (wet weight) obtained, weighed, dried for 96 h at 55°C, re-weighed for DM, and composited by steer. Dried samples of hay, orts, and feces were ground as described above.

On d 20, each steer was intra-ruminally pulse-dosed with 5 g of Co-EDTA in a 150-ml aqueous solution. The Co marker was administered throughout the rumen by injecting through a stainless steel probe with a perforated tip. Ruminal fluid (approximately 100 mL) was collected by suction strainer immediately prior to dosing and at 3, 6, 9, 12, 18, and 24 h post-dosing. Ruminal fluid pH was measured immediately after collection. Twenty milliliters was stored (-20°C) for later analysis of Co concentration and 5 mL was acidified with 1 mL of 25% (wt/vol) meta-phosphoric acid and stored (-20°C) for subsequent analysis of VFA and NH3-N. Frozen (-20°C) ruminal samples were prepared for analysis by thawing, centrifuging, and collecting the supernatant. Cobalt was analyzed by atomic absorption using an air/acetylene flame.

Ground samples of forages and SBM were composited by period and daily orts composited by steer (within period) on an equal weight basis (5% as-fed). Feed, orts, and feces were analyzed for DM and OM, N, and NDF and ADF. Feed, orts, feces, and ruminal particulate samples were analyzed for IADF (IADF recovery was 102 ± 4%).

Data were analyzed as a 4 × 4 Latin square using the GLM procedure of SAS. The model included period, steer, and treatment. Because the treatment structure consisted of a 2 × 2 factorial, orthogonal contrasts were used to partition specific treatment effects. Contrast statements include: 1) C3 vs C4 forage; 2) supplemented vs unsupplemented; 3) contrast 1 × contrast 2. Ruminal pH, NH_3-N, and VFA data, collected at the fixed times after feeding, were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included steer, period, treatment, time, and treatment × time. In addition, steer × period × treatment were used to specify variation between steers (using the RANDOM statement). Steer × period × treatment were used as the SUBJECT and autoregression (AR1) used as the covariance structure. The same contrasts noted above were used to partition the treatment sums of squares. If no treatment × day interaction is detected ($P > .10$) measurements were averaged and the treatment means compared as described above.

Experiment 2: Influence of CP Supplementation of C3 versus C4 Forage on Efficiency of Nitrogen Use by Lambs

Four wethers (38 ± 1 kg BW kg) were used in a 4 × 4 Latin square design. Wethers were provided continuous access to fresh water and the same low-quality C3 or C4 forage used in Exp. 1 (Table 1). Forage was provided at 120% of the previous 5-d average intake in two equal portions (0700 and 1700), with feed refusals from the previous day determined before the 0700 feeding. A trace mineral salt mix was provided daily. In addition, an intramuscular injection of vitamins A, D, and E was administered to each lamb at the onset of the trial to safeguard against deficiency. Treatments were the same as described in Experiment 1. The quantity of supplemental CP provided was 0.19% of BW/d (CP basis). Wethers were randomly allotted to treatments and housed in individual metabolism crates within an enclosed barn with continuous lighting.

Experimental periods were 20 d, with DMI determined on d 14 through 18. In addition, samples of forages, SBM, and orts were collected on d 14 to 18 and dried at 55°C for 48 h. On d 16 to 20, total fecal and urine output were collected. Urine was composited daily by wether (50% of total; weight basis) and stored at 4°C. Sufficient 6 *N* HCl (approximately 25 mL) was added to urinals daily to maintain urine pH < 5. A sub-sample of each daily fecal sample (7.5%; weight basis) was dried at 55°C for 96 h for calculation of fecal DM. On d 16 to 20, 10 mL of blood was collected from the jugular vein 4 h after feeding using vacutainers containing EDTA. Blood samples were centrifuged and plasma harvested and stored (-20°C). Dried samples were ground through a Wiley mill (1-mm screen). Samples of ground forages and SBM were composited by period and daily orts composited by lamb (within period) on an equal weight basis (10% as-fed). Feed, orts, and fecal samples were analyzed for DM and OM and NDF and ADF. Feed, orts, fecal, and urine samples were analyzed for N. Plasma samples were assayed for urea-nitrogen using a UV/VIS spectrophotometer.

Data were analyzed as described above. Plasma urea-N was analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included lamb, period, treatment, day, and treatment × day. In addition,

lamb × period × treatment was used to specify variation between animals (using the RANDOM statement). Lamb × period × treatment was used as the SUBJECT and autoregression was used as the covariance structure. The same contrasts noted above were used to partition treatment sums of squares.

Results and Discussion

Experiment 1

We noted CP supplementation × forage type interactions ($P < 0.01$) for forage and total DMI, N intake, and NDF intake by steers (Table 2). In each instance, the C4 forage had decreased overall intake and intake increased more with CP supplementation compared with the C3 forage. For example, forage DMI averaged 19.2 and 24.5 g/kg BW for steers consuming C4 and C3, respectively. Also CP supplementation increased C4 forage intake by 47% compared with 7% with C3. This may help explain some of the apparent inconsistencies reported in the literature for forage intake in response to CP supplementation. It is generally believed that CP supplementation of low-quality forage (< 7% CP) will increase forage intake up to 100%. This assumption has been based almost exclusively on research with C4 forages (McCollum and Galyean, 1985; DelCurto et al., 1990; Köster et al., 1996). However, forage intake has not been reported to increase in most, if not all, of the studies with CP supplementation of low-quality C3 forages (Horney, et al., 1996; Mathis et al., 2000; Bohnert et al., 2002).

Digestibility of DM responded similarly to intake, with a CP supplementation × forage type interaction ($P = 0.05$; Table 2) in which DM digestibility averaged approximately 47 and 52% and increased 12 and 9% with CP supplementation for C4 and C3, respectively. Neutral detergent fiber digestibility tended ($P = 0.07$) to be greater for C3 compared with C4 forage, while N and NDF digestibility increased with CP supplementation ($P < 0.03$). Diet digestibility has been reported to increase with CP supplementation of low-quality forage (Horney et al., 1996; Bohnert et al., 2002). We are aware of no data that has compared the in vivo digestibility of low-quality C3 and C4 forage; however, Foster et al. (1996) noted that NDF and ADF in vitro digestibility of C3 forages was greater than C4 forages sampled at the same time throughout the year.

Ruminal fluid and particulate dynamics were affected by forage type and supplemental CP. Ruminal liquid fill was greater ($P < 0.01$) for C3 than C4 (311 and 234 mL/kg BW, respectively; Table 2) and was not affected by CP supplementation ($P = 0.28$), whereas liquid dilution rate increased with CP supplementation ($P = 0.03$) and for C3 compared with C4 ($P < 0.01$). A CP supplementation × forage type interaction ($P = 0.02$) was noted for liquid retention time, with CP supplementation decreasing retention time from 15.3 to 11.7 h (24%) with the C4 and from 9.7 to 9.1 h (6%) with the C3 forage. Ruminal IADF fill was not affected by CP supplementation or forage type ($P > 0.54$); however, we did observe a CP supplementation × forage type interaction ($P = 0.02$) for IADF passage rate; C4 averaged 1.6 and C3 averaged 2.0 %/h with CP supplementation increasing passage rate by 46 and 10% for C4 and C3, respectively.

Ruminal NH_3-N responded with a CP supplementation × forage type interaction ($P = 0.02$; data not shown). Ammonia-N averaged 1.1 and 1.4 mM for C4 and C3, respectively, while providing supplemental SBM increased ruminal NH_3-N from 0.64 to 1.5mM with C4 forage and from 0.52 to 2.26 mM with C3 forage. Total VFA were greater with CP supplementation ($P = 0.03$; 79.4 vs 71.1 mM; data not shown) and tended to be greater for C3 vs C4 ($P = 0.11$; 78.0 vs 72.4 mM). Interestingly, the molar proportion of Ac was lower with C3 compared with C4 ($P < 0.01$) and Pr was greater ($P < 0.01$; data not shown). Consequently, The Ac:Pr was lower with C3 than C4 ($P < 0.01$; 3.9 vs 5.4), suggesting greater energetic efficiency with the C3 forage.

Experiment 2

Forage and total DMI by lambs showed a tendency ($P = 0.06$) to be greater with C3 compared with C4 forage (Table 2), with total DMI increasing with CP supplementation ($P < 0.01$). It is worth noting that there tended to be a CP supplementation × forage type interaction ($P = 0.11$) for both forage and total DMI, similar to that observed in Exp. 1 (C3 forage intake decreased 5% with CP supplementation and C4 intake increased 8%). Likewise, DM digestibility had a CP supplementation × forage type interaction in which digestibility averaged approximately 49% for C4 and 51% for C3, with CP supplementation increasing digestibility by 18 and 7%, respectively.

Nitrogen intake was increased with CP supplementation ($P < 0.01$; Table 2). Also, N intake was greater for C3 compared with C4 forage ($P = 0.01$) because of greater forage intake and greater forage CP concentration with C3 (6.3 vs 5.7%; Table 1). Similarly, plasma urea-N was greater with CP supplementation ($P < 0.01$; 5.8 vs 2.6 mM) and for C3 compared with C4 ($P < 0.01$; 4.8 vs 3.6 mM; data not shown). Fecal and urinary N excretion was increased ($P < 0.01$) with CP supplementation, and fecal N increased for C3 compared to C4 ($P = 0.02$). Nevertheless, efficacy of N use (N balance, N digestibility, and digested N retained) by lambs was not effected by forage type ($P > 0.34$), while N balance and N digestibility were greater with CP supplementation ($P < 0.01$).

In summary, these data indicate that intake and digestibility of the C3 and C4 forages in the current study were not similar and, more importantly, the physiological response of ruminants to supplemental protein depends, in part, on the cell wall structure of the basal diet. Therefore, further research comparing other low-quality C3 and C4 forages is warranted to determine if the observed responses in the current study are indicative of differences in utilization of low-quality C3 and C4 forages by ruminants.

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Table 1. Feedstuff[a] nutrient content (DM basis)

Nutrient,%	C3	C4	SBM
Exp. 1			
CP	6.3	5.7	52.6
OM	90.5	93.8	92.6
NDF	66.4	69.8	13.0
ADF	36.2	36.6	5.3
IADF	19.0	19.1	2.5
Exp. 2			
CP	6.3	5.7	51.8
OM	90.0	93.2	92.6
NDF	68.1	69.7	14.8
ADF	35.8	35.5	5.2

[a] C3 = cool season forage (bluegrass straw); C4 = warm season forage (tall grass-prairie hay); SBM = soybean meal.

Table 2. Intake, digestibility, ruminal dynamics, and efficiency of N use by ruminants consuming low-quality cool-season (C3) and warm-season (C4) grass hay with or without soybean meal (CP) supplementation

	Treatment					P-Value[b]		
Item	C4	C4+CP	C3	C3+CP	SEM[a]	CP vs No CP	C4 vs C3	CP × Type
Exp. 1 – Steers								
DMI, g/kg BW								
Forage	15.6	22.9	23.7	25.3	0.6	<0.01	<0.01	<0.01
Soybean meal	0.0	1.7	0.0	1.7				
Total	15.6	24.6	23.7	27.0	0.6	<0.01	<0.01	<0.01
N Intake, g/kg BW	0.147	0.356	0.228	0.385	0.007	<0.01	<0.01	<0.01
NDF Intake, g/kg BW	10.8	16.0	15.6	16.9	0.5	<0.01	<0.01	<0.01
Digestibility, %								
DM	42.8	51.8	49.7	54.2	0.9	<0.01	<0.01	0.05
N	28.4	54.5	37.5	55.2	3.5	<0.01	0.21	0.27
NDF	43.5	50.0	48.0	52.7	1.7	0.02	0.07	0.61
Ruminal Liquid								
Fill, mL/kg BW	220	249	306	316	16	0.28	<0.01	0.56
Dilution Rate, %/h	6.5	8.7	10.5	11.0	0.5	0.03	<0.01	0.13
Retention Time, h	15.3	11.7	9.7	9.1	0.5	<0.01	<0.01	0.02
Ruminal IADF[c]								
Fill, g/kg BW	9.5	9.3	9.6	9.1	0.5	0.55	0.92	0.79
Passage Rate, %/h	1.3	1.9	1.9	2.1	0.06	<0.01	<0.01	0.02
Exp. 2 – Lambs								
DMI, g/kg BW								
Forage	25.8	27.8	29.5	28.2	0.9	0.69	0.06	0.11
Soybean meal	0.0	3.6	0.0	3.6				
Total	25.8	31.4	29.5	31.8	0.9	<0.01	0.06	0.11
DM Digestibility, %	44.7	52.8	48.9	52.4	0.5	<0.01	0.01	0.01
Daily N Intake, g/kg BW	0.246	0.558	0.288	0.577	0.008	<0.01	0.01	0.21
Fecal N, g/kg BW	0.159	0.195	0.183	0.214	0.007	<0.01	0.02	0.72
Urine N, g/kg BW	0.065	0.221	0.080	0.261	0.017	<0.01	0.15	0.50
N Balance, g/kg BW	0.022	0.143	0.025	0.102	0.019	<0.01	0.35	0.30
N Digestibility, %	35.3	65.2	36.5	63.0	1.15	<0.01	0.68	0.20
Digested N Retained, %	23.4	39.2	23.2	27.9	9.64	0.33	0.57	0.59

[a] n = 4.

[b] CP = CP supplementation; Type = forage type.

[c] IADF = indigestible ADF.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

SHEEP GRAZING WHEAT SUMMER FALLOW AND THE IMPACT ON SOIL NITROGEN, MOISTURE, AND CROP YIELD

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ABSTRACT. In typical dryland farming areas of Montana, annual precipitation is not sufficient for annual harvest of small grains. Summer fallow in alternate years, is a common method of conserving soil moisture to produce a crop in the following season. Current methods of fallow management are primarily mechanical tillage and spraying with herbicides. Although these methods are effective, they are expensive, making fallow management the highest variable cost associated with dryland grain production. The objectives of this study were to compare the impact of grazing small grains stubble with sheep, as a fallow management tactic, against traditional management practices of chemical and mechanical fallow across two cropping systems on wheat grain yield and soil nitrate-nitrogen and moisture. Fallow management treatments (chemical, mechanical, graze) were imposed, in a randomized complete block split-plot design, on spring wheat-fallow and winter wheat-fallow cropping systems in a 6 ha 45 plot study at Montana State University's Fort Ellis Research Center near Bozeman, MT. Data on treatment impact on wheat yield and soil nitrate-nitrogen and gravimetric water concentration were recorded. Wheat yields did not differ between cropping system ($P > 0.50$). Soil nitrate-nitrogen ($P > 0.43$) and percent gravimetric water ($P > 0.06$) within either spring wheat-fallow or winter wheat-fallow cropping systems did not differ among fallow treatments at 0-15 cm, 15-30 cm, and 30-60 cm soil depths. This study demonstrates that grazing sheep on winter or spring wheat stubble and associated summer fallow does not negatively impact soil nitrate-nitrogen, percent gravimetric water, or subsequent crop yield.

Key Words. Small ruminant, Grain yield, Sustainable agriculture, Cereal stubble, Soil

Introduction

In dryland grain farming areas of Montana annual precipitation frequently is insufficient for profitable annual cereal cropping. Instead, a crop-summer fallow system is used to increase soil moisture and available nitrate-nitrogen (NO_3) for subsequent crop growth. Current methods of fallow management are primarily mechanical tillage and spraying with herbicides (i.e., chemical fallow). These methods are effective but expensive, making fallow management the highest variable cost associated with dryland grain production (Johnson et al., 1997). One of the greatest challenges for fallow management is the prevention of weed growth without increasing soil erosion (Fenster, 1997). Much research has been done on mechanical and chemical management of summer fallow (Anderson, 1999); however, no data are available on the effects of substituting grazing livestock for chemical or mechanical weed management.

Sheep can graze both dormant perennials crops (i.e., alfalfa) and small grains fallow to manage both pest insect and weed densities (Goosey et al., 2004, 2005; Hatfield et al., 2007a, b). Additionally, weaned lamb production is feasible on weedy barley (Thomas et al. 1990) and wheat stubble (Brand et al. 1999). Mullholland et al. (1976) suggested that cereal stubble that contained some green plant material offered an acceptable grazing resource for wethers and dry ewes at stocking rates of 4.25 sheep/ha for 11 weeks (330 sheep d/ha). Targeting sheep and small grains production in a mutually beneficial partnership has the potential to reduce production costs for both enterprises.

The objective of this study was to determine the impact of grazing small grains stubble with sheep, as a fallow management tactic, against traditional management practices of chemical-fallow and mechanical-fallow in two farming systems: 1) spring wheat-fallow and 2) winter wheat-fallow), on wheat grain yield and soil NO_3 and moisture.

Materials and Methods

Research was conducted from 2004 to 2006 at Montana State University's Fort Ellis Experiment Station, near Bozeman, Montana (Universal Transverse Mercator (UTM) 12 501833E, 5056909N; North American Datum (NAD) 1927) (Fig. 1). Fallow treatments were imposed on 0.14 ha plots in a randomized complete block split-plot design with cropping system as a main-plot factor and fallow management as a sub-plot factor. Each phase of each rotation was represented every year (Fig. 2). Sub-plot fallow treatments were: 1) Graze Fallow (i.e., grazing sheep), 2) Chemical Fallow (i.e., herbicides), and 3) Mechanical Fallow (i.e., tillage). Treatments were imposed on two main-plot cropping systems: 1) Spring wheat-fallow, 2) Winter wheat-fallow. Spring wheat

plots were seeded to 'McNeal'. Winter wheat plots were seeded to 'Promontory'. Data presented here are a small part of a much larger study.

Fig. 1. 2004 Aerial photograph of study site.

Fallow even S wheat odd	101	Mech Fallow	Fallow even S wheat odd	201	Chem Fallow	Fallow even S wheat odd	301	Graze Fallow
Fallow even W wheat odd	102	Mech Fallow	Fallow even W wheat odd	202	Chem Fallow	Fallow even W wheat odd	302	Graze Fallow
W wheat even Fallow odd	103	Mech Fallow	W wheat even Fallow odd	203	Chem Fallow	W wheat even Fallow odd	303	Graze Fallow
S wheat even Fallow odd	104	Mech Fallow	S wheat even Fallow odd	204	Chem Fallow	S wheat even Fallow odd	304	Graze Fallow
S wheat even S wheat odd	105	Mech Fallow	S wheat even S wheat odd	205	Chem Fallow	S wheat even S wheat odd	305	Graze Fallow
S wheat even Fallow odd	106	Graze Fallow	S wheat even Fallow odd	206	Mech Fallow	S wheat even Fallow odd	306	Chem Fallow
W wheat even Fallow odd	107	Graze Fallow	W wheat even Fallow odd	207	Mech Fallow	W wheat even Fallow odd	307	Chem Fallow
S wheat even S wheat odd	108	Graze Fallow	S wheat even S wheat odd	208	Mech Fallow	S wheat even S wheat odd	308	Chem Fallow
Fallow even S wheat odd	109	Graze Fallow	Fallow even S wheat odd	209	Mech Fallow	Fallow even S wheat odd	309	Chem Fallow
Fallow even W wheat odd	110	Graze Fallow	Fallow even W wheat odd	210	Mech Fallow	Fallow even W wheat odd	310	Chem Fallow
S wheat even Fallow odd	111	Chem Fallow	S wheat even Fallow odd	211	Graze Fallow	S wheat even Fallow odd	311	Mech Fallow
Fallow even S wheat odd	112	Chem Fallow	Fallow even S wheat odd	212	Graze Fallow	Fallow even S wheat odd	312	Mech Fallow
W wheat even Fallow odd	113	Chem Fallow	W wheat even Fallow odd	213	Graze Fallow	W wheat even Fallow odd	313	Mech Fallow
S wheat even S wheat odd	114	Chem Fallow	S wheat even S wheat odd	214	Graze Fallow	S wheat even S wheat odd	314	Mech Fallow
Fallow even W wheat odd	115	Chem Fallow	Fallow even W wheat odd	215	Graze Fallow	Fallow even W wheat odd	315	Mech Fallow

Fig. 2. Fallow management treatment allocation where all farming system phases (i.e., spring wheat-fallow or winter wheat-fallow) are represented in all (even and odd) calendar years.

Treatment Description.

Chemical, mechanical, and graze fallow treatments were imposed during either the broadleaf weed and cereal volunteer plant seedling and vegetative stages. On average, chemical and mechanical fallow was done 3 times and graze fallow was done 4 times per fallow period.

Chemical Fallow. Plots were treated with Gly Star Plus® (glyphosphate, N-[phosphonomethyl] glycin) at the rate of 1.17 liters/ha mixed with 1.75 liters/ha of dimethylamine salt of dicamba. Wilbur-Ellis R-11® surfactant was used at a rate of 0.3 l/ha. Herbicide application was done at 8 km/hr and 40 psi using an engine powered (Briggs & Stratton Corporation, Milwaukee, WI) Broyhill® Stadium 302.8 liter sprayer with a 3.05 m spray boom mounted on a utility vehicle.

Mechanical Fallow. Mechanical fallow was completed using a John Deere 740 tractor pulling a John Deere 100 toolbar equipped with cultivators tilling soil to an approximate depth of 15 cm.

Graze Fallow. Graze fallow was done using a variety of class and age of western white faced sheep that, within a fallow period, were randomly assigned to fenced plots at the beginning of each grazing session. Stocking rates ranged from 29 to 153 sheep days/ha (Goosey et al., 2005; Hatfield et al., 2007 a, b, c). Water and white salt were available ad-libitum. Each grazing session ended when approximately 6.5 kg/plot or less green weeds and volunteer cereal plants remained.

Sample Collection.

Cropping System Yield. Yield was determined by harvesting ripe grain (i.e., <12% kernel moisture) from each plot using a Case International 1420 Combine. Total kg of harvested grain was recorded from each plot and yield (kg/ha) was determined.

Cropping System Soils. Two soil core samples were taken per plot to measure soil nutrient profiles and soil moisture. A sample consisted of all soil from a 2.5 cm diameter soil probe. Each sample was divided into three separate sub-samples by depth: 1) 0-15 cm, 2) 15-30 cm, and 3) 30-60 cm. Sub-samples were composited by depth within each plot. Immediately after sample extraction, soils were placed in air-tight, lined sampling bags, sealed and returned to the laboratory. Samples (soils and bags) were weighed to determine wet weight, then dried at 105ºC for 3 weeks and weighed again to determine dry weight. Gravimetric water content was calculated as [(wet weight – dry weight)/dry weight] (Gardner, 1986). Dried samples were analyzed for nitrate-nitrogen NO_3 concentrations (Mulvaney, 1996).

Statistical Analysis.

The experimental design was a randomized complete block, arranged in a split-plot design, with three replicates. Data were analyzed within each cropping system. The model included the effects of year, fallow treatment, and year by fallow treatment interaction. The GLM procedures of SAS (SAS Institute, 2003) were used to compute least squared means for fallow treatment effects.

Results

Year by fallow treatment interactions were not detected ($P > 0.16$) for any variable tested therefore data were combined and analyses were recalculated using only the effect of treatment across year.

Grain yields within either spring or winter wheat cropping system did not differ ($P > 0.50$) among fallow treatments (Table 1). No differences were detected

among treatment for NO_3 ($P > 0.43$) or percent gravimetric water ($P > 0.06$) within either spring wheat-fallow or winter wheat-fallow cropping systems at 0-15 cm, 15-30 cm, or 30-60 cm soil depths (Table 1). Gravimetric water concentrations vary between treatments in the spring wheat-fallow cropping system (15-30 cm) by 1.1% and 2.2 % in the winter wheat-fallow system (0-15 cm). Although not different these tendencies, particularly during drought years, could become apparent.

Discussion

No published studies exist examining the relationship between cereal fallow managed by sheep grazing and subsequent crop yields. Our data indicates that, over two years (one full crop-fallow rotation), yield was not compromised by sheep grazing when compared to chemical and mechanical fallow management.

Hatfield et al. (2007c) reported, in an eight site study, no detrimental effects on soil nutrient profiles (K, EC, NO_3, OM, P, pH) from sheep grazing cereal fallow compared to tillage and burning. Our results concur with Hatfield et al. (2007c) regarding NO_3. Additionally, Hatfield et al. (2007c) indicates no consistent changes in soil bulk density from grazed, tilled and burned cereal fallow plots. Goosey et al. (2005) also reported no differences in soil bulk density or gravimetric water concentration between grazed and non-grazed plots. Results from the current study concur with those of Goosey et al. (2005).

Hatfield et al. (2007b) documented that sheep grazing was as effective as mechanical tillage for managing weeds in winter wheat stubble. However, this study did not include summer fallow Weed growth in cereal fallow can reduce subsequent wheat yields by 509 to 1525 kg/ha (Greg, 1981), largely through their use of soil moisture. Therefore, sheep grazing, with the intent of killing actively growing weeds, should have positive effects on soil moisture, assuming bulk density and water infiltration are not adversely impacted.

Over the first two years of our study, sheep grazing winter and spring wheat-fallow has demonstrated equal conservation of soil NO_3 and gravimetric moisture, the two primary components associated with crop yield in the semiarid northern Great Plains, compared to traditional fallow management practices. However, differences among grazing and cropping systems, as a whole, may require additional years for treatment differences to become apparent. This research needs to be conducted over a longer time period to investigate any long-term changes.

Implications

Compared to other forms of fallow management sheep grazing fallow has the potential to be a viable alternative. Although soil NO_3 and gravimetric water concentrations were not adversely impacted, a need exists for additional research addressing the economic considerations associated with these types of grazing systems. Currently a need exists for long-term 'enterprise-level' research addressing the economics of sheep grazing fallow.

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Table 1. Grain yield[1], soil nitrogen[1] and soil moisture[1] in 2005 and 2006 of winter and spring wheat plots summer fallowed by mechanical, chemical, and sheep grazing

	Mechanical[5] (M)	Chemical[6] (C)	Grazed[7] (G)	S.E.	M vs. G *P*-value	C vs. G *P*-value
	Spring Wheat-Fallow					
Yield (kg/ha)[2]	3423	3446	3246	201	0.54	0.50
NO_3 Nitrogen 0-15 cm (ppm)[3]	18.5	17.9	20.2	3.12	0.71	0.63
NO_3 Nitrogen 15-30 cm (ppm)[3]	4.1	5.0	5.1	1.02	0.49	0.93
NO_3 Nitrogen 30-60 cm (ppm)[3]	4.9	6.2	4.0	1.86	0.74	0.43
% Gravimetric[4] Water 0-15 cm[3]	20.3	20.3	21.5	0.68	0.20	0.20
% Gravimetric[4] Water 15-30 cm[3]	12.7	12.6	13.7	0.37	0.11	0.07
% Gravimetric[4] Water 30-60 cm[3]	12.3	12.1	11.4	0.37	0.11	0.21
	Winter Wheat-Fallow					
Yield (kg/ha)[2]	3082	2808	2890	323	0.67	0.87
NO_3 Nitrogen 0-15 cm (ppm)[3]	19.2	23.1	18.4	5.25	0.91	0.53
NO_3 Nitrogen 15-30 cm (ppm)[3]	5.2	4.4	3.9	0.97	0.35	0.70
NO_3 Nitrogen 30-60 cm (ppm)[3]	2.7	3.1	3.1	0.88	0.75	0.96
% Gravimetric[4] Water 0-15 cm[3]	23.3	22.2	21.1	0.71	0.06	0.31
% Gravimetric[4] Water 15-30 cm[3]	13.1	12.6	12.1	0.49	0.13	0.40
% Gravimetric[4] Water 30-60 cm[3]	11.8	11.4	11.5	0.43	0.61	0.94

[1]No year x treatment interactions were detected $P > 0.16$.
[2]7 September 2005; 28 August 2006.
[3]27 September 2005; 29 September 2006.
[4]Gravimetric Water = (Wet Weight – Dry Weight / Dry Weight) X 100.
[5]Shallow tillage (10cm) conducted with John Deer 740 tractor pulling a John Deer 100 toolbar
[6]Chemical fallow conducted with Gly Star Plus® at the rate of 1.17 liters/ha mixed with 1.75 liters/ha of dimethylamine salt of dicamba.
[7] Graze fallow: completed using a variety of class and age of western white faced sheep. Stocking rates ranged from 29 to 153 sheep days/ha

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

VISUAL OBSTRUCTION: WEIGHT TECHNIQUE FOR ESTIMATING PRODUCTION ON NORTHWESTERN BUNCHGRASS PRAIRIE RANGELANDS[1]

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ABSTRACT: The estimation of standing crop is important in the management of rangeland resources. Direct measurements by clipping, drying, and weighing of herbaceous vegetation are time-consuming and labor-intensive. Therefore, non-destructive methods for efficiently and accurately estimating standing crop are needed in rangeland forage management. We assessed a visual obstruction (**VO**) technique to estimate standing crop (**SC**) of northwest native bunchgrass communities at The Nature Conservancy's Zumwalt Prairie Preserve in northeastern Oregon. This method involves obtaining a height-density index by measuring the height of a pole that is obscured by vegetation when viewed from the side. Five hundred seventy six plots (0.5 m^2) were subjected to VO measurement; and subsequently, all vegetation within a plot was clipped to ground level. Only current year's crop was taken. Regression analysis was used to evaluate the relationships of VO to standing crop, with standing crop as the dependent variable. Total standing crop was 1261 ± 51 $kg \cdot ha^{-1}$ and mean of VO measurement was 12.8 ± 0.4 cm for vegetation in the study site. By growth habit of plants, standing crops were 688 ± 26, 13 ± 26, 416 ± 26, and 144 ± 26 $kg \cdot ha^{-1}$ for grasses, grasslikes, forbs, and shrubs, respectively, and all growth habits differed from each other ($P < 0.01$). A positive ($P < 0.01$) linear relationship occurred between VO and SC measurements, however, correlation was low with only 46% of the variation in standing crop being attributable to VO (y, $kg \cdot ha^{-1}$ = 270.58 + 77.66x, cm; $r^2 = 0.46$, n = 576). In heterogeneous mid-height bunchgrass communities like the Zumwalt Prairie Preserve, the VO technique will not accurately predict standing crop although many wildlife investigators will still find it useful for describing vegetative structure in these communities. Consequently, we recommend that, if considering VO as a surrogate for SC, investigators should calibrate VO technique against clip plots to evaluate applicability to their situation.

[1] The project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2006-35101-16572, The Nature Conservancy and Oregon State University's Eastern Oregon Agricultural Research Center. We thank Uranbileg Daalkhaijav, Samuel Wyffels, and Amartuvshin Daalkhaijav for their assistance with the field work. Our gratitude extends to Phil Shephard and Angie Freeman who assisted us with many logistical details while on the study area.

Key Words: Biomass, Non-destructive Technique, Rangelands, Robel Pole, Visual Obstruction

Introduction

The measurement of rangeland herbage standing crop is important in the management of multiple uses such as livestock production, wildlife food and cover, and soil protection against erosion. For decades, a common technique to obtain standing crop dry weight estimates has been the standard clip and weigh technique (Cook and Stubbendieck, 1986), which consists of clipping herbage of known area, and then the clipped herbage is oven dried and weighed. This technique is destructive, labor-intensive, and requires considerable time. In an attempt to overcome these problems, several non-destructive techniques, such as the biometer (Pearson and Miller, 1972), the Massey grass meter (Holmes, 1974), the Ellinbank pasture meter (Earle and McGowan, 1979), and the rising plate meter (Michell and Large, 1983; Gabriels and Van Den Berg, 1993) have provided estimates of standing crop with high degrees of accuracy. However, they were not designed to measure vegetation visual obstruction (VO), a height-density measurement that relates to habitat suitability for grassland wildlife (Fontaine et al., 2004; Winter et al., 2005; Lueders et al., 2006). If the VO technique could be used to simultaneously estimate forage availability for livestock grazing management and status of habitat conditions for various grassland wildlife species, both researchers and managers would have a useful tool that could be used with inter-disciplinary collaboration.

Robel et al. (1970) and Vermeire and Gillen (2001) concluded that in tallgrass prairie, standing crop dry weight estimates can be indirectly obtained using measured VO in regression models. Visual obstruction technique also has been used to predict standing crop (r^2 = 0.88) for the sandy lowland sites on Nebraska sandhills (Benkobi et al., 2000). Likewise, Harmoney et al. (1997) and Ganguli et al. (2000) found that VO measurement were accurate predictors of standing crop in Iowa pastures (r^2 = 0.63) and in the shortgrass prairie (r^2 = 0.85) of Texas, respectively. However, in other ecosystems, such as marshes in Upper Texas, VO was only able to explain 35% of the variance in clip plot standing crop (Whitbeck and Grace, 2006). In another study, Volesky et al. (1999) reported that VO measurement would probably not be useful in a double-sampling technique for prediction of

total standing crop on upland range sites in the Nebraska Sandhills.

The accuracy and precision of the VO technique for predicting standing crop thus appears to vary from ecosystem to ecosystem. The performance of the VO as a predictor of aboveground productivity in bunchgrass community ecosystem is limited. Our objective therefore was to determine the potential of a VO technique in assessing standing crop on bunchgrass prairie in northeastern Oregon.

Materials and Methods

Study Site

The study was conducted from late June to late July 2006 on the Zumwalt Prairie which is the last large remnant of the northwest bunchgrass ecosystem type (Tisdale, 1982) community. Our study area within the prairie was The Nature Conservancy's (**TNC**) Zumwalt Prairie Preserve (lat 45°34′N, long 122°57′W) which is located near Enterprise, Ore., in northeastern Oregon. Elevations of the study area ranged from 1340 to 1460 m and topography was relatively flat with rolling hills (7% slopes on average). The area receives around 330 mm of precipitation annually (30-year average) with a distinct dry period in July and August. Precipitation is bimodal; falls in spring as localized thunderstorms and in winter as snow. Long-term average annual temperature (30-year average) is 6.4°C, and ranged from -2.8°C (December) to 17.1°C (July). Precipitation and temperature data (NOAA 1957-1987) were from the Enterprise, Ore. weather station (Station ID: 352672) at 1163 m elevation located northwest (<30 km) of the study site. Soils are mostly shallow to moderate deep silt loams with localized areas of shallow and very shallow rocky soils (USDA NRCS, TNC unpublished data). Idaho fescue (*Festuca idahoensis* Elmer), prairie Junegrass (*Koeleria macrantha* [Ledeb.] J.A. Schultes), bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Löve), and Kentucky bluegrass (*Poa pratensis* L.) are the dominant grasses, while western yarrow (*Achillea millefolium* L. var. *occidentalis* DC.), tall annual willowherb (*Epilobium brachycarpum* K. Presl), twin arnica (*Arnica sororia* Greene), corn speedwell (*Veronica arvensis* L.), and old man's whiskers (*Geum triflorum* Pursh) are the dominant forbs. Silky lupine (*Lupinus sericeus* Pursh) and slender cinquefoil (*Potentilla gracilis* Dougl. ex Hook.) were the primary subshrub/shrub species, while dwarf rose (*Rosa gymnocarpa* Nutt.), Nootka rose (*Rosa nutkana* K. Presl.), and common snowberry (*Symphoricarpos albus* [L.] Blake) were occurring occasionally. Further descriptions of vegetation characteristics of the study site can be found in Darambazar et al. (2007). The study site has been utilized >50 years as spring/summer pasture for cattle rotational grazing (P. Shephard, personal communication) at a moderate intensity along with abundant native ungulates and other grassland wildlife.

Experimental Design

This study was conducted as part of a larger, multi-disciplinary study examining the effects of livestock stocking rates on this grassland food web. The experimental design for the larger study is a randomized complete block design with one factor (livestock grazing) and four grazing treatment levels (livestock densities). In 2006, fences were erected around four blocks (Block A, B, C, and D) of land 160 ha in size, and within each block, four contiguous paddocks were partitioned, each 40 ha in size. The data summarized here are based on the pre-treatment sampling of vegetation that was conducted in these paddocks during 2006 (treatments will not occur until 2007). Within each paddock, we established a set of 36 sampling points which were uniformly distributed along a grid of 6 north (**N**)-south (**S**) transects (columns) and 6 east (**E**) - west (**W**) transects (rows) that traversed each paddock. Transects were approximately 90 m apart. Sampling points were then located 1.5 m from the intersection of each N-S and E-W transects. This resulted in 144 sampling points for each block, for a total of 576 sampling points available for direct comparisons between the 2 techniques.

Visual Obstruction

Visual obstruction and clipped standing crop data were collected from each sampling point in late June to late July 2006. Two 2-person teams carried out field work of this study. The approach described by Robel et al. (1970) was used to measure VO and a 0.5 m^2 (0.5 × 1 m) rectangular frame or plot was used to clip and harvest standing crop. Equipment used for VO measurement was similar in design to that used by Robel et al. (1970). Our equipment consisted of 2 poles (60 and 100 cm, reading and sighting pole, respectively) that were connected by a 4-m nylon cord attached to the top of each pole. The reading pole was painted in white and marked at each decimeter, with 0.5-dm increments in red. The bands were numbered in ascending order beginning with 1 at the bottom. One person positioned the reading pole vertically in the center of a 0.5 m^2 (0.5 × 1 m) rectangular frame. A second person, the observer, would place the sighting pole at a distance of 4 m from the center of the frame. Looking from a height of 1 m, the observer would read the number of the lowest band not obstructed by vegetation. At each sampling point, 4 VO measurements were recorded, 1 for each cardinal direction. The four VO measurements were averaged for each sampling point and multiplied by 10 to convert to centimeters (Volesky et al., 1999). Every 3 days of fieldwork, observers rotated between their duties to minimize potential individual observer's biases.

Standing Crop

After VO measurement, all vegetation within the rectangular frame was clipped at ground level. All clipped samples were separated by live and dead materials, the latter of which was discarded. Live material (standing crop) further separated by botanical species, was oven dried at 60°C for 48 h and weighed. Total standing crop of each sampling point was determined by summing the aboveground biomass of all species removed from each plot and expressed in $kg \cdot ha^{-1}$. Plant nomenclature and separating plants by growth habit throughout this paper follows the recommendations of the

USDA Natural Resources Conservation Service (USDA, NRCS, 2007).

Data Analysis

Regression analysis was used to evaluate the relationships of VO to standing crop, with standing crop as the dependent variable using REG procedures of SAS (SAS 2002). Individual sampling points were considered as observations. Prediction accuracy of the developed regression model was checked at the 0.05 level ($P < 0.05$). A variety of transformations (LOG, LOG10) were compared to attain maximum variance explanation. Residuals were also examined to evaluate assumptions of normality and homogeneity of variance. Statistical analysis was conducted with non-transformed values. Least squares means of standing crop, as well as standing crop summed by growth habit, and visual obstruction measurements data were generated and separated using MIXED procedure of SAS and were considered different at the 0.05 level ($P < 0.05$).

Results and Discussion

The total standing crop of the experimental site was 1261 ± 51 kg·ha^{-1} during 2006. By plant growth habit, standing crops were 688 ± 26, 13 ± 26, 416 ± 26, and 144 ± 26 kg·ha^{-1} for grass, grasslike, forb, and shrub plants, respectively (Figure 1). Biomass of plant growth habits differed from each other ($P < 0.05$).

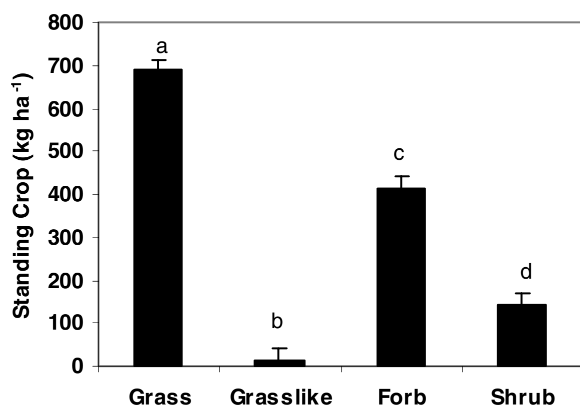

Figure 1. Standing crop by growth habit of bunchgrass communities on the Zumwalt Prairie Preserve in northeastern Oregon. Data are presented as the mean ± SEM. Bars with different letters are different at $P < 0.05$.

Mean VO was 12.8 ± 0.4 cm. Neither VO measurement nor SC differed ($P > 0.05$) by blocks. The results of the ANOVA tests associated with the regression analysis indicate that the relationship between standing crop and VO is significant ($P < 0.001$, $F = 490.54$, n = 576). However, when data were pooled from all sampling points, VO was only able to explain 46% of the variance in standing crop (Figure 2). Relations of VO and SC were slightly different with experimental blocks. In particular, VO was able to explain 54%, 52%, 35%, and 43% of the variance in standing crop, for block A (y, kg·ha^{-1} = 40.09 + 100.84x, cm; $r^2 = 0.54$, $F = 165.24$, n = 144), block B (y, kg·ha^{-1} = 248.19 + 79.12x, cm; $r^2 = 0.52$, $F = 153.80$, n = 144), block C (y, kg·ha^{-1} = 314.09 + 71.30x, cm; $r^2 = 0.35$, $F = 76.30$, n = 144), and block D (y, kg·ha^{-1} = 479.58 + 59.06x, cm; $r^2 = 0.44$, $F = 109.24$, n = 144), respectively.

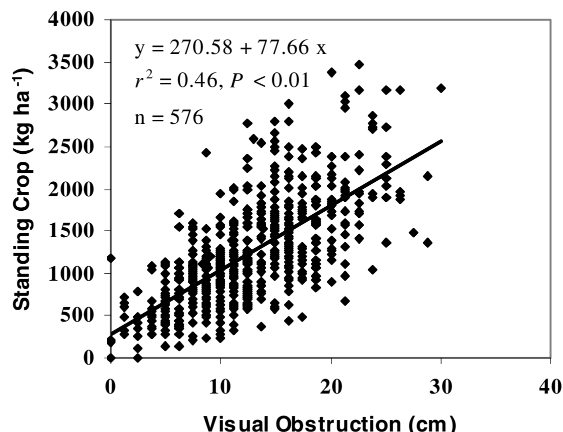

Figure 2. Relationship between standing crop (y) and visual obstruction measurements (x) of northwestern bunchgrass communities on the Zumwalt Prairie Preserve in northeastern Oregon.

Given the wide range of micro-environmental conditions across our study site, the spatial, and vegetation structural heterogeneity present in bunchgrass prairie ecosystems, and the inherent differences in the VO and SC techniques, r^2 values 0.46 between the 2 techniques are probably quite reasonable and acceptable. There are several possible different aspects (or a combination of several reasons) why VO was not a good predictor of current year's standing crop in bunchgrass communities on Zumwalt Prairie Preserve.

Vermeire and Gillen (2002) believe that plant stature and morphology are the primary factor controlling the volume measured by visual obstruction. In a companion study, Darambazar et al. (2007) found that the vegetations throughout the study sites were relatively heterogenic and a diverse mixture of plants. In particular, abundance of mid-height or taller plants like silky lupine and slender cinquefoil were high. In lesser but still significant extent contributed dwarf rose, Nootka rose, and common snowberry. For plants, three-dimensional distribution on the plant is often highly varied. For instance, these plants usually have medium-height, relatively tiny stem, but large, widely-spreading leaves on the top. And their contribution to total standing crop expected to be greater than that of their contribution to VO measurement. Therefore, stratification of data in different vegetation groups before regression determination may also be expected to result in different relations.

As mentioned previously, when we clip sampling plots, we discarded dead material and hence it did not contribute to total standing crop. Whereas, in VO measured before vegetation clipping, plant dead material

can contribute to VO measurement (Vermeire and Gillen, 2001; Whitbeck and Grace, 2006). Also, Whitbeck and Grace (2006) documented that inclusion of the percent dead variable in the VO regression raised the percentage of explained variance in standing crop from 35% to 55%. They further discussed that results indicate that magnitude of dead material presence did compromise the ability of the VO technique to predict standing crop efficiently. While during the current study, there was significant amount of standing dead material at our study site.

Additional potential confounding influence is likely that, surface microtopographic variance (e.g., zoogenic micro-hills, micro-terraces and hummock) might also make even more difficult to accurately predict SC through VO. Finally, we conclude that a complex and interacting set of management and environmental factors must be considered when measuring, predicting, or managing rangelands through visual obstruction technique.

Implications

Results obtained from the current study show that the VO technique is not accurate in predicting the current year's standing crop across the diverse plant communities of northwestern bunchgrass prairie areas such as those found on the Zumwalt Prairie Preserve. Visual obstruction is a valuable technique for evaluating the amount of plant cover as it relates to wildlife species and may have use to managers as a rough estimate of standing crop, especially when used to compare grassland communities of similar structure across space or the same communities across time. However, we recommend that in all cases, investigators should calibrate VO technique against clip plots to evaluate its accuracy in specific situations.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

RESPIRATORY ELIMINATION OF SELENIUM IN SHEEP GIVEN THE ACCUMULATOR PLANT *SYMPHYOTRICHUM SPATHULATUM* (WESTERN MOUNTAIN ASTER)

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ABSTRACT: Selenium (Se) is a necessary mineral required by mammals and poultry. If toxic amounts are ingested, expired air becomes a potentially important, but poorly investigated, route of elimination. A study was performed to evaluate respiratory toxicokinetics of Se in sheep. Sheep were gavaged with the accumulator plant *Symphyotrichum spathulatum* at Se equivalent doses of 0, 2, 4, 6 or 8 mg/kg BW. As positive controls an additional two sheep were gavaged with purified sodium selenite at 4 mg Se/kg BW and two sheep were gavaged with purified selenomethionine (Se-Met) at 8 mg Se/kg BW. Expired air samples were collected prior to dosing and at 1, 2, 4 and 8 hrs post dosing. Samples were collected from both sheep in the control, selenite and Se-Met groups and from 4 sheep in each of the plant-Se treatment groups. The air Se concentrations of the Se-Met group were statistically higher ($P < 0.05$) than all other groups at each time point of collection. The selenite, 2 and 4 mg plant-Se/kg BW groups all had peak concentrations at the 2 hr collection time. The 8 mg plant-Se/kg BW group showed a linear increase in respiratory Se concentration through 8 hours. The 6 mg plant-Se/kg BW group peaked at 1 hour, then dropped and peaked again at 4 hours and finally dropped between 4 and 8 hours. At 8 hours, the 8 mg plant-Se/kg BW group was significantly higher ($P < 0.05$) than all other groups. The elimination profile for Se-Met was dissimilar to any of the other treatments, with greater than 20 times the concentration of Se in the expired air than the high dose plant Se or the selenite treatments. The 4 mg selenite and 4 mg plant Se had similar elimination profiles, although the 4 mg plant Se had significantly greater ($P < 0.05$) concentrations at 2, 4 and 8 hrs. The total dose of the plant Se appreciably altered the elimination profile. These findings indicate that both dose and chemical form of Se affect respiratory elimination kinetics.
Keywords: Selenium, Sheep, Respiratory kinetics

Introduction

The trace mineral selenium (**Se**) is essential to mammals and poultry. However there is a narrow margin of safety between deficiency and toxicity when supplementing Se. Several cases of accidental Se overdose have been documented by Gabbedy and Dickson (1969), Lambourne and Mason (1969), Janke (1989) and Kyle and Allen (1990). Acute poisoning with Se results in vomiting (in monogastrics), dyspnea and death (Selenium, 2005). Lesions are often seen affecting the liver, lungs and heart muscles.

Certain species of plants can accumulate large amounts of Se and are referred to as Se-accumulator plants (Selenium in Nutrition, 1983). Accumulator plants such as *Astragalus*, *Aster, Symphyotrichum,* and others can contain several hundred to several thousand parts per million Se. If ingested, these plants cause Se poisoning in livestock. Over 500 sheep deaths were documented over a 4 year period due to ingestion of plants and water containing large amounts of Se in southeastern Idaho on reclaimed phosphate mining sites (Fessler et al., 2003).

After entering the body, Se compounds are converted to hydrogen selenide, which is either utilized by the body or methylated to form excretion products (Finley 2005). The first methylation step gives rise to methyselenol that is again methylated to form dimethylselenide. This can be furthur methylated to trimethylselenonium ion. Trimethylselenonium ion is the most common metabolite excreted in the urine (Barceloux, 1999). After large amounts of Se have been ingested, the methylation reaction of dimethylselenide to trimethylselenonium ion becomes overloaded because this is the rate-limiting step (Tiwary et al. 2005). Dimethylselenide is then excreted through respiration. Jiang et al. (1983) observed dimethylselenide to be the major compound excreted in expired air following selenite exposure and dimethyldiselenide to be the major compound excreted in expired air following selenomethionine exposure. The objective of this study was to evaluate the respiratory kinetics of Se from the accumulator plant, *Symphyotrichum spathulatum* (Western Mountain Aster), in sheep.

Materials and Methods

Twenty-two sheep weighing between 23 and 29 kg were randomly assigned to the following study groups: control, sodium selenite, selenomethionine (**Se-Met**) and 2, 4, 6, or 8 mg plant-Se/kg BW. Two animals were assigned to each of the control, selenite and Se-Met groups. Four animals were assigned to each level of plant Se. The selenite group received 4 mg Se/kg BW as purified sodium selenite (United States Biochemical Corp., Cleveland, OH). The Se-Met group received 8 mg Se/kg BW as purified Se-Met (Sigma-Aldrich Co., St. Louis, MO). These two groups served as positive controls in the study. The treatments were prepared for each individual animal and administered through an intraruminal gavage, followed by a

*This research was supported by the Utah Agricultural Experiment Station, Utah State University, Logan, Utah
Approved as journal paper no. 7882

flush of water to make certain each animal received the full assigned dose.

Air samples were collected as described previously by Tiwary et al. (2005) (see figure 1). Air was collected into 3 L tedlar bags (SKC Inc., Eighty Four, PA). Following collection, air was pumped across a labeled charcoal filter (SKC Inc., Eighty Four, PA) using a Gilian pump (Sensidyne Inc., Clearwater, FL) set at 1 L/min. The pump was allowed to run for 2 min to allow 2 L of air to cross the filter.

Figure 1. Air collection apparatus showing funnel around sheep muzzle attached to nonrebreathing tee, which is attached to 3 L tedlar bag.

Air was collected prior to dosing and at 1, 2, 4 and 8 h post dosing from all animals. All charcoal filters were capped tightly and stored prior to analysis.

Tiwary et al. (2005) found a 50:50 ratio of ethanol to water to be the best extraction solvent for expired Se. They found the highest concentration of Se was extracted when using 3 mL of solvent in each of two extraction procedures. We performed two extractions using 3 mL of 50:50 ethanol to water on all samples.

Each charcoal filter contains two compartments of charcoal and each compartment was analyzed separately. The end of the charcoal tube was broken and the charcoal from each compartment was placed into separate, labeled 15 mL polypropylene, metal-free tubes (CPI International, Santa Rosa, CA). Three milliliters of the ethanol, water solvent was added to each tube and the tubes were capped tightly. The tubes were rotated on a shaker for 2 h at 200 rpm. Following rotation, the tubes were centrifuged for 10 min at 500 x g. One milliliter of supernatent was removed to another labeled 15 mL metal-free tube containing 9 mL of 5% nitric acid water. The charcoal from Compartment 1 was then blotted dry and was returned to the original tube. A second extraction was performed identically to the first extraction on Compartment 1 of all samples. Standards were prepared in the same matrix. Samples and standards were analyzed by Inductively Coupled Plasma-Mass Spectrometry on an ELAN 6000 (PerkinElmer, Shelton, CT). Only one extraction was performed on Compartment 2 because it is used as a breakthrough compartment. Compartment 2 from all samples contained no detectable Se.

Means and general linear models procedures of SAS (SAS Inst., Inc., Cary, NC) were used to evaluate data. A setting of $\alpha = 0.05$ was used to establish significance unless stated otherwise. The means function was used to separate significance versus insignificance between the treatment groups.

Results and Discussion

Se has a distinct odor when eliminated in expired air (Selenium in Nutrition, 1983). Faint odor was observed in all plant treatment groups, but occurred most often in the 6 and 8 mg plant-Se groups between 2 and 4 h. The strongest odor observed occurred in the Se-Met group. Tiwary et al. (2005), recorded 'Strong' odor in the 4 mg Se-Met group at all time points. Surprisingly, in the present study, the odor of the 8 mg Se-Met group was recorded as 'Obvious,' but not 'Very Strong.'

Prior to dosing, no group means were significantly different from each other. All samples were below the detection limit of 0.01 µg prior to dosing.

The mean Se-Met respiratory elimination was significantly different from all other groups at all time points post-dosing with concentrations more than 20 times greater (Table 1). Following statistical evaluation comparing all groups, statistical evaluation was performed again without including the Se-Met group. At the 2 h collection, the selenite, 4, and 6 mg plant-Se groups were significantly different from the control ($P = 0.0866$) under the standard of $\alpha = 0.1$. At 4 h, the 6 mg plant-Se group was significantly different from the control, selenite, 2 mg and 4 mg plant-Se groups ($P = 0.0083$). Also at 4 h, the 8 mg plant-Se group was significantly different than the control and selenite groups. At 8 h, the 8 mg plant-Se group was significantly different than all other groups ($P = 0.0004$).

Treatment	0 h	1 h	2 h	4 h	8 h
Control	0.00	0.00	0.00	0.00	0.00
Selenite	0.00	0.11	0.14	0.02	0.02
Se-Met	0.00	5.84	4.50	2.69	0.81
2 mg/kg	0.00	0.05	0.09	0.07	0.03
4 mg/kg	0.00	0.10	0.18	0.08	0.07
6 mg/kg	0.00	0.19	0.16	0.20	0.07
8 mg/kg	0.00	0.06	0.09	0.14	0.22

Table 1. Mean selenium concentration (µg) from 1 L of expired air at each collection time point.

The Se-Met group elimination peaked at 1 h and gradually decreased through 8 h (Fig. 2). This agrees with Hirooka and Galambos (1966) and McConnell and Roth (1966) who found the majority of Se exhaled in 24 h following administration occurred during the first 6 h.

The selenite group peaked at 2 h and decreased drastically between 2 and 4 h (Fig. 3). The elimination curve then stayed consistent from 4 to 8 h. The mean of the 2 and 4 mg plant-Se groups peaked at 2 h and then gradually decreased (Fig. 4, 5). The peak of the 4 mg plant-Se (0.18) group was slightly higher than the peak of the 2 mg plant-Se group (0.09). Hirooka and Galambos (1966)

observed an increase in Se exhaled with each increase in dose of Se administered.

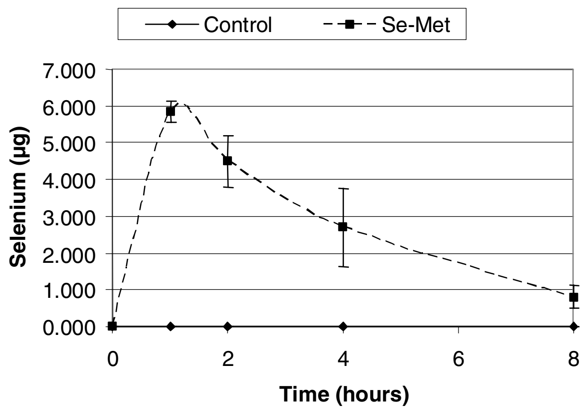

Figure 2. Mean respiratory elimination of selenium in 1 L of expired air from sheep dosed with Se-Met at 8 mg/kg BW and control sheep ± SEM.

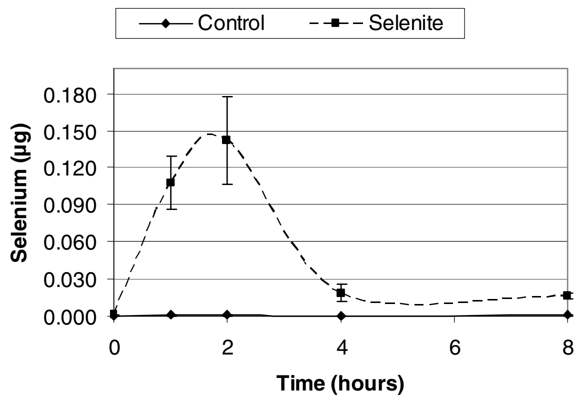

Figure 3. Mean respiratory elimination of selenium in 1 L of expired air from sheep dosed with sodium selenite at 4 mg/kg BW and control sheep ± SEM.

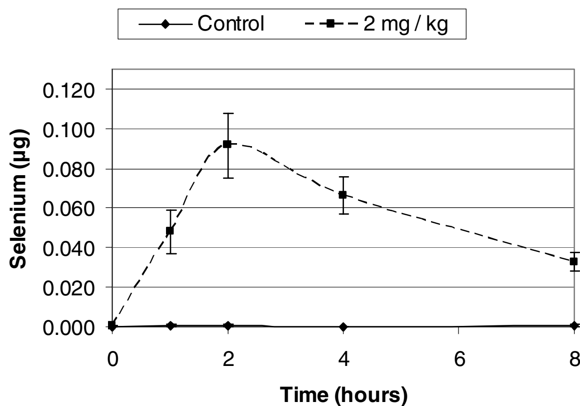

Figure 4. Mean respiratory elimination of selenium in 1 L of expired air from sheep dosed with 2 mg plant-Se/kg BW and control sheep ± SEM.

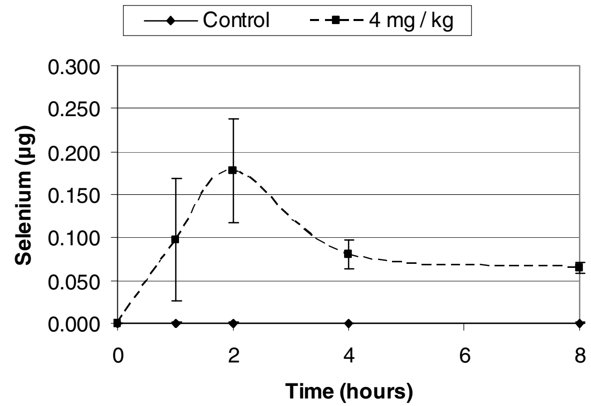

Figure 5. Mean respiratory elimination of selenium in 1 L expired air from sheep dosed with 4 mg plant-Se/kg BW and control sheep ± SEM.

The 6 mg plant-Se group peaked at 1 h, then dropped and peaked again at 4 h before a final decrease through 8 h (Fig. 6). The four animals in the 6 mg plant-Se group eliminated Se differently, which led to the two peaks in the mean of the group. One animal peaked at 1 h with 0.41 µg Se. Two other animals reached peak respiratory elimination at about 4 h. The final animal peaked at 1 h, dropped slightly at 2 h and peaked again at 4 h. The 1 h peak of this animal was 0.17 µg Se, the 4 h peak was 0.21 µg Se with a drop at 2 h to 0.11 µg Se. The differences in each of the individual animals explain the two peaks in the curve of the 6 mg plant-Se group. The first animal that peaked at 1 h had a much higher concentration than all other animals at that time point causing the mean curve to peak at 1 h. The other animals peaked at 4 h causing the mean curve to peak again at that time.

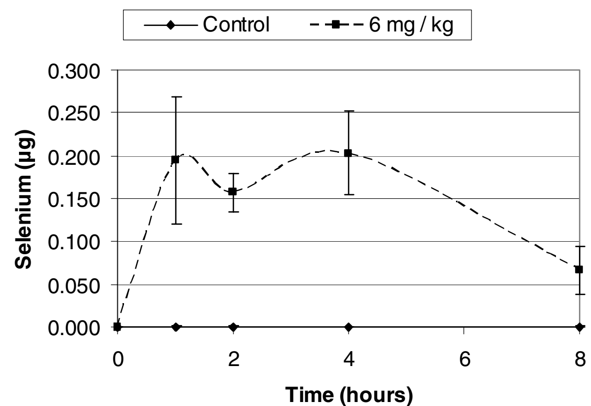

Figure 6. Mean respiratory elimination of selenium in 1 L expired air from sheep dosed with 6 mg plant-Se/kg BW and control sheep ± SEM.

The 8 mg plant-Se group increased linearly through 8 h (Fig. 7). One of the animals in the 8 mg plant-Se group reached a plateau at 4 h but the respiratory elimination did not drop prior to the 8 h collection. Two of the animals increased most rapidly through 2 h then only gradually increased through 8 h. The final animal saw a

drastic increase in respiratory Se elimination from 0.08 µg Se at 4 h to 0.31 µg Se at 8 h. This animal caused the mean curve to appear to increase linearly even though one animal reached plateau at 4 h and two of the animals only increased slightly through 8 h.

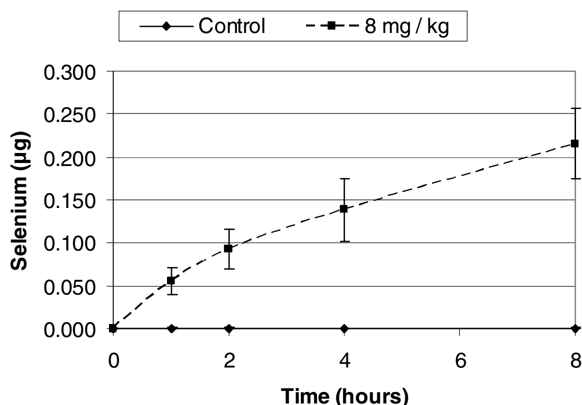

Figure 7. Mean respiratory elimination of selenium in 1 L expired air from sheep dosed with 8 mg plant-Se/kg BW and control sheep ± SEM.

The animals receiving Se-Met were given the same dose of Se as the highest plant Se group of 8 mg Se/kg BW. Se-Met is believed to be the most common chemical form of Se found in plants (Sors et al., 2005). The vast difference in the amount eliminated between the two groups indicates this particular plant contained Se in a different chemical form. Another possible explanation is the Se-Met in plants is not as bioavailable to sheep as the purified compound of Se-Met.

A similar study performed by Tiwary et al. (2005) observed the effects of varying amounts of purified sodium selenite and selenomethionine on respiratory elimination in sheep. At the same dose (4 mg Se/kg BW) and times (4 and 8 h), the selenite group reported here had only about 17% and 19% of the respired Se as that reported previously. The Se-Met group at the same dose (8 mg Se/kg BW) had about 62% of the content found by Tiwary et al. at 4 h and about 23% at 8 h.

The respiratory elimination of Se in sheep from the plant, *Symphyotrichum spathulatum*, is dependent upon dose. The highest dose of 8 mg plant-Se/kg BW caused Se to be eliminated up through 8 h and likely beyond. The lower doses caused a major elimination of Se in expired air early on, followed by a decrease in the amount eliminated.

Overall Se eliminated in expired air was a very small amount of the total administered dose. The largest animal was treated with 6 mg plant-Se/kg BW. The total dose this animal received was 174.0 mg Se. This animal peaked at 4 h with 0.21 µg Se eliminated at that point in 1 L of expired air. This is a small percentage of the total dose. Respiratory elimination occurs following ingestion of this plant, however only a small amount of Se is eliminated in expired air through the observed 8 h.

All groups appear to peak and decrease during the observed 8 h except the 8 mg plant-Se group. More research should be done to evaluate the respiratory elimination of 8 mg plant-Se beyond 8 h to see when respiratory elimination peaks and when elimination drops following administration of this particular plant.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

SPECIES COMPOSITION AND DIVERSITY ON NORTHWESTERN BUNCHGRASS PRAIRIE RANGELANDS[1]

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ABSTRACT: Management and conservation of rangelands are increasingly concerned with maintaining productivity, species composition, and diversity of native plant communities. We estimated aboveground annual productivity, species composition, and diversity of a native bunchgrass type community across 1152, 0.5 m^2 plots at The Nature Conservancy's Zumwalt Prairie Preserve in northeastern Oregon. Standing crop was estimated by clipping current year's crop to ground level and canopy cover was estimated visually as cover classes. The Shannon diversity index (*H*) was used to characterize species diversity in the study area. Across the study sites 186 plant species were observed, approximately 80% of which were native perennial species. Native bunchgrasses and perennials contributed nearly 80% to the total standing crop with 16% attributed to invading and/or introduced species. We found that the prairie was low in productivity but high in evenness of species abundance.

Key Words: Aboveground Biomass, Species Richness, Bunchgrass Community

Introduction

It is important to monitor the status of native bunchgrass prairie vegetation because of their high value for wildlife and the maintenance of the existing grassland remnants. In the Pacific Northwest, there has been concern that grazing of late-successional ecosystems may decrease plant species diversity on a local and regional scale and adversely affect rare, threatened, or endangered species. Collins (1992) found community aboveground production in more diverse grassland plots was more stable not only with respect to a rare, major perturbation, drought, but also with respect to more normal year-to-year variation in climate. Native vegetation is the best indicator of the potential productivity of a specific location. The measurement of rangeland herbage standing crop is important in the management of multiple uses such as livestock production, wildlife food and cover, and soil protection against erosion. Yet, herbaceous production in grasslands can be highly variable across years. In eastern Oregon, as Sneva (1982) determined, variability in rangeland productivity is linked to the amount and timing of precipitation received over the winter and early summer. Estimates of aboveground net primary production have been reported for many sites in the Central Grassland region as well as around the world (Lauenroth, 1979). However, few studies in northwestern North America and in Blue Mountain region of northwest have dealt specifically with composition, diversity, and aboveground productivity in prairie ecotypes. Plant species diversity in this study viewed at the alpha level that is the number and relative abundance of species within a particular habitat type (Whittaker, 1975). The objective of this study was to determine the productivity, species composition, and diversity of a bunchgrass prairie in northeastern Oregon.

Materials and Methods

Study Area

The study was conducted from late June to late July between 2004 and 2006 on the Zumwalt Prairie which is the largest remnant of the northwest bunchgrass ecosystem type (Tisdale, 1982). Our study area was within the prairie at The Nature Conservancy's (**TNC**) Zumwalt Prairie Preserve (lat 45°34′N, long 122°57′W) which is located near the city of Enterprise in northeastern Oregon (Damiran et al., 2007). The area is on a basalt plateau at an elevation of 1340 to 1460 m with little relief (mean slope = 7%) and receives around 330 mm of precipitation annually (30-year average) with a distinct dry period in July and August. Precipitation is bimodal, falling in spring as localized thunderstorms and in winter as snow. Long-term average annual temperature (30-year average) is 6.4°C, and ranged from -2.8°C (December) to 17.1°C (July). Precipitation and temperature data (NOAA, 1957-1987) were from the Enterprise, Ore. weather station at 1163 m elevation located northwest (<30 km) of the study area. Soils are mostly shallow to moderate deep silt loams with patchy influence of loess. Small areas of shallow and very shallow rocky soils occur on ridgetops and upper hillslopes (USDA, NRCS, TNC, unpublished data). The vegetation is dominated by native bunchgrasses that include Idaho fescue (*Festuca idahoensis* Elmer), prairie Junegrass (*Koeleria macrantha* [Ledeb.] J.A. Schultes), and bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Löve). The study area has grazed for over 100 yr and in the past >50 yr as

[1] The project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2006-35101-16572, The Nature Conservancy and Oregon State University's Eastern Oregon Agricultural Research Center. We thank Uranbileg Daalkhaijav, Samuel Wyffels, and Amartuvshin Daalkhaijav for assistance with field work; also our thanks to Phil Shephard for logistical support.

spring/summer pasture for cattle (P. Shephard, personal communication). Native ungulates (*Odocoileus hemionus*, *Cervus elaphus*) and Belding's ground squirrels (*Spermophilus beldingi*) are also common. Plant species nomenclature and separating plants by growth form, life span, and origin throughout this paper follow the recommendations of the USDA Natural Resources Conservation Service (USDA, NRCS, 2007).

Experimental Design

This study was conducted as a part of a larger, multi-disciplinary study examining the effects of livestock stocking rates on the grassland food web. There were four blocks of land 160 ha in size, and within each block, four contiguous paddocks were partitioned, each 40 ha in size. The data summarized here are based on the pre-treatment sampling of vegetation that was conducted in the blocks in 2004 and 2005 and in the paddocks in 2006. Within each paddock, we established a set of 36 sampling points. This resulted in for a total of 576 sampling points available for clipping for production, and 1152 for estimating canopy cover (two plots per sampling point).

Percent Cover

Canopy cover was measured in a rectangular frame (0.5 × 1 m, 0.5 m^2) on 1152 plots by ocular estimation (Daubenmire, 1959). Cover classes were: 0 = 0%, 1 = 0.01-1%, 2 = 1.1-5%, 3 = 5.1-25%, 4 = 25.1-50%, 5 = 50.1-75%, 6 = 75.1-95%, 7 = 95.1-99%, and 8 = 99.1-100% and converted to the midpoint percentage of the estimate. Canopy cover was estimated for each vascular plant species having canopy within plot boundaries. Cover values of species in the same functional group within the same plot were added together to create total cover values for these groups. The sum of cover values exceeded 100% due to canopy overlap. We estimated the amount of the soil surface falling into the following 7 categories: bare ground, herbaceous litter, woody litter >5 mm diameter, rock fragment >5 mm, bedrock, bryophyte, and lichen or other biological soil crust.

Standing Crop

We monitored herbaceous productivity of the prairie for 3 consecutive years during 2004, 2005, and 2006. We separated current year's crop from total standing crop to quantify year effects to annual productivity. Clipped standing crop data were collected from each sampling point at peak production, in late June of 2004 and 2005 and from late June to late July of 2006. All vegetation within the rectangular frame was clipped at ground level. Clipped samples were separated into live and dead materials, the latter was discarded. Live material (standing crop) was further separated by species, oven dried at 60°C, and weighed. Total standing crop of each sampling point was determined by summing the aboveground biomass of all species removed from each plot and expressed in $kg \cdot ha^{-1}$. Data by species were placed into functional groups based on plant growth form, life cycles, and origin (i.e., native or exotic).

Species Diversity and Frequency

Diversity was calculated using three indices: richness, the total number of species (*S*) tallied per 0.5 m^2 plot or site, heterogeneity (***H***), and evenness ($\boldsymbol{E_H}$) estimated by the Shannon-Wiener formula (Krebs, 1972), where, $H = -\Sigma\, p_i \ln\ p_i$ and p_i – the relative percentage of standing crop of individual species on each plot or site. Standing crop was used as a measure of species abundance in diversity calculations. Heterogeneity index considers both richness and evenness components of diversity; that is, the number of species and evenness of their abundance. Species frequency was calculated as the number of times (or plots) an individual species is present in the study sites.

Data Analysis

Standing crop and cover, as well as the measurements summed by growth form were generated and separated using MIXED procedure of SAS (SAS, 2002).

Results and Discussion

Frequency of Occurrence

Native grasses dominated the species composition of the study area; Idaho fescue, prairie Junegrass, and bluebunch wheatgrass constituted 84.2, 68.8, and 63.4% frequency of occurrence, respectively. In contrast, the most common introduced species was Kentucky bluegrass (*Poa pratensis* L.) at 43.4% frequency. Western yarrow (*Achillea millefolium* L. var. *occidentalis* DC.) (55.7%) was prevalent among forbs, followed by tall annual willowherb (*Epilobium brachycarpum* K. Presl) (47.7%) and twin arnica (*Arnica sororia* Greene) (47.4%). The shrub component was well presented by two subshrub species: silky lupine (*Lupinus sericeus* Pursh) (58.0%) and slender cinquefoil (*Potentilla gracilis* Dougl. ex Hook.) (44.8%). Dwarf (*Rosa gymnocarpa* Nutt.) and Nootka roses (*Rosa nutkana* K. Presl.) (1%) and common snowberry (*Symphoricarpos albus* [L.] Blake) (0.5%) occurred only occasionally.

Species Diversity

Heterogeneity (richness combined with equitability) of an ecosystem is important, but relative abundance or evenness of taxa is frequently viewed as more important to land resource managers than richness. In nature, biomass and number of individuals are almost never evenly distributed between species (Wilson et al., 1996). Evenness, as described Mulder et al. (2004), is the relative contribution of each species to the total biomass or number of individuals. A total of 186 vascular plant species was found across the study sites with 13 species being the average number per plot. Although the total number of plant species found in the study was higher than that was observed on the prairie of Minnesota (132 species), the number of species per plot was lower than those reported on the same prairie (15 to 18) and on the rough fescue grasslands of Montana (18 to 19) (Tilman, 1993; Short and Knight, 2003). A confounding feature about species richness according to West (1993) was that the actual number of species (***S***) present in the sampling universe is difficult to know because *S* is usually underestimated from subsampling, whereas equitability is usually overestimated. Shannon diversity index was 1.8 with evenness in species abundance being 0.7 for the study area. On Kansas native tallgrass prairie Shannon diversity indices were around 2.0 to 3.0 (Hickman et al., 2004; Gene Towne and Kemp, 2003). Almost 80% of the plant species found in our study were native perennial

species. Most notable on this grassland community was the remarkable diversity of forbs accounting for 119 species or 64% of the total number of species in the area. In addition, 36 or 19% of the total number of species were invading and/or introduced species.

Canopy Cover

Total canopy cover of vegetation was 104.6 ± 5%. The grass/grasslike component cover was highest (56.5 ± 3%), followed by the forb component (35.6 ± 3%), with shrubs contributing least (12.5 ± 3%) to the total canopy cover ($P < 0.05$). Difference in mean percent cover between the growth forms was as high as 21-23%. At individual species level, the most abundant species by canopy coverage was Idaho fescue (21.9%) followed by bluebunch wheatgrass (10.7%), and Kentucky bluegrass (5.8%). Old man's whiskers (*Geum triflorum* Pursh) contributed most (6.3%) to the forb cover with twin arnica (4.2%) and hoary balsamroot (*Balsamorhiza incana* Nutt.) (3.5%) also contributing to cover. Shrub component contained substantial canopy coverage of silky lupine (6.3%) and slender cinquefoil (4.8%). Stohlgren et al. (1998) found mean foliar cover of native species to be 28.7 to 36.3% and of exotic species 8.2 to 9.0% in the Central Grasslands. The ground surface layer is an important component of a habitat type (Table 1). The study sites had relatively high herbaceous litter and low lichen or biological crust, but no woody litter or bedrock. Litter could be readily separated according to whether it originated from the plants of the herbaceous or woody layers since in the prairie virtually all of the dead organic material overlying the soil was not decomposed due to dry conditions. The estimate for bare ground cover (16.6%) in this study was lower compared to that was observed on the shortgrass steppe of northeastern Colorado (30 to 70%) by Guenther and Detling (2003). Bare ground found on this rangeland was less than 30%, which has been suggested as the maximum acceptable level for adequate soil erosion protection (Hofmann and Ries, 1988). However, according to Johnston (1962) when bare ground is approximately 15% hydrologic changes such as reduced infiltration and increased runoff occur in mixed prairie and fescue grassland ecosystems.

Standing Crop

Standing crops on the prairie varied ($P < 0.05$) across years. The highest production (1928 ± 92 $kg \cdot ha^{-1}$) was obtained in 2005, whereas lower standing crops ($P > 0.05$) were measured in 2004 and 2006 (1381 ± 92 and 1262 ± 92 $kg \cdot ha^{-1}$, respectively). Graminoids contributed most to the total standing crop (701 ± 26 $kg \cdot ha^{-1}$), forbs were the next most abundant group with 416 ± 26 $kg \cdot ha^{-1}$ and shrubs produced 144 ± 26 $kg \cdot ha^{-1}$ ($P < 0.05$). Native perennials made up 79% of the total standing crop with introduced perennials contributing 12%. Annuals contributed less to total production with native and introduced species making up 3 and 4%, respectively. Species with the highest standing crop was Idaho fescue (217 $kg \cdot ha^{-1}$) with bluebunch wheatgrass producing the next highest amount (163 $kg \cdot ha^{-1}$). Kentucky bluegrass introduced a substantial amount (98 $kg \cdot ha^{-1}$) of herbage to total production. Contribution of old man's whiskers was also significant (123 $kg \cdot ha^{-1}$), with other forbs such as hoary balsamroot (35 $kg \cdot ha^{-1}$), twin arnica (26 $kg \cdot ha^{-1}$), and western yarrow (25 $kg \cdot ha^{-1}$) making in lesser amounts. At seven grassland sites in North America and Europe, Bakker et al. (2006) divided their experimental sites into two classes of low (0-300 $g \cdot m^{-2}$) and high (300-600 $g \cdot m^{-2}$) productivity. In doing so they refer to that the threshold of 300 $g \cdot m^{-2}$ corresponds roughly to the biomass above which light penetration to the soil surface is <5% and thus limiting to the establishment of many plant species (Huisman and Olff, 1998). The estimate for production on the bunchgrass prairie (125.5 $g \cdot m^{-2}$) in this study (2006) was essentially identical to what Bakker et al. (2006) found on the bunchgrass steppe of Utah (125 $g \cdot m^{-2}$). Production values for 2004 and 2006 were lower as compared to those reported on the rough fescue grasslands in southern Alberta (Willms and Rode, 1998) and for the Central Grassland region (Sala et al., 1988) but higher than that of a northwest bunchgrass site (Sims and Singh, 1978). Dry environments on infertile soils have low productivity and favor plants that compete well for both nutrients and water in the absence of herbivory (Olff and Ritchie, 1998). Furthermore, annual productivity by Ovington et al. (1963) is not synonymous with plant biomass or with gross changes in plant biomass from year to year and for a plant community it is composed of many different species and individuals of the same species which do not necessarily attain their greatest individual weights at the time of maximum community biomass.

Implications

Characteristics of the native bunchgrass prairie as measured by standing live crop, plant diversity, and cover as it relates to wildlife species could be incorporated into future livestock or wildlife and conservation management decisions. The study show that the current year's standing crop on the northwestern bunchgrass prairie can vary greatly from year to year and be lower than on the other grasslands. Percent bare ground and invading and/or introduced species found in this study should be of practical significance since they could be indicative of hydrologic and range condition changes occurring in the ecosystem. In respect to the larger study (food web study), the results from this study will be used to refine the study design on the stocking rates and provide a baseline analysis of vegetation production, canopy cover, and frequency, how these variables are influenced by cattle stocking rates, and for the study of vegetation-soils and vegetation-grazing relations.

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Table 1. Percent cover of the ground surface layer on the Zumwalt Prairie Preserve in northeastern Oregon for 2006 (n = 1152)

	Bare Ground	Herbaceous Litter	Woody Litter	Bryophyte	Lichen or Biocrust	Rock Fragment	Bedrock
Mean ± SEM	16.6 ± 1.7	32.9 ± 1.6	0	10.3 ± 0.8	1.1 ± 0.2	3.8 ± 0.6	0

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

Utilization of corn gluten meal by heifers as a self-fed supplement

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ABSTRACT: Utilization of a self-fed supplement that is effective at small amounts can minimize costs. Studies from NMSU have shown that it is possible to formulate and deliver a small supplement fed at 113.4 g/d to replace use of a cottonseed meal supplement fed at 454 g/d in gestating beef cows on winter range with minimal weight loss. Sixty-eight yearling heifers (303 ± 1.4 kg) were used in a completely randomized design with treatments in a 2X2 factorial arrangements to evaluate consumption of a self-fed corn gluten meal (**CGM**) formulated small supplement in comparison to an animal protein (**AP**) based small supplement. The duration of this study was 4 months beginning in July and terminating in November. The study was replicated across 4 pastures of which 2 had been aerially treated with tebuthiuron and 2 were not treated. Heifers were allowed supplements ad libitum while grazing piñon-juniper/blue grama rangelands. During the study CP content (OMB) declined (P<0.01) in hand plucked samples (11.1, 9.1, and 6.7 ± 0.9 % for 12-July, 16-Aug, 3-Oct, respectively). Supplement CP and estimated UIP content (P<0.01) on an as fed basis for CGM and AP were 27.6 ± 1.5 and 16.4 ± 0.6, 37.2 ± 1.5 and 21.4 ± 0.6, respectively. The calculated bypass protein value for each supplement was 59.4 and 57.5% of CP for the CGM and AP mix respectively. Supplements were formulated by weight with 50% protein source and 50% mineral. Supplement disappearance from feed tubs was recorded each week to calculate consumption per head. Cow body weights were recorded at initiation of treatments and at 28-d intervals. Daily consumption was not different (P=0.13) between the CGM and the AP (150.3 and 175.8 ± 3.8 g $head^{-1}·d^{-1}$, respectively). No differences (P=0.16) were found in total body weight gain. The pasture treatments did not influence consumption of either small supplement formula (P>0.5). Results from this initial study show that CGM small supplement mix can be used in place of an AP mix.

Key Words: Beef Cows, Protein Supplementation, Corn Gluten Meal, Animal Protein

Introduction

Previous studies conducted at NMSU have shown that low amounts of high undegradeable intake protein (UDP) fed in a small package may enhance utilization of nitrogen (Sawyer et al., 1998, Stalker et al., 2002; Sawyer et al., 2005). Thus, protein supplementation in a small quantity may have the potential to decrease production costs. Sawyer et al., (2005) found that feeding an animal protein (AP) mix as part of a small range supplement decreased feed cost by 53% compared to hand feeding a 36% CP range cube. Consequently, supplement costs can be minimized by reducing the amount of supplement required per animal. This can be accomplished by utilizing more concentrated protein sources that are used efficiently in potent formulas at smaller amounts. If providing small amounts of a self-fed effective supplement reduces the number of trips required and the amount of feed fed then, the costs of supplementation should be reduced with no change in animal productivity. Although previous studies have demonstrated that animal protein sources were effective in reducing delivery and feed costs while maintaining cow body condition objectives there are concerns about animal protein sources due to potential involvement with BSE (mad cow disease). Therefore concentrated plant based sources of UDP maybe more acceptable to the industry. The objectives of this study were to determine consumption of a self-fed supplement formulated with corn gluten meal protein mixed with salt and mineral and a self-fed supplement formulated with animal proteins mixed with salt and mineral with a secondary objective to determine the as fed crude protein content of the supplements (out of the feed tub and in the field) and estimate the potential ruminal protein bypass characteristics of the small supplements.

Materials and Methods

This study was conducted over a 17 week period from July to November at New Mexico State University's Corona Range and Livestock Research Center, Corona, NM. The ranch's elevation is 1900 m with an average annual precipitation of 400 mm. Rangeland at this study site is characterized as piñon-juniper woodland that is moderate to high density. Primary grass species are blue grama (*Bouteloua gracilis*) and wolftail (*Lycurus phleoides*) with minor components of sideoats grama (*Bouteloua curtipendula*), perennial threeawn (*Aristida spp*.), and galletagrass (*Hilaria jamesii*) (Knox et al., 1998).

Sixty-eight long yearling replacement heifers were used in a completely randomized design with treatments arranged in a 2x2 factorial to evaluate consumption of CGM added supplement (CGMS) compared to an AP added supplement (APS) and the influences of brush control

management on supplement intake. Heifers were randomly assigned to one of four pastures containing 130 ha. Two pastures were treated with Tebuthiuron in 1997 and two pastures were untreated. Treatments were then randomly assigned within pasture treatment which resulted in 2 replications per treatment. Cow weights were collected prior to pasture assignment, at the initiation of treatment application and at 28-d intervals thereafter until termination of the trial. Body condition scores (BCS, 1 = emaciated, 9 = obese) were assigned at initiation and termination of trial.

Ingredients for both supplements were mixed and bagged at the Corona Range and Livestock Research center. The APS supplement was produced on site by combining 2 parts salt-mineral mix with 1 part each of hydrolyzed feather meal and dried blood meal. The CGMS was composed of 1 part corn gluten meal and 1 part salt and mineral mix (Table 3). A grab sample of mixed supplement was collected at the time of mixing, and chemical analyses were conducted on these samples. Supplements were provided ad libitum in each of the four pastures and were placed in tubs at water. Delivery amounts were recorded each week so that total inputs could be determined and consumption per head by week could be calculated. Feed remaining in the open tub feeder was weighed once a week and subtracted from the amount of feed delivered the previous week. Consumption was calculated as grams of supplement that disappeared from the tub each week and was reported as supplement consumed per heifer per day. If supplement remaining in tubs was wet from precipitation it was allowed to dry before it was weighed to record supplement remaining weight. Consumption of APS and CGS were determined on a weekly basis. The initial composition of small supplement provided to tub feeders consisted of 2 parts mineral and 1 part protein mix to allow for a gradual change from salt and mineral. The ratio decreased as heifers accepted the protein mixture. Within 3 weeks they were consuming the final mixer. The final mix was 1 part mineral mix and 1 part protein mix. Heifers used in this study had experience consuming the a salt and mineral since birth. No other source of salt or mineral was provided.

Table 3. Composition of NMSU mineral

Calcium, maximum	11.5%
Phosphorus, minimum	8 %
Magnesium, minimum	2 %
Potassium	2 %
Copper	1000 ppm
Zinc	1000 ppm
Manganese	2500 ppm
Selenium	13 ppm
Vitamin A, units/lb	120,000

Forage quality was estimated from 1000 g of hand plucked samples at three different periods during the study in each of the four pastures. Samples were analyzed for DM, CP, ash (AOAC, 2000) and NDF (Van Soest et al., 1991). Samples of supplements were taken at the time of mixing and were analyzed for CP (AOAC, 2000) and CP solubility (Poos-Floyd et al., 1985) using a buffer and protease incubation. Each of the weekly supplement samples were composited into three samples that represented the following periods; July 12 to August 15, August 16 to October 2 and October 3 to November 3. Data were analyzed using GLM procedures of SAS using pasture as the experimental unit. A probability of less than 0.10 was considered significant.

Results and Discussion

Protein content of supplements The as fed crude protein content of CGMS was 27.6 ± 1.5% and the APS was 37.2 ± 1.5% (P<0.01). The in vitro protein solubility and protease degradability estimate of protein bypass was 16.4 ± 0.6 % as fed for the CGMS and 21.4 ± 0.6 % as fed for APS (P<0.01). The calculated protein bypass value for each mix was 59.4 and 57.5% for CGM and the AP mix respectively (Table 1).

Table 1. Crude protein and estimated undegradable intake protein analysis of small supplements.

Supplement	Crude protein % (as fed)	Estimated Bypass protein % (as fed)	Bypass protein % of crude protein
Corn gluten meal	27.6 ± 1.5*	16.4 ± 0.6*	59.4
Animal protein	37.2 ± 1.5	21.4 ± 0.6	57.5

* P<.01

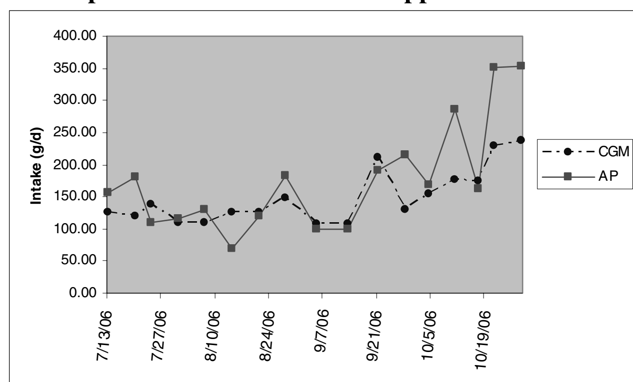
Small supplement consumption. The daily consumption of CGMS was 150.3 ± 3.8 g head^{-1}·d^{-1} which was not different from APS consumed at 175.8 ± 3.8 g head^{-1}·d^{-1} (P=0.13, Figure 1). However, heifers in both treatments consumed 32-55% over targeted intake of 113.4g.

Figure 1. Mean consumption of small supplement with Corn Gluten Meal or Animal Protein.*

N = 34. $P < 0.13$

Consumption of the CGMS was more consistent over the 17 week experimental period than the APS (Figure 2).

Figure 2. Weekly consumption of corn gluten meal and animal protein formulated small supplement



Brush control or non brush control pastures did not influence daily consumption (159.7 and 165 ± 3.7 g $head^{-1}\cdot d^{-1}$, respectively) of either supplement treatment (P>0.5). The similarity in supplement intake between the bush and non brush control pastures suggests that forage availability did not influence supplement intake. Donart 1998 (personal communications) showed that the brush treated pastures produced 4 time the herbaceous forage as the untreated pastures. The forage quality did not differ (P=0.25) over the course of the study between the two CGMS treated pastures and the two APS treated pastures (9.0 and 7.6 ± 0.9 % crude protein respectively). Forage from brush control treated pastures and non-treated pastures also had similar (*P* > 0.98) crude protein concentration (8.3 ± 0.9 % and 8.3 ± 0.9 % for brush controlled and non brush controlled, respectively). Over the duration of the study crude protein content declined (*P* < 0.01) in the hand plucked samples (11.1 ± 0.9 %, 9.1 ± 0.9 % and 6.7 ± 0.9 % for 7/12 to 8/15, 8/16 to 10/2 and 10/3 to 11/4 respectively). Ash content of the forage increased and neutral detergent fiber content remained high as the study progressed. During the study CP content (OMB) declined (P<0.01) in hand plucked samples (11.1, 9.1, and 6.7 ± 0.9 % for 12-July, 16-Aug, 3-Oct, respectively). Neutral detergent fiber (NDF) contents in July, August, and October were 69.6, 71.1, and 71.5 % OM basis respectively.

Type of supplementation did differentially influence (P <0.16) body weight or total weight gain (Table 2) of yearling heifers throughout the 17 week experimental period.

Table 2. Body weight and body condition responses of heifers to different supplementation strategies.

Item	CGMS	APS	SE[a]	P
Body Weight Responses				
July 10 BW, kg[b]	300	305	.98	0.98
August 8 BW, kg[c]	324	326	2.1	0.78
September 6 BW, kg	383	398	3.2	0.44
Final BW, kg[d]	398	408	1.9	0.16
Body Weight Changes				
August 8 BW, kg[c]	20.8.0	23.2.9	2.1	0.55
September 6 BW, kg[c]	60.2	63.2	5.3	0.44
October 23BW, kg	15.3	20.3	5.5	0.16
Total BW, kg[d]	96.3	106.7	--	--

[a]n = 15
[b]Cow weight at initiation
[c]Cow weight at 28 d intervals
[d]Cow weight change (Final – Initial)

Implications

In this initial study CGMS was an effective substitute for APS in a self-fed, small package supplement for long yearling replacement heifers while grazing 130 ha pastures having unlimited availability of forage. CGMS had only a small insignificant decrease in consumption and weight loss compared to APS.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECTS OF TALLOW SUPPLEMENTATION TO STEERS GRAZING WHEAT PASTURES ON GRAZING AND FEEDLOT FINISHING PERFORMANCE

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ABSTRACT: This experiment evaluated effects of tallow supplementation on steers grazing wheat pasture and subsequent feedlot performance. Forty-five English cross steer calves (initial BW 328 ± 14.8 kg) grazed 9 paddocks in a 41-d grazing study. Five steers grazed each paddock within BW class (light, medium, heavy) and supplement treatment. Steers received 1 of 3 supplements during the grazing period: 1) mineral pack (M) offered at 114 g/d, 2) M plus fiber as soybean hulls-wheat middlings (MF) offered at 0.50 % BW, and 3) MF plus tallow (MFT) offered at 0.625% BW. Supplements were offered daily (0800) to each grazing group (5 steers/group). Following the grazing period, each group of steers was assigned to an individual feedlot pen, and they were fed a common finishing diet for 56 d. During the grazing period, supplement intake differed ($P < 0.01$) as designed (113.1 g/d for M, and 0.48 and 0.59% of BW for MF and MFT, respectively). Grazing ADG was greater ($P = 0.05$) for steers receiving supplement compared with those receiving only M (0.90, 1.13, and 1.17 ± 0.06 kg/d, for M, MF, and MFT, respectively). During the feedlot period, ADG was not affected ($P = 0.26$) by the supplement received during the grazing period (1.57, 1.69, and 1.51 ± 0.07 kg/d for M, MF, and MFT, respectively). Dry matter intake was greater ($P = 0.10$) for M than for MF and MFT (14.5, 13.5, and 12.5 ± 0.06 kg/d, respectively). Steers receiving M were less efficient than those on MF and MFT as demonstrated by G:F ratio (0.109, 0.122, and 0.127 ± 0.007 for M, MF, and MFT, respectively). Treatments had no effect ($P \geq 0.41$) on hot carcass weight, marbling score, fat thickness, longissimus muscle area, internal fat, or yield grade. Treatments did not affect ($P > 0.90$) percentages of carcasses grading USDA prime or choice (66.7, 64.3, and 60.0 % for M, MF, and MFT, respectively). Tallow can be used for wheat pasture supplementation without adversely affecting performance. However, the performance response to tallow supplementation was similar to that of supplementing with highly digestible fiber.

Key Words: beef cattle, tallow supplementation, wheat pasture,

Introduction

Wheat pastures is a major forage for stocker calves in the south west United States during late fall, winter, and early spring. Livestock producers use wheat pasture because it is generally a high quality forage and cost of gain on wheat pasture is often lower than those of a conventional backgrounding program. Wheat forage is palatable and high in protein, energy, and minerals and low in fiber. It contains over 20% CP and over 70% DM digestibility (Mader, and Horn, 1986; Branine and Galyean, 1990). Stocker cattle can effectively utilize wheat pasture; they typically have daily gains of 0.7 to 0.9 kg. Wheat pasture grazing allows for maturation of muscle and bone while restricting fat deposition. However, an intramuscular lipid content of 3% is needed for acceptable beef palatability in the United States (Sarell and Cross, 1998). Fat deposition is restricted in animals grazing wheat pasture. Because cattle need to deposit intramuscular fat for acceptable beef palatability, cattle grown on forage before finishing require more days on feed and are also slaughtered at heavier weights to achieve acceptable carcass quality (Lewis et al ., 1990; Choat et al., 2003). Fat mass accumulation during the backgrounding period varied with energy intake (Owens et al., 1995). Increasing energy intake can increase the empty body fat: protein ratio (Byers, 1980; Old and Garrett 1987; Slabbert et al., 1992). Increasing rate of gain of cattle during winter wheat pasture grazing increases carcass and empty body fat content and reduces days on feed required in the feedlot (Hersom et al., 2004). Fat supplementation increases diet energy density, ADG, feed efficiency, and carcass fat deposition in feedlot cattle (Zinn and Plascencia, 1996). However, palatability problems as well as decreased fiber digestibility were associated with feeding fats to ruminants (Johnson and McClure, 1973), probably because of a toxic effect of long chain fatty acids on ruminal bacteria (Henderson, 1973). Because wheat pasture fiber content is low, fat supplementation may not have a mayor negative effect on fiber digestibility. Moreover, it may decrease production of methane produced by rumen fermentation, which could result in greater ruminal production of propionate (Zinn and Plascencia, 1996), and decreased energy loss (Czerkawski, 1973). Therefore the objective of this experiment was to evaluate effects of tallow supplementation on performance of steers grazing wheat pasture and subsequent feedlot performance.

Materials and Methods

Forty five steer calves and 10 pastures were used to evaluate effects of fat supplementation on performance of beef steers grazing wheat pasture. Five steers were assigned randomly, within BW groups (light, medium, and heavy) to each of 9 grazing groups. Some pastures were divided in 2 sections using electric fance to allow rotation of steers as required for irrigation of pastures. Each group grazed together for a 41-d period.

During this period (41-d), steers received 1 of 3 supplements during the grazing period: 1) mineral pack (M) offered at 114 g/d, 2) M plus fiber as soybean hulls-wheat middlings (MF) offered at 0.50 % BW, and 3) MF plus tallow (MFT) offered at 0.625% BW. The M supplement contained 7.9% wheat middlings and was designed to deliver 200 mg/steer/d of monensin. While MF

supplement contained 48.9% wheat middlings 40.8% soybean hulls and also designed to deliver 200 mg/head/day of monensin. Supplements were offered once a day (0800). Fallowing the grazing period, each group of steers was assigned to an individual feedlot pen, and they were fed daily (0700) a common finishing diet for 56 d. The finishing diet was 90% concentrate based on dry-rolled corn. Steers were weighed at d 1, 28, and 56 to determine ADG. Orts were collected as needed to stimulate fresh feed intake and DM was determined to calculate DMI. On the final day of the finishing period steers were shipped to a commercial packing plant. Carcass data were obtained 2 d after slaughter. Measurements included HCW, LM area, marbling score, percentage of kidney, heart, and pelvic fat, and fat thickness measured between the 12th and 13th ribs.

Data were analyzed using the Mixed procedures of SAS (SAS Inst. Inc., Cary, NC). The model included effects of treatment as fixed effects and block as random effects. The distribution of carcasses grading USDA Choice and Select was analyzed using the GEN MOD procedure of SAS, for which the model statement included block and treatment.

Results and Discussion

Effects of tallow supplementation on grazing performance are presented on Table 1. Supplement intake differed ($P < 0.01$) as designed (113.1 g/d for M, and 0.48 and 0.59% of BW for MF and MFT, respectively). The MF supplement was consumed more readily than MFT. In general, cattle consumed the MF supplement in 10 to 30 min in the morning, whereas the MFT was consumed over a period of about 4 h in the morning (usually cattle on MFT cleaned the feeder by noon). Also, the M supplement was consumed throughout the day, especially after drinking water, and intake decreased as the wheat stage of maturity increased. Grazing ADG was greater ($P = 0.05$) for steers receiving supplement compared with those receiving only M (0.90, 1.13, and 1.17 ± 0.06 kg/d, for M, MF, and MFT, respectively). The supplementation program increased ADG by an average of 0.15 and 0.19 kg/d for MF and MFT, respectively, compared with M. These results agree with those found by Horn et al. (1995), who reported that ADG increased an average of 0.15 kg/d with fiber or starch supplementation. They found that steers grazing wheat pasture and supplemented with a mineral pack supplement gained 0.92 kg/d and those supplemented with highly digestible fiber supplement and starch supplement gained 1.08 and 1.05 kg/d, respectively.

Effects of tallow supplementation during wheat pasture grazing on feedlot finishing performance are presented on Table 2. Average daily gain was not affected ($P = 0.26$) by the supplement received during the grazing period (1.57, 1.69, and 1.51 ± 0.07 kg/d for M, MF, and MFT, respectively). Dry matter intake was greater ($P = 0.10$) for M than for MF and MFT (14.5, 13.5, and 12.5 ± 0.06 kg/d, respectively). Steers receiving M were less efficient than those on MF and MFT as demonstrated by G:F ratio (0.109, 0.122, and 0.127 ± 0.007 for M, MF, and MFT, respectively). Our results do not agree with those of Choat et al. (2003) who reported that increasing rate of gain during winter grazing negatively affected feedlot finishing performance. However, rate of gain during winter grazing was 0.29 kg/d for steers grazing native range and 1.03 kg/d for steers grazing winter wheat. This large difference in rate of gain might have set the steers grazing winter native range for a compensatory gain during the feedlot, while the smaller difference observed in our study was probably not enough to express compensatory gain.

Effects of tallow supplementation during wheat pasture grazing on carcass characteristics of finishing steers are presented on Table 3. Treatments had no effect ($P \geq 0.41$) on HCW, marbling score, fat thickness, LM area, internal fat, or yield grade. Treatments did not affect ($P > 0.90$) the percentage of carcasses grading USDA prime or choice (66.7, 64.3, and 60.0 % for M, MF, and MFT, respectively). Our results disagree with Choat et al. (2003) and Hersom et al. (2004) who reported greater marbling for steers at faster rate of gain during winter grazing (wheat pasture vs. dormant native range grazing).

Implications

Tallow can be used in supplements to improve weight gain of cattle grazing wheat pasture. However, the improvement in weight gain is not great enough to affect marbling and other carcass characteristics at the finishing phase of beef production.

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Table 1. Effects of tallow supplementation on grazing performance of beef steers grazing winter wheat pasture

	Supplements[1]					Contrast[2]	
Item	M	MF	MTF	SE	P	S vs. M	F. vs. T
Initial BW, (Kg)	328	330	328	14.8	0.24	0.36	0.15
Final BW, (Kg)	384	390	389	15.8	0.38	0.20	0.80
ADG, (Kg)	0.98	1.13	1.2	0.05	0.10	0.04	0.64
Supplement Intake, (kg/d)	0.11	1.6	1.9	0.06	0.01	0.01	0.005
Supplement Intake, (%/BW)	0.035	0.48	0.59	0.003	0.01	0.01	0.01

[1]Supplement Type: M = 113.5 g of mineral supplement (Table 1); MF = mineral supplement plus soybean hulls-wheat middlings offered at .50% BW; MFT = mineral supplement plus soybean hulls-wheat middlings offered at .50% BW and tallow offered at 0.125% BW.
[2]S vs. M = contrast of steers receiving supplement (fiber or fiber plus tallow) vs. reciving only mineral pack

Table 2. Effects of tallow supplementation during winter wheat grazing on feedlot performance of beef steers

	Supplements[1]					Contrast[2]	
Item	M	MF	MTF	SE	P	S vs M	F vs T
Initial BW, (Kg)	384	330	328	14.8	0.24	0.36	0.15
Final BW, (Kg)	384	390	389	15.8	0.38	0.20	0.80
ADG (kg/d)							
d 0 – 28	1.58	1.57	1.64	0.07	0.76	0.73	0.53
d 28 – 56	1.57	1.70	1.51	0.07	0.26	0.69	0.13
d 0 – 56	1.57	1.63	1.58	0.06	0.75	0.67	0.55
Daily DMI, (Kg)							
d 0 – 28	12.2	11.7	11.6	0.51	0.61	0.38	0.73
d 28 - 56	16.9	15.2	13.4	0.81	0.08	0.05	0.18
d 0 – 56	14.5	13.4	12.4	0.60	0.16	0.10	0.30
Gain to feed ratio							
d 0 – 28	0.13	0.13	0.14	0.009	0.42	0.34	0.38
d 28 – 56	0.90	0.11	0.11	0.006	0.10	0.04	0.82
d 0 – 56	0.11	0.12	0.13	0.007	0.12	0.06	0.46

[1]Supplement Type: M = 113.5 g of mineral supplement (Table 1); MF = mineral supplement plus soybean hulls-wheat middlings offered at .50% BW; MFT = mineral supplement plus soybean hulls-wheat middlings offered at .50% BW and tallow offered at 0.125% BW.
[2]S vs. M = contrast of steers receiving supplement (fiber or fiber plus tallow) vs. reciving only mineral pack

Table 3. Effects of tallow supplementation during winter wheat grazing on carcass characteristics of feedlot beef steers

	Supplements[1]					Contrast[2]	
Item	M	MF	MTF	SE	P	S vs M	F vs T
HCW, kg	347.2	344.9	344.3	7.12	0.84	0.58	0.91
Marbling score	440.7	420.5	416.7	12.78	0.41	0.21	0.84
Fat thickness, cm	1.19	1.20	1.09	0.18	0.72	0.74	0.49
LM area, cm^2	82.7	82.4	82.9	1.84	0.97	0.93	0.85
KPH, %	1.93	1.93	1.97	0.06	0.87	0.80	0.66
Yield grade	2.85	2.87	2.73	0.21	0.72	0.74	0.48
Choice, %[3]	66.7	64.3	60.0		0.93		

[1]Supplement Type: M = 113.5 g of mineral supplement (Table 1); MF = mineral supplement plus soybean hulls-wheat middlings offered at .50% BW; MFT = mineral supplement plus soybean hulls-wheat middlings offered at .50% BW and tallow offered at 0.125% BW.

[2]S vs. M = contrast of steers receiving supplement (fiber or fiber plus tallow) vs. receiving only mineral pack

[3]Distribution of Choice + Prime vs. Select + Standard carcasses did not differ among treatments (P = 0.93).

Proceedings, Western Section, American Society of Animal Science.

Vol. 58, 2007

INTERACTION OF FORAGE TYPE AND LEVEL ON CONDITIONING OF HEIFER CALVES FOR EXPORTATION.

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ABSTRACT: One problem of the cow–calf system in northern Mexico is the low weaning weight of the calves that are exported to USA. Post- weaning calf conditioning (30 – 60 d) before sale can be an alternative to improve exportation weight. The objective was to evaluate the effect of oat hay (OH) and corn stover (CS) at high (H) and low (L) levels on average daily gain (ADG), dry matter intake (DMI) and gain efficiency (GE) being the combinations oat hay high(OHH), oat hay low (OHL), corn stover high (CSH) and corn stover low (CSL). Fifty four weaned heifer calves Angus, Hereford and their crosses were used, averaging 210 d of age and 151 Kg initial body weight. Heifer calves were fed ad libitum with isoenergetic and isonitrogenous diets during 56 d. the calves were assigned to the groups with homogenous initial body weight, and randomly assigned to the treatment (OHH, OHL, CSH, and CSL; n= 13, 13, 14 and 14, respectively) and weighed every 14 d. In the last 14 d of the test, four calves of each treatment were randomly selected these were kept in individual stalls to measure DMI. Data for ADG was analyzed with PROC MIXED (SAS) in a 2x2 factorial arrangement and with initial weight as covariable. DMI and GE were analyzed with the PROC GLM. For ADG (Kg) the lineal response for each combination was different (P< 0.05) (OHH: 1.36± .052, OHL: 1.48± .052, CSH: 1.06± .O51, CSL: 1.25± .036) at the end of the test, means for final body weight were OHH 218.09± 1.73; OHL 218.41± 2.92; CSH 220.64± 2.88; and CSL 219.59± 2.88. For DMI (Kg) covariable and forage level effects were found (P< 0.05) (H, 6.043± 2.08 and L, 6.78± 2.08). For GE results (P>0.05) were: OHH 3.87± 0.33, OHL 4.06± 0.33, CSH 3.98± 0.33, CSL 4.59± 0.33. Higher ADG offer lower cost per kg gained and conditioning of heifer calves before exportation is an alternative to improve profitability of the cow calf system in Mexican cattle ranches.

KEYWORDS: Oat Hay, Corn Stover, Weaned Heifer Calves.

Introduction

One problem of the cow-calf system in northern Mexico is the low weaning weight of the calves that are exported to the United States, the system is the most important beef cattle activity in the State of Chihuahua. It is carried out in 17.5 million hectares of grassland and shrub land with a 990 thousand beef head inventory (Báez et al., 1999). The main product of the cow-calf system is weaned calves males and females that are exported, and the State of Chihuahua exports on the average 385,000 calves per year (Sánchez, 2006). In the State of Chihuahua during the agricultural season 2003-2004 306,600 hectares of oat were grown, 79.4% of this area was dedicated to forage production (INEGI, 2005), and during 2006 an area of 208,048 hectares were grown with corn (SAGARPA, 2007). Use of these forages for post-weaning calf conditioning (30-60 d) before sale is an alternative to improve exportation weight.

The objective was to evaluate the effect of oat hay (OH) and corn stover (CS) at high (H) and low (L) levels on average daily gain (ADG), dry matter intake (DMI) and gain efficiency (GE) fed to weaned heifer calves.

Materials and Methods

The study was conducted in Teseachi Experimental Ranch property of the University of Chihuahua, located in Namiquipa County, Chihuahua, México. Fifty four weaned heifer calves Angus, Hereford and their crosses were used, averaging 210 d of age and 151 Kg initial body weight. Prior to the beginning of the study the animals were vaccinated, treated for internal and external parasites, and vitamins A,D,E were applied; they had an adaptation period of 15 d. Heifer calves were fed ad libitum with isoenergetic and isonitrogenous diets (Table 1) during 56 d. They received either oat hay high (OHH), oat hay low (OHL), corn stover high (CSH) or corn stover low (CSL). The calves were assigned to the groups with homogenous initial body weight, and randomly assigned to the treatment (OHH, OHL, CSH, and CSL; n= 13, 13, 14 and 14, respectively) and weighed every 14 d. In the last 14 d of the test, four calves of each treatment were randomly selected these were kept in individual stalls to measure DMI (Schneider and Flatt, 1975).

Data for ADG was analyzed with PROC MIXED (SAS) in a 2x2 factorial arrangement and with initial weight as covariable. DMI and GE were analyzed with the PROC GLM.

Results and Discussion

For ADG (Kg) the lineal response for each combination was different ($P < 0.05$) and results were: OHH: 1.36± .052; OHL: 1.48± .052; CSH: 1.06± .O51; CSL: 1.25± .036). At the end of the test, means for final body weight were: OHH 218.09± 1.73; OHL 218.41± 2.92; CSH 220.64± 2.88; and CSL 219.59 ± 2.88; and an effect of the covariable was found ($P<.0001$).

Lineal response results indicate that combinations with forage at low levels were higher for each forage, OHL showed the higher ADG (Figure 1). On the other hand, OH in both levels had a higher ADG than CS. These results are similar to those reported by Gasser et al. (2006), these differences may be also explained due to the quantity and quality of the fiber in each forage. Forage intake responses resulting from CP supplementation may be related to basal forage quality (Lawler–Neville et al, 2006). In addition to forage diet level, forage source has an effect in nutrient digestibility, ruminal characteristics and animal productivity (Bárcena, 2002).

For DMI (Kg) only effects of covariable and forage level ($P < 0.05$) were found (H, 6.043± 2.08 and L, 6.78± 2.08), similar results were reported by Gasser et al. (2006). For GE results ($P>0.05$) were: OHH 3.87±0.33, OHL 4.06±0.33, CSH 3.98±0.33, CSL 4.59±0.33 (Table 2).

Even though both forages are common ingredients used by cattlemen in northern México and specifically in Chihuahua for conditioning weaned calves, oat hay because of its nutritive value is a better option for feeding this type of animals under short periods of feeding and high efficiency.

Implications

Higher ADG means lower cost per kg gained and conditioning of weaned heifer calves before exportation is an alternative to improve profitability of the cow-calf system in Mexican cattle ranches. Another advantage of conditioning weaned calves is that it could reduce the disease incidence and the animals will perform better during their growing and finishing phases once they arrive to the United States cattle operations.

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Table 1. Ingredients and chemical composition of diets

Item	Diets.			
	OHH	OHL	CSH	CSL
Oat hay (%)	35.0	24.88	0.0	0.0
Corn stover (%)	0.0	0.0	33	23.89
Cotton seed (%)	4.22	8.839	6.9	9.594
Turkey litter (%)	2.5	4.689	2.1	2.96
Fat oil (%)	3.6	2.687	3.6	1.765
Corn gluten (%)	0.292	1.99	8.25	4.679
Calcium carbonate (%)	0.49	1.061	0.75	0.75
Microfos 12:10 (%)	0.38	0.896	0.9	0.883
Sodium chloride (%)	0.22	0.448	0.23	0.20
PC (%)	14.579	14.5	14.47	14.5
NEm (Mcal/Kg)	1.840	1.853	1.847	1.853
NEg (Mcal/kg)	1.204	1.215	1.210	1.215
Ca (%)	0.624	0.627	0.637	0.627
P (%)	0.387	0.499	0.456	0.508

Table 2. Regression coefficients and estimate error values per treatment

Item	Treatment			
	OHH	OHL	CSH	CSL
ADG	1.36± 0.052	1.48± 0.052	1.06± 0.051	1.25± 0 .036
DMI	6.18± 0.293 [a]	6.97± 0.293 [a]	5.90± 0.296 [a]	6.59± 0.297 [a]
GE	3.87± 0.33 [a]	4.06± 0.33 [a]	3.98± 0.33 [a]	4.59± 0.33 [a]

[a] Represent the least square means and standard error values.

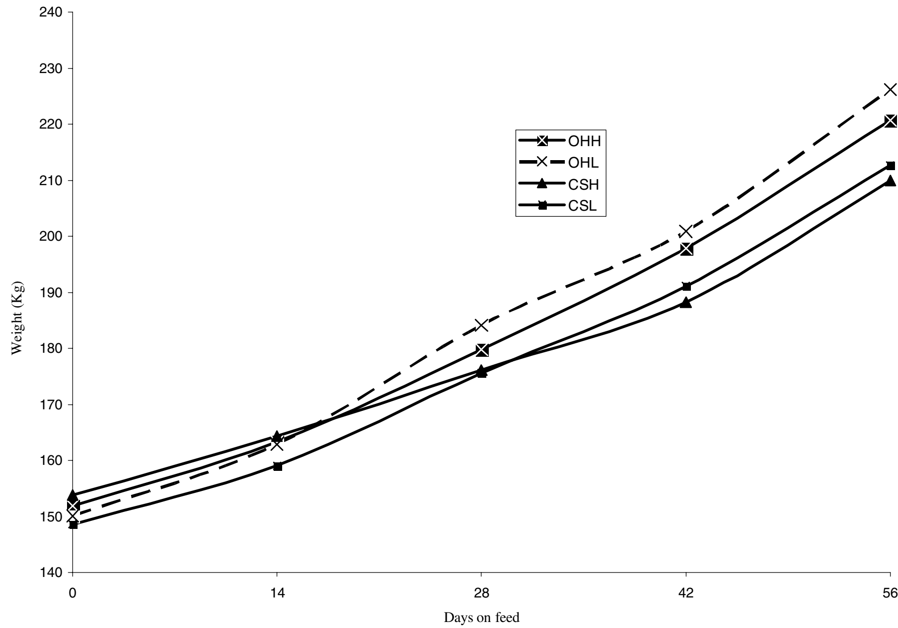

Figure 1. Body weight change (Kg) by treatment and days on feed

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECTS OF SUPPLEMENTAL GROUND FLAXSEED ON THE GROWTH PERFORMANCE OF STEERS GRAZING SUMMER NATIVE PASTURE IN THE NORTHERN GREAT PLAINS

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ABSTRACT[1]: The objective of this experiment was to evaluate the efficacy of supplemental flaxseed on increasing the growth performance of yearling steers grazing native range in the northern Great Plains. Eighteen Angus cross steers (avg initial BW 368 ± 4.6 kg) were rotationally grazed on native range starting in June until September. Steers were allotted to one of three treatments that were grazing only (CON); cracked corn and soybean meal (65.2% corn, and 32.4% soybean meal, and 2.0% dried molasses fed at 0.29% of BW; CRN); or ground flaxseed (98% ground flaxseed and 2.0% dried molasses fed at 0.18% of BW; FLX). Supplements were individually fed and formulated to be isonitrogenous and isocaloric on a TDN basis. The experiment consisted of three 29 d experimental periods. Starting on d 20 of each period, steers were bolused with 5g of TiO_2 after feeding and again 12 hours later until the end of the period. Starting on d 25 steers were fecal sampled at 0730 and 1930. Forage IVDMD was determined using six ruminally cannulated beef heifers allotted to similar dietary treatments and grazed with steers. There was a treatment × period interaction for forage intake (P = 0.007), ADG (P = 0.01) and feed efficiency (P < 0.001). Furthermore, cattle consuming FLX tended (P = 0.10) to consumer lower quality forage than CRN. Additional supplement tended to decrease (P = 0.11) forage intake compared to CON and CRN consumed more forage (P = 0.03) than FLX. However, ADG was greatest for supplemented steers (P < 0.001) compared to CON but did not differ (P = 0.47) between CRN and FLX. Feed efficiency was greater (P = 0.001) for supplemented steers and did not differ (P = 0.71) between supplemented groups. Supplemental flaxseed is an effective source of energy for improving growth performance of grazing steers.

Key Words: Beef cattle, Flaxseed, Grazing, Growth, Intake,

Introduction

In the northern Great Plains, stocker cattle can be a significant source of revenue. However, forage quality can often limit desired levels of production. Additional grain is often used to boost dietary energy density. However, corn-based supplements should not be fed at a level greater than 0.35% of BW in order to avoid poor utilization of forages (Vanzant et al., 1990).

Because fat has 2.25 times more energy than carbohydrates supplemental lipids boost the energy density of the diet. As with starch-based supplements, the level at which fat can be added to the diet is limited and should not exceed 6% (Whitney et al., 2000). Limited research is available regarding lipid supplementation to forage-based diets. Supplemental whole soybeans or soybean oil has been shown to have minimal effects on grazed forage digestibility (Brokaw et al., 2001) and have a positive impact on feed efficiency when fed with stored forages (Albro et al., 1993; Whitney et al., 2000). However, to our knowledge no data has been published on the effects of ground flaxseed supplementation on growth performance of grazing cattle. Our hypothesis was that supplemental flaxseed would increase the feed efficiency of beef steers grazing summer native pasture. Therefore, our objectives were to evaluate forage intake, masticate quality, and growth performance in beef steers grazing native pasture on the northern Great Plains.

Materials and Methods

Animals and diets

Eighteen Angus crossed steers (avg initial BW 368 ± 4.6 kg) and six ruminally and duodenally cannulated Angus beef heifers (avg. initial BW 340 ± 11.4 kg) were rotationally grazed on typical rangeland at the USDA-ARS Northern Great Plains Research Laboratory (46° 46` N 100° 50`W) from June 9, 2006 until September 1, 2006. The study site was comprised of three 12.1 ha pastures that contained 2,710 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 allowed to graze each pasture for 29 d and were rotated at the end of each sampling period to the next pasture. Cattle were randomly allotted to one of three dietary treatments being grazing only (**CON**); cracked corn and soybean meal (65.2% cracked corn, 32.4% soybean meal, and 2.0% dried molasses) fed at 0.29% of BW on a DM basis (**CRN**); or ground flaxseed (98% ground flaxseed and 2.0% dried molasses) fed at 0.18% of BW on a DM basis (**FLX**). Supplements were individually fed and formulated to be isonitrogenous and isocaloric on a TDN basis.

Starting on d 20 of each period, steers were bolused with 5 g of titanium dioxide twice daily. Cattle had free access to fresh water and trace mineralized blocks (American Stockman, North American Salt Co., Overland Park, KS). Cattle were weighed after a 12 h shrink at the beginning and end of each period and amount of supplement fed was adjusted according to BW changes. All experimental procedures were reviewed and approved by the Northern Great Plains Research Laboratory, Animal Care and Use committee.

[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.

Sampling and laboratory analysis

Starting on d 25, fecal samples were obtained from steers twice daily until the end of the period. Fecal samples were composited within animal, dried in a 55° C oven and ground through a 1 mm screen (Wiley mill; Thomas Hill and Sons, Philadelphia, PA). On the morning of d 22, rumen-fistulated heifers were evacuated of all of their rumen contents and were allowed to graze for 1 hour after which masticate was collected and processed as outlined by Brokaw et al. (2001). On d 29, approximately 800 mL of rumen fluid was removed from each heifer and transported to the laboratory for 48 h IVDMD using a DaisyII system (Ankom Technology Corp. Fairport, NY). Each heifer's masticate collected the previous week was incubated in her respective rumen fluid in an effort account for any variation dietary treatment had on rumen environment.

All feed, masticate and fecal samples were analyzed for DM and ash (AOAC, 1990). Nitrogen content of feed, masticate, 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 and feces were determined using an ANKOM 200 fiber analyzer (ANKOM Technology, Fairport, NY). Fecal samples were analyzed for titanium dioxide according to the procedures of Myers et al. (2005).

Statistical analysis

All data were analyzed using the MIXED model of SAS (SAS Inst. Inc., Cary, NC) as a split-plot in a completely random design. Supplementation was used as the main plot tested against animal within treatment (error a) and the effect of sampling period and the treatment × sampling period interaction was the subplot tested against residual error (error b). Single degree of freedom orthogonal contrasts were used to compare effects of Control vs. supplements (Corn and Flaxseed), as well as Corn vs. Flaxseed and sampling period effects were tested using orthogonal polynomial contrast (Steel and Torrie, 1980). The model included animal as the random variable.

Results

There was a treatment × period interaction (P = 0.01) for forage DM intake (Figure 1). Across treatment, CON forage intake tended (P = 0.11) to be higher compared to the supplemented cattle with CRN being greater (P = 0.02) than FLX. A quadratic response (P < 0.001) was observed for forage intake, with sampling period 2 having the lowest intake compared to sampling periods 1 or 3. Masticate IVDMD differed (quadratic, P < 0.001) across period (Table 1). Although there was no treatment × sampling period interactions (P = 0.53) observed for masticate IVDMD nor was there a difference (P = 0.13) between CON and supplemented cattle, flax-fed cattle did tend (P = 0.10) to consume lower quality masticate than CRN. A treatment × sampling period interaction (P = 0.01) was observed for masticate N content because CON had lower masticate N than either CRN or FLX in sampling period 2, but had higher masticate N in sampling period 3. Overall, dietary treatment did not influence (P = 0.25) masticate N content.

There was a treatment × sampling period interaction (P < 0.001) for apparent total tract DM, NDF, and N digestibility (data not shown). Apparent total tract DM digestibility was greater (P < 0.001) for CON versus supplemented cattle with CRN being greater (P < 0.001) than FLX. However, total tract NDF digestibility was lower (P < 0.001) for supplemented cattle and FLX was lower than CRN. Apparent total tract N digestibility was lower (P < 0.001) for CON versus supplemented cattle. However, across the supplemented treatments, apparent total tract N digestibility was higher (P < 0.001) for CRN than FLX.

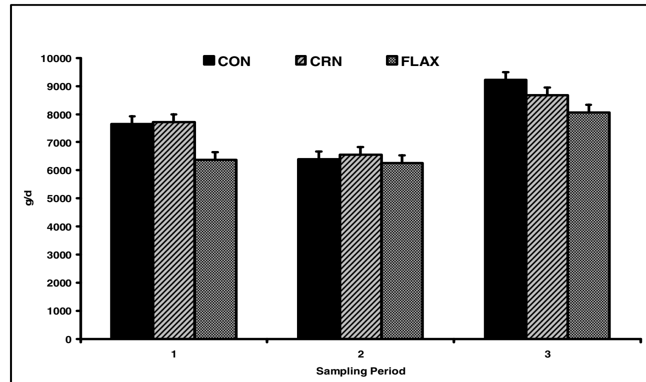

Figure 1. Effects of supplemental flaxseed on forage intake in beef steers grazing summer pasture in the northern Great Plains. (Treatment × sampling period P = 0.01; SE = 270.1)

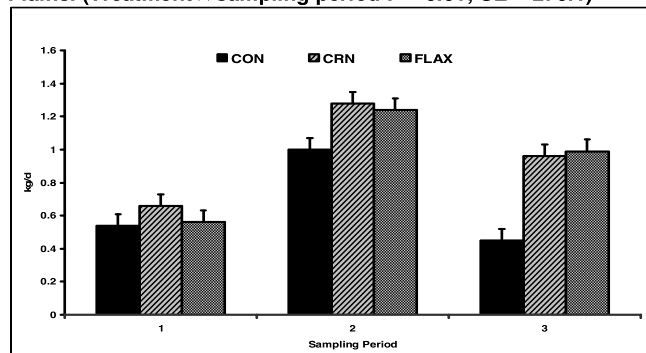

Figure 2. Effects of supplemental flaxseed on ADG in beef steers grazing summer pasture in the northern Great Plains. (Treatment × sampling period P = 0.01; SE = 0.99)

There was a treatment × sampling period interaction (P < 0.01) for steer ADG (Figure 2) and feed efficiency (data not shown). As expected, the increase in dietary energy increased total weight gain and ADG (P < 0.001) compared to unsupplemented cattle. Although, estimated forage quality was lower, animal performance was greatest (quadratic, P < 0.001) during sampling period 2.

Discussion

The significant interaction between treatment and sampling period reflects the reduction in forage intake for FLX compared to CON or CRN during sampling period 1. However, during the remainder of the summer grazing season, forage intake for FLX did not differ across treatment. Despite differences in forage quality during the summer grazing season, Brokaw et al. (2001) did not observe any treatment × sampling period interactions when beef heifers fed either no supplement or supplemental cracked corn or soybean oil grazed summer pasture. Overall, supplemental energy did not negatively impact forage intake compared to control, which was expected due

to the low level at which these energy supplements were fed. Others have shown that supplemental energy in the form of carbohydrates either increased (Matejovsky and Sanson, 1995) or decreased (Chase and Hibberd, 1987; Pordomingo et al., 1991) forage intake in grazing cattle. Fat supplementation has also been shown to not influence (Brokaw et al., 2001) or reduce (Pavan et al., 2007) forage intake in grazing cattle.

Brokaw et al. (2001) did not observe any differences between corn and corn oil supplemented heifers for total tract OM disappearance but did report differences across sampling period. Fieser and Vanzant (2004) observed a supplement type × forage maturity interaction. However, these authors reported a greater depression in apparent total tract OM digestibility when corn was supplemented to high quality forages. Whereas in the current experiment we observed that CON and CRN treatments did not differ for sampling periods 1 and 2 however, during sampling period 3, apparent total tract DM digestibility increased for unsupplemented control compared to CRN and FLX was lowest. This reduction in digestibility has been noted previously (Chase and Hibberd, 1987; Pordomingo et al., 1991). Our results also agree with Whitney et al. (2000) and Pavan et al. (2007) who documented a depression in forage digestion with supplemental fat.

The significant treatment × sampling period response appeared to manifest itself as time on trial elapsed. Specifically, during sampling period 1 all treatments had similar growth performance. However, starting in sampling period 2 treatments started to diverge with CON having lower gains and a slightly lower feed efficiency. Overall, treatment means indicate that supplementation improved animal performance. Cattle supplemented with ground flaxseed performed as well as cattle supplemented with corn. Although previous work has reported an increase in feed efficiency when fats were added to a forage-based diet (Whitney et al., 2000), no differences were noted between CRN and FLX.

Total BW gain, ADG, and feed efficiency was greatest for sampling period 2 despite a reduction in masticate quality, forage intake and total tract DM digestibility. This discrepancy could be due to the average temperature during sampling period 2 being the highest for the entire experiment (NDAWN, 2006). Therefore, daily forage intake may have been reduced with more grazing during the cooler night and early morning hours. In addition, masticate samples were collected in the late morning (1000), therefore based on differences noted by Holt and Hilst (1969) regarding diurnal variation in nonstructural carbohydrates in forages, perhaps by grazing at night cattle consumed a higher quality diet than was represented by the masticate. Also, although the steers were held off feed overnight before weighing, the lower digestibility of their diets in sampling period 2 may have resulted in additional digesta weight that was measured as artifact growth. Lastly, the potential errors associated with marker based values for intake and digestibility should be interpreted with caution.

In conclusion, ground flaxseed, appears to be a viable option for use as an energy supplement to steers grazing native range by improving growth performance without reducing forage utilization.

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Table 1. Effects of supplemental ground flaxseed on the quality of masticate selected by heifers grazing summer native pasture in the northern Great Plains (% of DM)

	Treatments[a]				CON	CRN	Sampling period[c]						Trt ×
Item	CON	CRN	FLX	SE[b]	vs. Supplement	vs. FLX	1	2	3	SE[b]	Linear	Quadratic	Period[d]
N	1.23	1.22	1.20	0.02	0.43	0.49	1.37	1.00	1.28	0.02	0.003	<0.001	0.01
NDF	73.3	73.0	74.1	0.57	0.73	0.26	72.5	77.6	70.3	0.57	0.04	<0.001	0.29
IVDMD	65.0	64.4	62.2	0.77	0.13	0.10	65.4	59.3	66.9	0.69	0.15	0.001	0.52
Total Fatty acid	1.54	1.6	1.6	0.03	0.14	0.89	1.69	1.43	1.65	0.03	0.34	0.001	0.97

[a]Cattle were allowed to graze native range pastures and were allotted to one of three dietary treatments being grazing only (**CON**); cracked corn and soybean meal (65.2% cracked corn, 32.4% soybean meal, and 2.0% dried molasses) fed at 0.35% of BW on an as-fed basis (**CRN**); or ground flaxseed (98% ground flaxseed and 2.0% dried molasses) fed at 0.20% of BW on an as-fed basis (**FLX**).
[b]n = 2
[c]The experiment consisted of three sampling periods (1 = June 9th to July 7th, 2006; 2 = July 7th to August 4th, 2006; 3 = August 4th to September 1st, 2006).
[d]Treatment × sampling period

Table 2. Effects of supplemental ground flaxseed on the growth performance of steers grazing summer native pasture in the northern Great Plains

	Treatments[a]				Control	CRN	Sampling period[c]						Trt ×
Item	CON	CRN	FLX	SE[b]	vs. Supplement	vs. FLX	1	2	3	SE[b]	Linear	Quadratic	Period[d]
DM intake, g/d													
Forage	7759	7677	6914	227.2	0.11	0.02	7260	6420	8668	155.9	<0.001	<0.001	0.01
Total	7759	8893	7645	219.8	0.07	0.001	7871	7064	9362	152.2	<0.001	<0.001	0.01
Forage intake, g/kg of BW	18.9	18.2	16.5	0.55	0.02	0.04	18.6	15.3	19.6	0.65	0.01	<0.001	0.001
Total intake, g/kg of BW	19.0	21.1	18.3	0.54	0.29	0.001	20.3	16.9	21.2	0.37	0.01	<0.001	0.001
NDF intake	5656	5772	5295	164.4	0.55	0.05	5375	5114	6234	112.7	<0.001	<0.001	0.001
N intake	97.2	136.3	111.0	2.53	<0.001	<0.001	121.1	87.3	136.2	1.81	<0.001	<0.001	<0.001
Total tract digestibility, % of intake													
DM	65.0	68.7	64.9	0.14	<0.001	<0.001	67.5	62.2	68.9	0.08	<0.001	<0.001	<0.001
NDF	68.7	68.0	65.9	0.18	<0.001	<0.001	68.0	64.9	69.7	0.18	<0.001	<0.001	<0.001
N	56.6	68.1	64.1	0.58	<0.001	<0.001	67.5	53.8	64.5	0.41	0.99	<0.001	<0.001
Performance													
Gain, kg	19.1	28.1	26.9	1.13	<0.001	0.47	16.9	34.1	23.1	1.13	0.001	<0.001	0.01
ADG, kg/d	0.66	0.97	0.93	0.04	<0.001	0.47	0.58	1.17	0.80	0.04	0.001	<0.001	0.01
F:G[e]	14.6	10.1	9.8	0.91	<0.001	0.71	14.2	6.28	14.0	0.81	0.79	<0.001	<0.001

[a]Cattle were allowed to graze native range pastures and were allotted to one of three dietary treatments being grazing only (**CON**); cracked corn and soybean meal (65.2% cracked corn, 32.4% soybean meal, and 2.0% dried molasses) fed at 0.35% of BW on an as-fed basis (**CRN**); or ground flaxseed (98% ground flaxseed and 2.0% dried molasses) fed at 0.20% of BW on an as-fed basis (**FLX**).
[b]n = 6
[c]The experiment consisted of three sampling periods (1 = June 9th to July 7th, 2006; 2 = July 7th to August 4th, 2006; 3 = August 4th to September 1st, 2006).
[d]Treatment × sampling period
[e]kg of total DM intake / kg of gain

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

REPRODUCTIVE PERFORMANCE OF HEIFERS OFFERED AD LIBITUM OR RESTRICTED ACCESS TO FEED FOR A 140-D PERIOD AFTER WEANING [1]

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ABSTRACT: Reproductive performance was evaluated in heifers born in 4 years that were randomly assigned to either control (fed to appetite; n = 268) or restricted (fed at 80 % of that consumed by controls adjusted to a common BW basis; n = 263) feeding during a 140-d postweaning trial, beginning about 2 mo after weaning at 6 mo of age. Heifers were fed a diet of 64 % corn silage, 23 % alfalfa and 13 % of a protein-mineral supplement (DM basis). Restricted fed heifers consumed 26 % less feed over the 140-d trial and had lower ADG (0.48 vs. 0.66 kg/d; P < 0.001) than control heifers. After the trial, heifers were combined and subjected to an estrous synchronization protocol. Heifers were artificially inseminated at about 14 mo of age and then exposed to bulls for the remainder of a 51-d breeding season. Differences in BW of restricted and control fed heifers persisted (P < 0.01) throughout the prebreeding period (316 vs. 338 kg at approximately 13.5 mo of age) and subsequent grazing season (404 vs. 414 kg at about 19.5 mo of age), but ADG from the end of the 140-d trial to 19.5 mo of age was greater (P < 0.01) in restricted heifers than control heifers (0.49 vs. 0.42 kg/d). The proportion of heifers attaining puberty by 14 mo of age was less (P < 0.01) in restricted (58 %) than control fed heifers (69 %). Means of age at puberty were adjusted to reduce bias from differences in proportions of animals that attained puberty, assuming age of puberty to be normally distributed. Adjusted age at puberty was greater in restricted heifers than control heifers (418 vs. 398 d; P < 0.05). Mean BW at puberty, predicted from regression of BW on age, was less (P < 0.01) in restricted (317 kg) than control (337 kg) heifers. Pregnancy rate from AI did not differ (P = 0.3; overall mean = 50 %) due to feed level. Final pregnancy rate averaged 87 and 91 % for restricted and control heifers, respectively (P = 0.15). Accounting for differences in pregnancy rate, amount of harvested feed provided per pregnant heifer was reduced 22 % with the level of restriction implemented in this study.

Key Words: Heifer development, Pregnancy, Puberty

Introduction

Development of replacement heifers at sufficient rates of growth to ensure puberty prior to breeding is critical for efficient beef cattle production. In many production settings, forage conditions are inadequate to support sufficient rates of development during the postweaning period, requiring additional supplemental feed resources that increase cost of production. Thus, producers are faced with the challenge of balancing feed resources to achieve optimal development goals, while minimizing cost of production by not overextending feed inputs. Guidelines were established several decades ago for developing replacement heifers to ensure attainment of puberty at an age that permits calving at 2-yr of age (reviewed in Patterson et al., 1992). However, recent research provides evidence that input of harvested feed can be reduced without major adverse affects on reproductive performance by altering pattern of gain (Freetly et al., 2001) or by feeding to lighter target weights than those typically recommended (Funston and Deutscher, 2004); thereby reducing expense of raising heifers.

The present research is a portion of a long-term project to evaluate the influence of 2 levels of nutritional input during heifer development and winter supplementation on lifetime productivity. Objectives of this research were to evaluate the reproductive performance of heifers offered either ad libitum or restricted access to feed during the postweaning period.

Materials and Methods

All research protocols used in this study were approved by our institutional Local Animal Care and Use Committee. Heifers used in this study were a stable composite population (**CGC**; ½ Red Angus, ¼ Charolais, ¼ Tarentaise). Heifers represent a randomly selected population produced by mating CGC dams and sires (n = 42) with consideration given to minimize inbreeding, but without emphasis on production traits. Heifers were born during a 4-yr period. Pertinent dates, total numbers of heifers assigned to each treatment in each year and other information on heifers used in the study are provided in Table 1. After weaning, heifers were stratified into groups based on weaning weight and were randomly assigned to 1 of 4 (Yr 1) or 1 of 22 to 24 pens (Yr 2, 3 and 4). In Yr 1, heifers were group fed with 26 or 27 heifers/pen. Heifers in Yr 2, 3, and 4 were individually fed in pens that contained 6 individual feed bunks equipped with electronic Calan gates (American Calan, Northwood, NH). Heifers were allowed a minimum of 1 mo for adaptation to experimental pens (all years) and to become trained to the head gates (Yr 2, 3 and 4). During this time, heifers were allowed ad libitum access to the test diet fed once daily. In Yr 1, pens were randomly assigned to receive either control (n=2) or restricted (n=2) level of feeding. In Yr 2, 3 and 4, heifers were randomly assigned within pens to either a control or restricted level of feeding for a 140-d trial. Feed restriction was initiated when heifers were approximately 8 mo

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of age and 227 $\pm$ 21 kg BW (Table 1). Control heifers were fed to appetite and restricted heifers were fed at 80 % of that consumed by controls adjusted to a common BW basis, as determined at 4-wk intervals. Composition of the diet fed during each year is shown in Table 2. Weight of feed offered was recorded daily. Orts were removed from the feed bunk and weight recorded as necessary to ensure that fresh feed was provided for each heifer on a daily basis.

Measures of BW were recorded at initiation and conclusion of the 140-d study (ages shown in Table 1), and at approximately 28-d intervals throughout the study. These measures of BW were collected prior to feeding, and were used to adjust feed level of restricted heifers 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]. Measures of BW were also made at about 1 mo (initiation of estrous synchronization) and 7.5 mo (about Dec 1) after the end of the restriction period.

Blood samples were collected from the tail vein at 9- to 11-d intervals beginning at approximately 11.5 mo of age and ending at approximately 14 mo of age. Serum from these samples was used to determine circulating concentrations of progesterone as an indicator of pubertal status. Concentrations of progesterone were determined directly without extraction by solid-phase RIA (Coat-a-Count kit; Diagnostic Products Corp., Los Angeles, CA) as reported previously (Bellows et al., 1991). Week in which puberty occurred was defined as the first week that serum concentration of progesterone exceeded 1 ng/mL. Average BW of heifers at week of puberty was predicted from the regression of BW on age.

At the end of the 140-d trial, heifers were combined and given ad libitum access to the same diet, under drylot conditions for approximately 50 d to allow for estrous synchronization and AI. At approximately 14 mo of age (30 to 40 d after end of restriction), heifers were weighed and subjected to an estrous synchronization protocol. In Yr 1, 2 and 3, heifers were subjected to the CO-Synch + CIDR protocol with timed AI of heifers not detected in heat by 48-72 h. Heifers in Yr 1 were observed for estrus and AI for a total of 31 d, whereas heifers in Yr 2 and 3 were only subjected to AI during the 2 to 3 d after the PGF injection given in the estrous synchronization protocol. In Yr 4, a single injection of PGF was given on d 7 of an 11-d AI breeding period. After AI, heifers were placed on native range and exposed to bulls for the remaining duration of a 48- to 53-d breeding season. Heifers were evaluated for pregnancy by transrectal ultrasonography using a 5 MHz transducer approximately 1 mo after AI and again at about 1 mo after bull removal. Date of AI, estimated age of fetus at pregnancy diagnosis, and date of calving (available for yr 1, 2 and 3) were used to predict the day of the breeding season that conception occurred. For yr 1, 2 and 3, date of calving was used to calculate number of days from onset of breeding to calving.

Data were analyzed with the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Body weight, ADG, puberty, pregnancy and day of conception data were analyzed using a model that included age of dam (2, 3, 4, or 5 and older) and age of heifer at onset of the study as covariates; fixed effects of year and treatment, and the interaction of these fixed effects. Because observations of pubertal status ended before all heifers attained puberty, observed mean ages and weights at puberty were biased downward depending on the percentage pubertal. To reduce this bias in comparing means differing in the percentage pubertal, means for age at puberty were adjusted assuming age at puberty to be normally distributed. Following Dickerson and Hazel (1944), the SD of the observed sample is related to the SD of the non-truncated distribution (s) by an adjustment factor equal to $1/(1\text{-}i^{z} + iZ)^{0.5}$; where i is the expected standardized deviation from the true mean of the observed mean derived from the proportion attaining puberty and Z = the deviation in SD units from the true mean at the point of truncation. The mean of a sample from the truncated normal distribution is: Xt + h*s/p; where h = height of the ordinate of the standard normal curve at the point of truncation defined by the proportion selected (p). An additional analysis was performed to evaluate effects of pubertal status at 14 mo of age on subsequent reproduction traits and to determine if BW differed among animals classified by pubertal status. The model for this analysis included pubertal status at 14 mo of age (yes or no), year and treatment, and the interactions among these factors. Least square means and largest SE are presented, unless specified otherwise.

Results and Discussion

Restricted fed heifers consumed 26% less feed over the 140-d trial resulting in a 26 kg lighter ($P < 0.001$) BW at the end of the trial (Figure 1). Off test BW also differed due to year, being heavier ($P < 0.01$) in the first 2 yr of the study than the last 2 yr (Figure 1). The ADG over the 140-d trial was reduced ($P < 0.001$) by restricted feeding, with the magnitude of reduction differing over the years ($P < 0.001$ for interaction of year and treatment). Differences in BW of restricted and control fed heifers persisted ($P < 0.01$) throughout the pre-breeding period (316 vs. 338 ± 2 kg at approximately 13.5 mo of age, Figure 2) and subsequent grazing season (404 vs. 414 ± 2 kg at about 19.5 mo of age). Although ADG was reduced during feed restriction, ADG from end of the 140-d trial to 19.5 mo of age was greater ($P < 0.01$) in restricted heifers than control heifers (0.49 vs. 0.42 ± 0.005 kg/d, Figure1).

Proportion of heifers that became pubertal by 14 mo of age varied ($P < 0.001$) over the 4 yr of the study, but was less ($P < 0.01$) in restricted than control fed heifers (Table 3). Assuming age of puberty to be normally distributed, means for age at puberty of each treatment by year classification were adjusted to reduce bias from differences in proportions of animals that attained puberty. Adjusted mean age at puberty was greater in restricted heifers than control heifers (418 vs. 398 ± 4 d; $P < 0.05$, Table 3). Regression of BW on age was used to estimate BW of individual heifers at time of puberty, which differed ($P < 0.01$) due to treatment and year (5th column Table 3). In addition, the adjusted mean age at puberty and the mean ADG for each treatment within year grouping was used to predict mean BW at which all heifers within a treatment by year classification would be pubertal (6th column Table 3). The predicted BW at puberty was less ($P < 0.01$) in restricted (317 kg) than control (337 kg) heifers. Predicted BW at puberty ranged from 53 to 67 percent of the expected mature cow BW

(558 kg at BCS 5 for cows 5 yr old and older in this herd; Table 3). With the exception of control heifers from Yr 1, in which almost all heifers attained puberty, pubertal heifers were heavier than pre-pubertal heifers at the off tests and pre-breeding BW measures, with magnitude of difference varying due to treatment and year ($P < 0.05$ for 3-way interaction of pubertal status, treatment and year; Figure 2). Collectively these results show that restricted heifers attained puberty at lighter BW, albeit at an older age.

Pregnancy rate from AI tended to be influenced by the interaction of year and treatment ($P = 0.11$; Figure 3), where AI pregnancy rates did not differ due to feed level in Yr 1 and 2, but a reduction was observed in feed restricted heifers in Yr 3 ($P = 0.11$) and 4 ($P < 0.05$). Final pregnancy rate averaged 87 and 91 % for restricted and control heifers, respectively ($P = 0.15$; Figure 3). For heifers that became pregnant, predicted day of the breeding season that conception occurred was influenced by the interaction of year and treatment ($P = 0.06$; Figure 3). In Yr 1, average day of conception was earlier ($P < 0.001$) in restricted than control heifers. However, there was a tendency for average day of conception to be later in restricted than control heifers from Yr 3 ($P = 0.14$) and Yr 4 ($P = 0.1$). Number of days from onset of breeding to calving (available for Yr 1, 2 and 3) did not differ due to treatment ($P = 0.74$; 294 ± 14 d) or the interaction of year and treatment ($P = 0.22$).

Differences in off test and pre-breeding BW (BW for restricted heifers from Yr 1 and 2 equal to or greater than BW of controls from Yr 3 and 4; Figure 1 and 2), pubertal status and the ability of the estrous synchronization protocols used to induce puberty likely contribute to the interactions of year and treatment noted above for AI pregnancy rate and day of conception. Heifers that had not achieved puberty by 14 mo of age had lower AI pregnancy rates (31 ± 7 %) than heifers that were pubertal (56 ± 3 %). Average day of conception tended to be influenced ($P = 0.12$) by a 3-way interaction among pubertal status, year and treatment, where heifers that had not reached puberty took longer to conceive in Yr 1, 3 and 4, but not Yr 2, and the magnitude of difference in day to conception between pubertal and non-pubertal heifers was greater in restricted than control heifers. An interaction of pubertal status and treatment was observed ($P = 0.02$) for final pregnancy rate, where a reduction in pregnancy rate was observed in restricted heifers that had not achieved puberty (70 ± 5 %) when compared to restricted heifers that had achieved puberty (92 ± 8 %), or control heifers that had (95 ± 8 %) or had not (92 ± 3 %) achieved puberty. It can be interpreted from these results that non-pubertal heifers from the control group were closer to becoming pubertal at time of breeding than the non-pubertal heifers from the restricted group.

Implications

The level of restriction imposed in this study was predicted to result in a 20-d delay in puberty and a reduction in pregnancy rate was observed in restricted fed heifers that failed to reach puberty prior to breeding, which resulted in a 4 % reduction in overall pregnancy rate. After accounting for differences in pregnancy rate, a 22 % reduction in harvested feed provided per pregnant heifer was obtained with the level of restriction implemented in this study. In addition, restricted heifers achieved greater ADG during the grazing season than control fed heifers. Differences in market values of heifer calves and open heifers will impact potential economic advantages of restricted feeding.

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Table 1. Animal numbers and averages for other descriptive data on heifers from each of the 4 yr of the study

	Yr 1	Yr 2	Yr 3	Yr 4
Year of birth	2002	2003	2004	2005
Restricted, n	53	62	82	66
Control, n	52	63	84	69
Birth, d of yr (±15)	101	103	95	95
Wean age, d	167	178	172	190
On test date	11/20	12/2	12/9	12/5
On test age, d	226	232	248	244
On test BW, kg	239	212	235	224
Off test age, d	363	373	387	385
Age at AI, d	423	422	429	419

Table 2. Composition (%, DM basis) of diets fed during the 140-d feeding period within each of 4 years

	Yr 1	Yr 2	Yr 3	Yr 4
Corn silage	52	67	67	68
Alfalfa	38	18	18	17
Supplement[1]	10	15	15	15
DM	47.5	36.1	36.8	37.3
CP	13.3	15.1	15.1	17.1

[1]Containing protein and mineral.

Table 3. Proportion (%) of restricted (Rest) and control (Cont) heifers from each year that achieved puberty by 14 mo, and predicted age (d), BW (kg), and percentage of mature BW (MBW) at time of puberty

Trt	Yr	%[1]	Age, d[2]	BW, kg[1,3]	Pred BW, kg[1,2]	% MBW[4]
Rest	1	92	386	303	300	54
Cont	1	98	365	330	334	60
Rest	2	29	454	334	356	64
Cont	2	41	437	366	374	67
Rest	3	80	391	292	320	53
Cont	3	90	386	314	315	57
Rest	4	27	440	294	317	57
Cont	4	48	406	310	323	58

[1]Differs due to treatment (P < 0.01) and year (P < 0.001).
[2]Adjusted to account for differences in proportions reaching puberty in each year by treatment classification (P < 0.05 for interaction of year and treatment). Pred BW =predicted mean BW of heifers at estimated age of puberty.
[3]BW of heifers that achieved puberty.
[4] (Pred BW/558kg) x 100 = percentage of expected mature BW at which heifers are predicted be pubertal.

Figure 1. Growth of heifers from 4 different years that were provided ad libitum (Control) or Restricted access to feed during a 140-d trial during the postweaning period. Top panel: BW at the end of the trial (*P* for treatment < 0.001; *P* for year <0.001). Middle panel: ADG for the 140-d trial (*P* for treatment by year <0.001). Bottom panel: ADG of heifers from end of the feeding trial to beginning of December, when heifers were managed together on pasture (*P* for treatment by year <0.001).

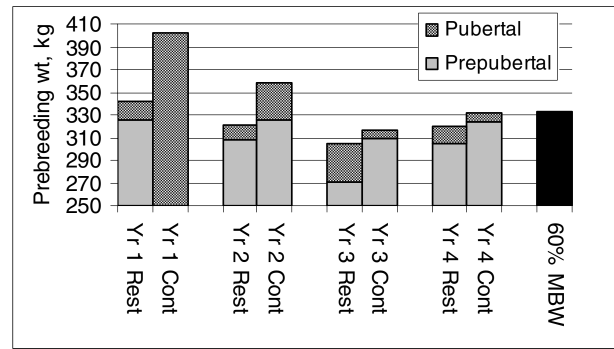

Figure 2. BW of heifers at 2 wk prior to breeding, 1 mo after a 140-d feeding trial in which of heifers were provided ad libitum (Cont) or restricted (Rest) access to feed during the postweaning period. The trial was replicated over 4 yr. Heifers BW are sub-classified by pubertal status (see legend) within each year by treatment classification (*P* = 0.06 for interaction of year, treatment and pubertal status). Bar on right side of graph depicts BW equivalent to 60% of the expected BW of mature cows in this herd.

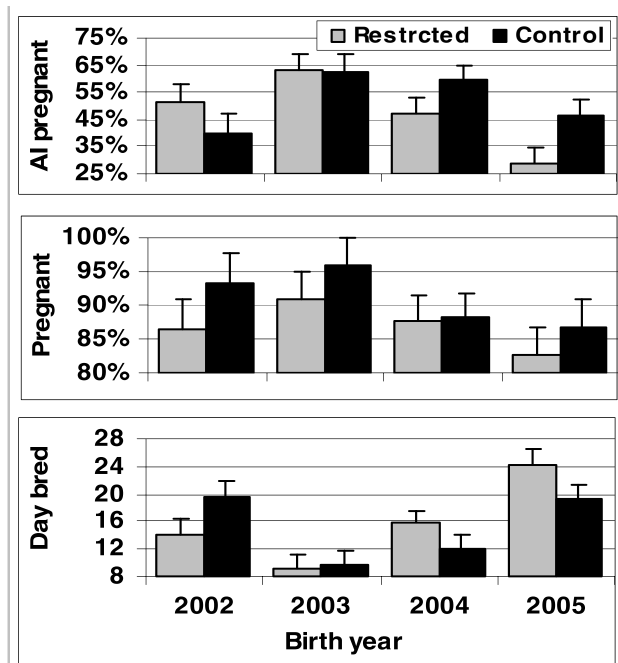

Figure 3. Reproductive performance of heifers from 4 different years (x-axis) that were provided ad libitum (Control) or Restricted access to feed during a 140-d trial during the postweaning period. Top panel: AI pregnancy rate (*P* = 0.12 for treatment x year). Middle panel: Overall pregnancy rate (P = 0.15 for treatment). Bottom panel: Day of breeding season when conception occurred (*P* = 0.06 for treatment x year). Restricted heifers conceived earlier (P < 0.001) than controls for 2002, and later for 2004 (*P* = 0.14) and 2005 born heifers (*P* = 0.1).

Proceedings, Western Section, American Society of American Science
Vol. 58, 2007

GROWTH AND ATTAINMENT OF PUBERTY IN HEIFERS FROM COWS SUPPLEMENTED WITH LINSEED MEAL DURING EARLY LACTATION

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ABSTRACT: Preliminary studies in our group suggested diets during early lactation for beef cows may influence puberty onset and offspring growth. This study examined the effects of supplementing cows with phytoestrogen rich linseed meal (LSM) during lactation on heifer calf growth and reproduction. Immediately after parturition, multiparous cow-calf pairs were allotted randomly to one of 12 pens, with six pens supplemented with LSM and six pens fed a control pellet consisting primarily of dried distillers grain plus solubles (CON). Both supplements were pelleted, isocaloric, and isonitrogenous and were offered prior to feeding alfalfa hay each day for the first 60 d of lactation. Heifer calves (n = 91) were followed from birth to 315 d of age. Birth wt, sixty d BW, and actual weaning weights were recorded, and adjusted weaning wt was calculated. On d 229.2 ± 1.5 of age and every 14 d until d 313.2 ± 1.5 of age, heifers were weighed, and venous blood samples were collected for progesterone analysis. Visual body condition score (BCS) was taken on ~d 229, 287, and 313. Birth weights were not different between groups and there was no effect of treatment on d 60 wt (P > 0.20; 39.5 ± 1.1 and 99.7 ± 2.2 kg, respectively). There was a tendency (P = 0.11) for heifers from LSM cows to have a greater adjusted weaning wt compared to heifers from CON cows (254.3 vs 243.0 ± 5.2 kg). Heifer BCS was not affected (P = 0.22) but heifers from LSM cows were heavier (P = 0.04) than heifers from CON cows (292.4 vs 286.1 ± 2.4 kg). Previous studies indicated that some heifers start to cycle at 7 mo of age, however in the current study only 27 heifers attained puberty by the completion of the trial (14 CON and 11 LSM; P = 0.40). Initial data regarding LSM supplementation during early lactation indicates that LSM does not appear to impact onset of puberty in heifer calves. However, it appears that LSM supplementation may enhance wt gain without influencing BCS of heifer calves. Further research is needed to explain the effects of phytoestrogens on growth and development of livestock.
Key words: phytoestrogen, linseed meal, cattle

Introduction

North Dakota is the national leader in flax production (USDA, NASS 2006). Linseed meal (LSM) is a byproduct of flax where the oil has been removed and is utilized in livestock diets. Tou and coworkers (1998) found that rat dams fed 10% flaxseed during gestation and lactation influenced reproductive parameters in female offspring such as decreased age to puberty and lengthened estrous cycle. Preliminary studies (Martin et al., 2006) have indicated that maternal nutrition may have an effect on the developing offspring in beef cattle. Maternal diets during lactation influenced heifer weaning wt (Martin et al., 2006).

We hypothesized that 10% LSM supplementation of the maternal diet during early lactation would influence calf growth and reproductive development.

Materials and Methods

Animal and Diets

The North Dakota State University Institutional Animal Care and Use Committee approved care and use of animals.

Upon parturition (d 0), cow-calf pairs were randomly assigned to one of two treatments: 1) 10% LSM pelleted supplement or 2) a control supplement, consisting primarily of dried distillers grain plus solubles (CON). Supplements were formulated to be isonitrogenous and isocaloric and alfalfa hay was fed ad libitium (Table 1). Pelleted supplements were offered (2.2 kg per hd/d) until d 60 of lactation. Animals were allotted into 1 of 12 pens, with six pens supplemented with LSM and six pens fed the CON pellet. Diets were formulated to provide nutrients for ~658 kg lactating mature cow as suggested by the National Research Council (NRC, 2000). Calves were weaned on d ~207 of age on average. Heifer calves (n = 91) were followed from birth to 315 d of age. At 229.2 ± 1.5 of age and every 14 d until d 313.2 ± 1.5 of age, heifers were weighed, and a blood sample was collected. Blood samples were immediately placed on ice and serum was stored at -20°C until assayed for progesterone (P_4). Visual body condition score (BCS; 1-9 scale; Wagner et al., 1988) was taken on ~d 229, 287, and 313. Heifers were managed similarly and fed as suggested by the National Research Council (NRC, 2000), throughout the course of the study.

Analysis of plasma and assays

Serum samples were analyzed for progesterone concentrations by competitive chemiluminescent immunoassay (Immulite 1000, Siemens, Los Angeles, CA). Heifers were considered to have attained puberty when P_4 concentrations serum levels where higher than 1.0 ng/ml. The intra- and inter-assay coefficients of variation were 4.8 and 8.9% respectively.

Statistical Analysis

Data were analyzed using the general linear model of SAS (V.9.1; SAS Inst. Inc., Cary, NC). Effects of lactational diet on birth wt, d 60 wt, weaning wt, adjusted weaning wt, final wt, BCS, and progesterone levels were analyzed.

Results and Discussion

Growth Performance

There were no differences in birth wt between treatment groups before the beginning of the trial. There was no effect of treatment on d 60 wt (*P* = 0.20). There was a tendency (*P* = 0.11) for heifers from LSM cows to have a greater adjusted weaning wt compared to heifers from CON cows (Table 3). Heifer BCS was not affected by treatment (*P* = 0.22) but heifers from LSM cows were heavier (*P* = 0.04) than heifers from CON cows (Table 3). This is in contrast to Tou et al. (1998), where females from rat dams consuming linseed meal were lighter when puberty was reached. Further Ward and coworkers (2001) reported no difference in ADG in rat females born form linseed meal fed and control animals.

Progesterone Assays and Puberty

Serum progesterone levels were not different between treatments (*P* = 0.85). Previous studies (Martin et al., 2006), demonstrated heifers may start to cycle at 7 mo of age, however in the current study only 27 heifers (30%) attained puberty by the completion of the trial (14 CON and 11 LSM; *P* = 0.40). Initial data regarding LSM supplementation during early lactation indicates that LSM does not appear to impact onset of puberty in heifer calves. These results agree with Ward et al. (2001), where 10% flaxseed or the purified lignan precursor were fed to rats during lactation and no significant difference in onset of puberty or length of estrous cycle were found in offspring. In contrast, Tou et al. (1998) reported that 10% supplemented flaxseed in rat dam diets fed during gestation and lactation decreased age to puberty, lengthened the estrous cycle, and resulted in persistent estrus in offspring. Moreover, Wright and coworkers (2002) reported octylphenol an estrogenic substrate, injected in pregnant ewes had ewe lambs with advanced puberty and first progesterone rise in compared with the control group.

Summary

In summary LSM supplemented at 10% in maternal diet during early lactation did not affect onset of puberty. Supplementation did have a tendency to increase adjusted weaning wt in heifer offspring, indicating a possible anabolic response of the LSM supplement. However further research is needed to determine effects of LSM on livestock during development.

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Table 1. Percent formula composition of supplements fed to beef cows.

Item	CON	LSM
	------------% DM------------	
Distillers Grain	65.7	-
Sunflower Meal	34.3	-
Corn Grain	-	20.2
Linseed Meal	-	79.8

[a]Supplements were formulated to be isocaloric and isonitrogenous.
[b]Supplement offered at 2.2 kg/hd/d.

Table 2. Percent formulation alfalfa hay, silage and supplements fed to beef cows.

Item	CON	LSM
	---------% As Fed---------	
Alfalfa Hay	13.8	13.8
Corn Silage	79.9	79.0
Corn Grain	-	1.30
Dry Distillers Grain	4.2	-
Linseed Meal	-	5.1
Sunflower Meal	2.2	-

[a]Supplements were formulated to be isocaloric and isonitrogenous.

Table 3. Effect of Supplements on Growth Performance

	Treatment			
Item	CON[a]	LSM[b]	SE	*P* value
Weight, kg				
Birth wt	39.1	40.7	1.38	0.40
d 60 wt	97.9	102.3	4.21	0.20
Wean wt	234.8	242.7	9.00	0.31
ADJ WW	242.5	253.8	7.20	0.11
Final wt	286.1	292.4	6.40	0.04
BCS (1-9)				
d 229	5.7	5.8	0.05	0.22
d 287	5.4	5.4	0.05	0.22
d 313	5.9	5.9	0.05	0.22

[a]Control (CON) n = 49

[b]Linseed Meal (LSM) n = 39

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

Concentrations of glucose, NEFA, thyroxine, and triiodothyronine in primiparous, anestrous, suckled beef cows exposed to bulls[1]

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ABSTRACT: The objective of this experiment was to determine if bulls affect metabolic factors in postpartum, anestrous, suckled beef cows. The null hypotheses tested in this experiment were that concentrations of glucose, NEFA, thyroxine (T4), triiodothyronine (T3), and T3:T4 ratios do not differ between cows exposed to bulls or steers. Primiparous, crossbred cows were 67 ± 3.5 d (± SE) postpartum at the start of the experiment. Cows were stratified by BW, BCS, calf BW, calving date, sex of calf and dystocia score. Cows were assigned randomly to be either exposed to bulls (EB; n = 8) or steers (ES; n = 8) 5 h daily for 9 d (D 0 to 8). On D 0, cows in each treatment were halter-restrained in individual adjacent stalls housed in similar but separate open-air sheds. Bulls (n = 2) and steers (n = 2) were contained in the immediate vicinity of cows, unrestrained and allowed free access to and contact with the frontal aspects of cows. Cows were fitted with indwelling jugular catheters 2 d before D 0. Blood samples were collected daily from each cow at 15-min intervals for 6 h from 1000 to 1600 h. The 5-h exposure period began 1 h after the start of the intensive bleeding period. Glucose was assayed at Times 15 and 225 min on D 2 and 8; and, T3 and T4 were assayed at Time 225 min only each day. NEFA were assayed at Times 15 and 300 min after the start of intensive bleeding period from D 0 to 8. Glucose concentrations did not differ between EB and ES cows on D 2 and 8 or Times 15 and 225 min, and averaged 81.0 ± 6.2 mg/dL. However, mean NEFA concentration was greater ($P < 0.05$) in ES cows (0.262 ± 0.024 mmol/L) than in EB cows (0.197 ± 0.020 mmol/L) over the course of the experiment. Concentrations of T4, T3, and T3:T4 ratios did not differ between EB and ES cows during the experiment. In conclusion, bulls did not appear to influence systemic glucose and thyroid hormones in primiparous, postpartum, suckled beef cows. However, it appears that adipose metabolism in postpartum beef cows may be affected by the presence of bulls.

Key words: bulls, NEFA, postpartum

INTRODUCTION

Resumption of luteal function in primiparous, anovular, suckled beef cows after calving is accelerated if cows are exposed to bulls (Custer et al., 1990) or excretory products of bulls (Berardinelli and Joshi, 2005). Olsen et al. (2006) found that cortisol concentrations increase in cows exposed to bulls. Cortisol is intimately involved with metabolic regulation of energy utilization (Hadley and Levine, 2007). There is the possibility that the biostimulatory effect of bulls to accelerate resumption of ovulatory activity involves metabolic changes in cows that are induced by changes in cortisol concentrations. The objective of this study was to determine if cows exposed to bulls alters systemic glucose, NEFA, and thyroid hormones concentrations. The specific hypothesis tested in this experiment were that systemic concentrations of glucose, NEFA, thyroxine (T4), triiodothyronine (T3), and T3:T4 ratios do not differ in postpartum, anestrous, suckled beef cows exposed daily for 5 h to bulls or steers for 9 d.

MATERIALS AND METHODS

This experiment was conducted at the Montana State University Livestock Teaching and Research Center, Bozeman. Animal care, handling, and protocols used in this experiment were approved by the Montana State University Institutional Large Animal Care and Use Committee.

Animals and Treatments

Sixteen spring-calving 2-yr-old Angus X Hereford primiparous, suckled beef cows, 2 mature Angus X Hereford bulls, and 2 yearling Angus X Hereford steers were used in this experiment. Cows and calves were maintained in a single pasture and had no contact with bulls or their excretory products during pregnancy and from calving until the start of the experiment.

Average calving date for these cows was Feb. 4, 2006. At the start of the experiment (D 0) cows averaged 67 ± 3.5 d postpartum. One week before the start of the experiment cows were stratified by body weight, BCS, calf birth weight, calving date, sex of calf and dystocia score and assigned randomly to two treatments: exposed to bulls (EB, n = 8) or steers (ES, n = 8) 5 h daily for 9 d (D 0 to 8).

[1] This study was supported by Award No. 2007-35203-17743, NRI Competitive Grants Program, CSREES, and the USDA, and the Montana Agric. Exp. Sta.. This is a contribution to Multistate Research Project, W1112, Reproductive Performance in Domestic Ruminants.

Facilities

Cows were housed within pens in separate lot areas at the Bozeman Livestock Teaching and Research Center. Pens within the north lot were used for maintaining EB cows while pens within the south lot were used for maintaining ES cows. During the daily sample collection and exposure period cows were moved into individual stalls within open-air sheds adjacent to pens that housed cows in each treatment. Sheds were similar in structure, area and light density. Light density within sheds was tested using a Minolta Autometer Pro Photometer and tarps were used to manipulate the light density so that cows in each sampling area were exposed to the same amount of light. During daily sample collections and exposure periods cows were halter-restrained within aligned (side-by-side) stalls, and bulls or steers were contained in front of cows. Bulls and steers were unrestrained and allowed to eat, roam, and come into contact with the frontal aspect of cows. Excretory products of bulls and steers were not removed from the exposure area throughout the experimental period.

Nutrition

Cows had free access to good quality, chopped mixed-grass alfalfa hay, and any pasture grasses that were available before the start of the experiment. Once cows and calves were moved into pens they were given free access to the same hay, 0.5 $kg \bullet hd^{-1} \bullet d^{-1}$ cracked barley, water, and a trace mineral-salt supplement. The TDN of the diet exceeded the NRC requirement for lactating beef cow with a mature weight of 545 kg by approximately 18% (NRC, 1996). Bulls were fed 0.5 kg of cracked barely and good quality, chopped mixed-grass alfalfa hay. Steers were fed a finishing ration that consisted of 70% concentrate and 30% roughage throughout the experiment.

Intensive Blood Sampling Procedures

Two days before the start of the experiment, each cow was fitted with an indwelling jugular catheter. Blood samples (~7 mL) were collected daily from each cow at 15-min intervals (starting at Time 0 and ending at 360 min) for 6 h (1000 to 1600 h) beginning 1 h before the 5-h exposure period each day during the 9 d period (D 0 to 8). Serum was harvested for each sample and stored at −20°C. Blood samples were collected by the same technician for cows in each treatment throughout the experiment. A total of 143 (3.9%) samples were not collected during this experiment due to non-cooperative animals, coagulation within catheters, or catheter replacement.

Metabolite and Hormone Assays

Glucose concentrations in serum for Times 15 and 225 min on D 2 and 8 were determined using a commercially available, hand-held glucose/ketone monitor (Precision Xtra™, MediSense/Abbott Laboratories, Bedford, MA). In order to compensate for assay drift, high and low serum pools were repeated 5 times every 20 samples. Mean high and low pool concentrations were 135.8 and 56.5 mg/dL with an inter-assay CV of 4.5 and 6.3%. Concentrations of NEFA at Times 15 and 300 min on all days were quantified with a commercially available enzymatic-colorimetric assay (HR Series NEFA – HR [2], Wako Diagnostics, Richmond, VA). Inter- and intra-assay CV were 5.8 and 4.6% for a serum pool that contained 0.415 mmol/L; and, 14 and 5.6%, respectively, for a serum pool that contained 0.100 mmol/L. Thyroxine (T4) and triiodothyronine (T3) concentrations at Time 225 min on each day were assayed using solid-phase RIA kits (Coat–A-Count; Diagnostic Products Corp., Los Angeles, CA). The intra-assay CV for serum pools that contained 155 and 105 ng/mL of T4 were 5.8 and 4.9%, respectively. The intra-assay CV for serum pools that contained 10.3 and 4.5 ng/mL of T3 were 11.2 and 24.5%, respectively.

Statistical Analyses

Glucose and NEFA concentrations were analyzed by separate ANOVA for a completely random design using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). The model included treatment, time, day, animal within treatment, and all possible interactions. Animal within treatment variance component was used to test the effect of treatment. Means were separated by Bonferroni multiple comparison tests. Thyroxine, T3, and T3:T4 ratio concentrations were analyzed by separate ANOVA for a completely random design using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). The model included treatment, day, animal within treatment, and all possible interactions. Animal within treatment variance component was used to test the effect of treatment. Means were separated by Bonferroni multiple comparison tests.

RESULTS

Stratification factors, days form calving to start of the experiment, cow BW at start of the experiment, calf BW, BCS of cows, calf sex ratio, and dystocia score did not differ ($P > 0.30$) between EB and ES cows (Table 1).

There was no interaction among or between treatments, days, and times for glucose concentrations. Glucose concentrations did not differ between Times 15 and 225 min, or D 2 and 8 or between EB and ES cows (Table 2). There was no interaction among or between treatments, days, and times for NEFA concentrations. Concentrations of NEFA did not differ between Times 15 and 300 min or among days (0 to 8). However, NEFA concentrations were greater ($P < 0.05$) in ES cows (0.262 ± 0.024 mmol/L) than in EB cows (0.197 ± 0.020 mmol/L) over the course of the experiment (Table 2). There was no interaction between treatments and days for concentrations of T4 and T3, or T3:T4 ratios. Concentrations of T4 and T3, and T3:T4 ratios did not differ among days or between EB and ES cows (Table 2).

DISCUSSION

In the present experiment we evaluated concentrations of glucose on D 2 and 8 at Times 15 and 225 min, NEFA concentrations every day at Times 15 and 300 min, and T4, T3 concentrations and T3:T4 ratios every day at Time 225 min. Blood samples were collected every 15 min for 6 h daily for 9 d. Concentrations of glucose, NEFA, T4, and T3 for a full collection period were evaluated in 1 or 2 cows and on 1 or 2 days chosen randomly. We found that the patterns of glucose, NEFA, T4, and T3 concentrations did exhibit pulsatile rhythms, in fact they exhibited a random pattern of change throughout the sampling period. Thus, we randomly chose D 2 and 8 and Times 15 and 225 min to evaluate changes in glucose concentrations: Times 15 and 300 min for all days for NEFA concentrations and Time 225 on all days for T4 and T3.

Exposing anestrous, suckled beef cows to bulls for 5 h daily over a period of 9 d did not influence glucose, T4, T3 concentrations and T3:T4 ratios. However, exposing anestrous cows to bulls decreased concentrations of NEFA compared to concentrations of NEFA in cows exposed to steers. This result of the present study for NEFA concentrations in suckled cows exposed to bulls is quite different from the result reported by Landaeta-Hernandez et al. (2004). They found that concentrations of NEFA were 33% higher in suckled cows exposed to bulls than in non-exposed suckled cows. The reason for this difference is not clear; however, the data reported by Landaeta-Hernandez et al. (2004) may have been confounded by the fact that more bull exposed cows had resumed ovulatory activity by the end of the experiment than non-exposed cows. This may have been due to an increase in cortisol concentrations that are associated with peri-ovulatory events of resumption of ovulatory activity in postpartum cows (Humphreys et al., 1983). A well-known catabolic affect of cortisol is the liberation of NEFA and amino acids from skeletal muscle and adipose tissue to be used as substrates for gluconeogenesis in the liver (for review see, Hadley and Levine, 2007). Whereas, in the present study all samples were collected in anestrous cows that had not resumed ovulatory activity and this difference may be related to NEFA concentrations in anestrous cows compared to those of cows during the peri-ovulatory period.

The primary objective of this experiment was to determine if exposing anestrous, suckled beef cows to bulls alters glucose, NEFA, and thyroid hormones that may directly or indirectly affect carbohydrate and lipid metabolism. This idea was based on the following observations that alterations in carbohydrate and lipid metabolism can either delay or accelerate the resumption of ovulatory activity in postpartum, anestrous, suckled beef cows (for reviews see, Randel, 1990; Short et al., 1990). Exposing postpartum cows to bulls increased systemic cortisol concentrations relative to cows not exposed to bulls (Olsen et al., 2006). Cortisol is a hormone known to influence carbohydrate and lipid metabolism (for review see, Hadley and Levine, 2007). Recently, Landaeta-Hernandez et al. (2004) reported that systemic concentrations of NEFA were greater in postpartum cows exposed to bulls than in cows not exposed to bulls. One could hypothesis that the biostimulatory effect of bulls to reduce the interval of postpartum may be mediated by inducing changes in cortisol concentrations which in turn may directly affect metabolic processes of postpartum, anestrous, suckled beef cows. This in turn could alter the hypothalamic release patterns of GnRH stimulating follicular development and ovulation. Thus, the affect of bull exposure on anestrous cows appears to be related to a decrease in catabolism of adipose tissue and may be involved in the mechanism whereby bulls accelerate the resumption of ovulatory activity.

IMPLICATIONS

Reproductive efficiency increases as the interval of postpartum anestrous decreases. Exposing postpartum, anestrous cows to bulls accelerates the resumption of ovulatory activity and may be used as a strategy to increase reproductive efficiency. The mechanism by which bulls decrease the postpartum, anestrous interval may be related to changes in metabolic regulation of adipose tissue. This is the first report wherein the presence of bull alters a metabolic processes related to adipose metabolism in postpartum, anestrous cows. Additional research is necessary to determine the mechanism of this effect.

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Table 1. Number of cows per treatment and least square means for days from calving to start of the experiment, cow BW at the start of treatment, calf birth weight (BW), BCS, calf sex ratio, and dystocia score for primiparous, suckled beef cows exposed to bulls (EB) or exposed to steers (ES)

	Treatment			
Variable	EB	ES	SEM[a]	*P* value
n	8	8		
Day postpartum[b]	67	67	3.5	0.94
Cow BW (kg)	526	531	36	0.76
Calf BW (kg)	31	32	3.5	0.61
BCS[c]	5.3	5.3	0.23	0.92
Calf sex ratio[d]	0.50	0.63	0.25[e]	0.62
Dystocia Score[f]	1.1	1.0	0.16	0.34

[a]SEM = Standard error of the mean.

[b] Days from calving to start of the experiment.

[c]BCS; 1 = emaciated, 9 = obese.

[d]Calf sex ratio = ratio of male to female calves, 1 = male and 0 = female. Tested with χ^2 analysis.

[e] χ^2 value.

[f]Dystocia Score: 0 = No assistance to 5 = Caesarean section.

Table 2. Least squared means of glucose, NEFA, thyroxine (T4), and triiobothyronine (T3) concentrations for primiparous, suckled beef cows exposed to bulls (EB) and steers (ES)

	Treatment			
Variable	EB	ES	SEM[a]	*P* value
n	8	8		
Glucose, mg/dL[b]	81.5	82.4	6.2	0.79
NEFA, mmol/L	0.197	0.262	0.099	0.06
T4, ng/mL[c]	17.7	14.0	7.9	0.15
T3, ng/mL[c]	9.5	8.8	3.5	0.41
T3:T4 ratio	0.68	0.79	0.36	0.25

[a]SEM = Pooled standard error of the mean.

[b]Glucose concentrations for samples obtained at Time 15 and 225 min on Days 2 and 8.

[c]T4 and T3 concentration for samples obtained at Time 225 min on each day of the experiment.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

Cortisol concentration patterns during acclimatization to facilities and protocols necessary for intensive blood sampling in primiparous, postpartum, suckled beef cows [1]

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ABSTRACT: The objective was to evaluate of cortisol concentration patterns in postpartum, anestrous, suckled beef cows during acclimatization to protocols necessary for intensive blood sampling. The null hypotheses were that characteristics of cortisol patterns do not differ throughout a 9 d intensive bleeding protocol. Body weight, BCS, dystocia score of cows, days postpartum, and calf BW were 532±37 kg, 5.3±0.23, 1.1±0.25, 66.8±3.5 d and 32±3.5 kg, respectively, at the start of the experiment. Before the start of the experiment, cows were maintained on pasture. Cows (n=8) were fitted with indwelling jugular catheters 2 d before the start of the experiment. Cows were halter-restrained in individual stalls for 6 h daily over 9 d (D0 through 8). Blood samples were collected from each cow at 15-min intervals from 1000 to 1600 h each day. Serum was assayed for cortisol using RIA. Characteristics of cortisol concentration patterns (mean, baseline, pulse frequency, amplitude, duration, and area under pulses) were analyzed by ANOVA. Cortisol concentrations were greater (P<0.05) on D0 (8.38 ng/mL) and D1 (4.41 ng/mL) than D2 through 8 (2.25 ng/mL). There was no change (P>0.05) in cortisol concentrations from D2 through 8. Baseline and area under pulses decreased (P<0.05) from D0 to D2 and did not differ (P>0.10) from D2 through 8. Pulse amplitude was higher (P<0.05) on D0 than D2 through 8. Furthermore, pulse amplitude was higher (P<0.05) on D1 than D8; however, pulse amplitude did not differ from D 2 through 8. Pulse duration was higher (P<0.05) on D0 and 1 than D2 through 8. Pulse frequency did not differ (P>0.10) from D0 to 8. In conclusion, it appears that acclimatization of postpartum, suckled beef cows to protocols necessary for intensive blood sample collection occurs within a 48-h period for cortisol concentrations, thereafter, cortisol patterns stabilize and are maintained.

Key words: cortisol, acclimatization, blood sampling

[1] This study was supported by Award No. 2007-35203-17743, NRI Competitive Grants Program, CSREES, and the USDA, and the Montana Agric. Exp. Sta.. This is a contribution to Multistate Research Project, W1112, Reproductive Performance in Domestic Ruminants.

INTRODUCTION

It is well understood that factors that change environments and activities can cause various physiological effects in cattle. Stress inducing factors in cattle such as changes in environmental conditions, management, and handling, as well as transportation and isolation can all be related to an increase in circulating cortisol concentrations, a stress hormone. Apple et al. 2005 reported that restrained and isolated calves exhibited an increase in cortisol concentrations. Furthermore, Negrão et al. 2004 reported that cortisol increases in response to milking in both the plasma and saliva of adult cows. Therefore, one physiological indication of a stress response appears to be an increase in mean circulating cortisol concentrations. However, the length of time needed for cows to adapt to intensive blood sampling procedures is not known.

The question we asked in these experiments was, "Does the intensive bleeding protocols used by our laboratory have an effect on circulating cortisol concentrations in postpartum anestrous beef cows?" The objective of this experiment were to determine whether exposure to personnel, facilities, and handling involved with our intensive bleeding protocols influences mean and temporal patterns of cortisol concentrations. We tested the null hypotheses that mean cortisol concentrations and characteristics of temporal patterns of cortisol concentrations patterns do not change over time in cows exposed to experimental facilities, handling, and laboratory personnel.

MATERIALS AND METHODS

Animals and Treatments

Eight spring-calving two-yr-old Angus X Hereford primiparous suckled beef cows were used in this experiment. Cows and calves were maintained in a single pasture during pregnancy and from calving until the start of the experiment. Average calving date for these cows was Feb. 4, 2006. At the start of the experiment (D 0) cows averaged 67 ± 3.5 d postpartum. One week before the start of the experiment, cows' body weight, BCS, dystocia score of cows, and calf BW were 534 ± 38 kg, 5.3 ± 0.23, 1.1 ± 0.25, and 32 ± 4.1 kg, respectively. Animal care, handling, and protocols used in this experiment were approved by the Montana State University Institutional Large Animal Care and Use Committee.

Facilities

Cows were housed within pens at the Bozeman Livestock Teaching and Research Center. During daily sample collection and exposure period cows were moved into individual stalls within open-air sheds adjacent to their pen and were halter-restrained within sequential, aligned (side-by-side) stalls.

Nutrition

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

Intensive Blood Sampling Procedures

Two days before the start of the experiment, each cow was fitted with an in-dwelling jugular catheter. Blood samples were collected daily from each cow at 15-min intervals (starting at time 0 and ending at time 360) for 6 h (1000 to 1600 h) each day during the 9 d period (D 0 to 8). At each interval catheters were cleared of saline and then blood was collected using Sarstedt 7 mL collection tubes. Catheters were then flushed using sterilized physiological saline solution (0.9%). Blood samples were refrigerated overnight, and then centrifuged at 4°C. Serum was harvested from each sample and stored at −20°C. Blood samples were collected by the same technician throughout the experiment. A total of 143 (3.9%) samples were not collected during this experiment due to non-cooperative animals, coagulation within catheters, or catheter replacement.

Cortisol Assays

Cortisol concentrations in serum were assayed in duplicates by solid-phase RIA kits (Diagnostic Products Corp., Los Angeles, CA) validated for bovine serum in our laboratory. Intra- and interassay CV were < 10% for pools of postpartum cow sera that contained 135 ng/mL and 25 ng/mL of cortisol, respectively.

Characteristics of Cortisol Patterns

Temporal cortisol concentration patterns exhibited a pulsatile rhythm in all animals throughout the experiment. Characteristics of temporal patterns of cortisol concentrations included baseline, pulse frequency, amplitude, duration, and area under pulse. Baseline was determined by estimating the overall pattern of cortisol release. This was done by visually estimating the lowest possible values on each side of a discernable pulse. A pulse was defined as any point greater than two SD's above the baseline. Pulse frequency was defined as the number of pulses per day. Pulse amplitude was the highest concentration observed within a pulse. Pulse duration was defined as the total time between baseline points on each side of a discernable pulse. Areas under pulses were measured by trapezoidal integration of cortisol concentration by time using EasyPlot® (Version 4.0.5, MIT, Spiral Software, USA).

Statistical Analyses

Cortisol concentrations were analyzed by ANOVA for a completely randomized design using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). The model included Day, Time, Animal within Day, and the Day by Time interaction. Animal within Day was the variance component was used to test the effect of Day. Means were separated by Bonferroni multiple comparison tests using the PDIFF procedure of SAS.

Characteristics of cortisol concentration patterns were analyzed by ANOVA for a completely randomized design using PROC GLM of SAS. The model included Day and means were separated by Bonferroni multiple comparison tests using the PDIFF procedure of SAS.

RESULTS

Cortisol concentrations were greater ($P < 0.05$) on D 0 (8.38 ng/mL) and D 1 (4.41 ng/mL) than D 2 through 8 (2.25 ng/mL). There was no change ($P > 0.05$) in cortisol concentrations from D 2 through 8.

Cortisol baseline, area under pulses was decreased ($P < 0.05$) from D 0 to D 2 and did not differ ($P > 0.10$) from D 2 through 8. Cortisol pulse amplitude was higher ($P < 0.05$) on D 0 than D 2 through 8. Cortisol pulse amplitude was also higher ($P < 0.05$) on D 1 than D 8, however did not differ from D 2 through 8. Average pulse duration was higher ($P < 0.05$) on D 0 and 1 than D 2 through 8. Cortisol pulse frequency did not differ ($P > 0.10$) from D 0 to 8.

DISCUSSION

Many stressors increase circulating cortisol concentrations, particularly in dairy cows. Milking and calf suckling may have an effect on both plasma and salivary concentrations of cortisol (Negrão et al., 2004). Cows exhibited a rise in cortisol concentrations within 30 min after the start of milking (Negrão et al., 2004). Furthermore, cortisol concentrations in postpartum beef cows rise within 15 min after the initiation of suckling (Dunlap et al., 1981). These data indicate that evacuation of milk from the udder results in an increase of circulating cortisol concentrations in lactating dairy and beef cows. Other stressors that influence cortisol concentrations are handling and transportation. Boandl et al. (1989) reported that injecting of anesthetic, and dehorning caused an increase in cortisol concentrations in Holstein calves.

Furthermore, cows that are transported to a new location for a period of 4 to 6 h have higher cortisol concentrations during transportation than cows that are not transported to new location during the same time period (Merrill et al., 2007).

The focus of this study was to determine the effects of intensive blood sampling protocols on cortisol concentrations performed in facilities that were unfamiliar to cows and while cows were separated from calves and not allowed to suckle. We found that mean cortisol concentrations fell from 8.38 ng/mL on D 0 to 2.25 ng/mL on D 2 through 8. In a study where lactating dairy cows were handled daily, housed in a controlled environment, and intensively bled by automated serial sampling cortisol concentrations ranging between 2 and 6 ng/mL (Lefcourt et al., 1993). Furthermore, Munksgaard and Simonsen (1996) collected blood samples via indwelling catheters at 30-min intervals, from 0800 to 1530 in Friesian dairy cows and found that mean cortisol concentrations ranged from 0.5 and 4 ng/mL. In our study mean cortisol concentrations of cows from D 2 to 8 are comparable to that of cows which are familiar to stanchions and human handling such as those used by Munksgaard and Simonsen (1996). These data indicate that cortisol concentrations fell from high concentrations to normal levels after the first 2 days of the present study after cows had become familiar to the blood sampling procedure, personnel, and handling.

In the present study, characteristics of cortisol concentration patterns (mean, baseline, pulse amplitude, area under pulses, and pulse duration) differed during the first 2 days; whereas, characteristics of cortisol did not differ among D 2 trough 8. This may have been due to a decrease in stressors associated with handling and intensive blood sampling protocols. Cows that are subjected to high-anxiety situations, i.e. restrained in chutes, have higher cortisol concentrations than cows in low-anxiety situations (not restrained); (Bristow and Holmes, 2006). Furthermore, bulls that have higher cortisol concentrations are found to have a significant positive correlation between cortisol concentrations and exit velocity from chutes (Curley et al., 2006). Together these data indicate that anxiety is linked to high cortisol concentrations in bovine. However, decreasing anxiety by acclimating cows to facilities and procedures may decrease cortisol response to stressors. Cows that are acclimated to stanchions before blood sampling protocols have lower cortisol concentrations than cows not acclimated to stanchions (Echternkamp, 1984). The results of the present study suggest that anxiety of cows decreased and cows became acclimated to a 6-h intensive blood sampling protocol within a 48 h after initiation of the protocol.

Cortisol pulse frequency was not affected by the intensive blood sampling protocol used in this experiment. Wagoner and Oxenreider (1973) reported that cortisol concentrations exhibited a diurnal pattern with a nadir (5.3 ng/mL) occurring between 1800 and 0200 hrs and a zenith (7.4 ng/mL) occurring between 0200 and 1800 hrs in primiparous Holstein cows catheterized 2 d before sampling. However, it appeared that cortisol fluxuates in a pulsatile rhythm throughout the day. If one examines the data of Wagner and Oxenreider (1973) between 1000 to 1600 h pulses occurred at a frequency of 0.75 pulses/h, which is slightly different than the mean pulse frequency in our study (0.86 pulses/hr) within that time frame. This difference could be because cows used in the present study were housed in open-air sheds while dairy cows used by Wagoner and Oxenreider (1973) were housed in milking stanchions. Furthermore, Lefcourt et al. (1993) reported that cows housed within controlled environmental chambers exhibit cortisol pulse cycle of 120 min. Therefore, the differences in cortisol pulse frequency may have been because the cows used in the present study were subjected to changes in environmental conditions.

In conclusion, mean cortisol concentrations were higher during the first 2 days of the intensive blood sampling period than the remainder of the experiment. Furthermore, characteristics of cortisol concentration patterns were differed at the start of the experiment but stabilized within 48 h and were maintained thereafter. Therefore, it appears that postpartum, suckled beef cows acclimatize to intensive blood sampling protocols and procedures within 48 h after the start of a 9-d intensive sampling period and should be considered when evaluating stress and factors associated with stress in postpartum beef cows.

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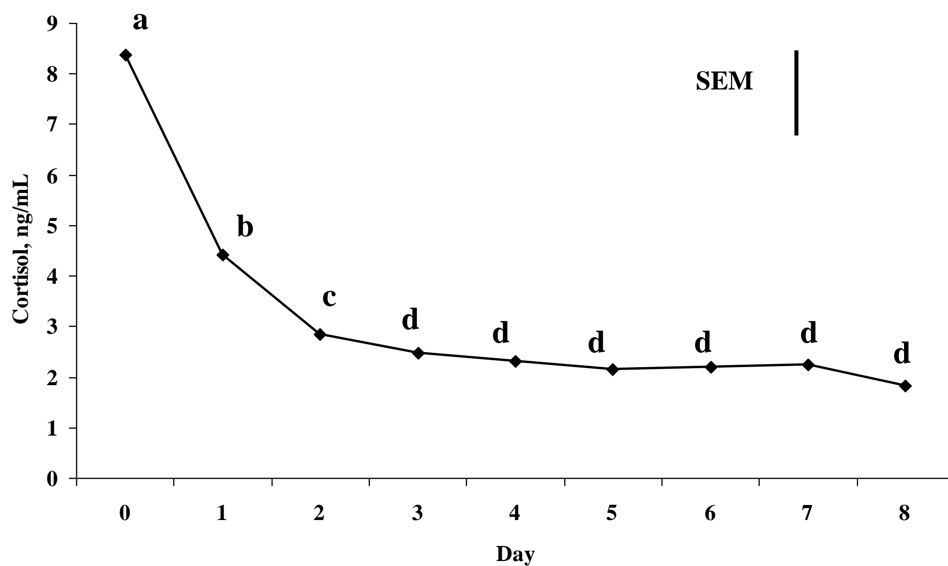

Figure 1. Least squared means of cortisol concentrations for cows (n = 8) by day of experiment. The vertical bar represents the pooled standard error of the mean (SEM). Points that lack a common letter differ ($P < 0.05$).

Table 1. Least squared means for baseline, pulse amplitude, area under pulses, pulse duration, and pulse frequency for temporal cortisol release patterns of cows during an intensive bleeding protocol over a 9-d period.

	Day										
Variable	0	1	2	3	4	5	6	7	8	SEM[a]	*P* value
Baseline[b]	1.91	0.54	0.26	0.06	0.28	0.07	0.18	0.31	0.05	0.56	< 0.05
Pulse amplitude[b]	15.37	10.68	5.57	4.13	5.33	5.11	5.90	5.63	3.54	4.14	< 0.05
Area under pulses[c]	502.1	319.1	175.1	114.7	153.6	157.9	170.5	164.0	122.0	152.6	< 0.05
Pulse duration[d]	78.5	79.5	64.9	52.0	63.9	65.5	59.6	60.4	60.5	16.1	< 0.05
Pulse frequency[e]	0.73	0.70	0.85	0.97	0.90	0.83	0.97	0.88	0.93	0.22	0.17

[a] SEM = Standard error of the mean.

[b] Baseline and pulse amplitude of cortisol in ng/mL

[c] Area under pulses expressed in cortisol concentration (ng/mL) by duration of pulses (min).

[d] Pulse duration of cortisol concentrations in minutes

[e] Pulse frequency measured in cortisol pulses/hr

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

Comparison of using 7- or 14-d CIDR treatments in an estrous synchronization protocol that included PGF2α, timed AI and GnRH in primiparous, suckled beef cows.[1]

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ABSTRACT: The objective was to compare the estrous synchronization response and AI pregnancy rates of primiparous, suckled beef cows using protocols that included controlled internal drug release devices (CIDR) for either 7 or 14 d, $PGF_{2\alpha}$ (PG), timed AI (TAI) and GnRH. We tested the hypotheses that the estrous synchronization response after PG injection and AI pregnancy rates do not differ between cows synchronized using a CIDR for either 7- or 14-d. Cows were stratified by calving date, calf BW, sex of calf, BW, BCS, and presence of a corpus luteum. Cows were then assigned randomly to receive a CIDR for 7 (CIDR7; n=25) or 14 d (CIDR14; n=25). Each CIDR14 cow received a CIDR on D -31 (D0 = d of PG injection); CIDR were removed 14-d later (D -17) from these cows. Cows received the CIDR14 treatment 74 d (SE; ± 18 d) after calving. Each CIDR7 cow received a CIDR on D -7, 25 d after the CIDR14 cows received a CIDR. These cows were given a CIDR 99 d (SE; ± 18 d) after calving. CIDR were removed from CIDR7 cows on D 0 and each CIDR14 and CIDR7 cow was injected intramuscularly with PG. Cows were observed for estrus during the next 60 h from 0600 to 2400 h daily. Cows that exhibited estrus within 60 h after PG were bred by AI 12 h later. Cows that did not exhibit estrus by 60 h were TAI at 72 h after PG and given GnRH (100 ug/cow). The proportion of cows that exhibited estrus after PG was greater (P<0.05) and the interval from PG to estrus was shorter (P<0.05) for CIDR7 cows than for CIDR14 cows. More (P<0.05) CIDR7 cows (60%) were bred by AI 12 h after PG than CIDR14 cows (20%), whereas, more (P<0.05) CIDR14 cows were bred TAI at 72 h after PG than CIDR7 cows. Overall AI pregnancy rates did not differ between CIDR7 (80%) and CIDR14 (72%) cows. These results indicate that using a CIDR for 7 d with PG given upon of removal of CIDR yields a superior estrous synchronization response compared to that of using a CIDR for 14 d followed 17-d after PG. However, both CIDR protocols yield similar and acceptable AI pregnancy rates when combined with TAI and GnRH in primiparous beef cows.

Key words: CIDR, estrous synchronization, cows

[1]This study was supported by the Montana Agric. Exp. Sta. and is a contributing project to Multistate Research Project, W1112, Reproductive Performance in Domestic Ruminants.

Introduction

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

Estrous synchronization protocols that include progestins are effective in synchronizing estrus and improving AI pregnancy rates in primiparous beef cattle (Lucy et al., 2001). One of the most common progestin-based protocols that can be used to synchronize estrus in postpartum cows includes the use of a controlled internal drug release device (CIDR) followed by $PGF_{2\alpha}$ 7 d later, before or at the time of CIDR removal (Larson et al., 2006). An estrous synchronization protocol that includes CIDR for 14 d followed by $PGF_{2\alpha}$ 17 d later can successfully synchronize estrus and yield acceptable AI pregnancy rates in beef heifers (preliminary evidence from our laboratory). The question we asked in this study is "Can one expect an acceptable estrous synchronization response and AI pregnancy rates in an estrous synchronization protocol that includes a CIDR for 14 d followed by $PGF_{2\alpha}$ 17 d later in primiparous, suckled beef cows?"

The objective was to compare the estrous synchronization response and AI pregnancy rates of primiparous, suckled beef cows using protocols that included CIDR for either 7 or 14 d, $PGF_{2\alpha}$, timed AI (TAI) and GnRH. We tested the hypotheses that the estrous synchronization response after $PGF_{2\alpha}$ injection and AI pregnancy rates do not differ between cows synchronized with a CIDR for either 7- or 14-d.

Materials and Methods

Animals and Treatments

Cows were housed at the Montana State University Livestock Teaching and Research Center, Bozeman. Animal care, handling, and protocols used in these experiments were approved by the Montana State University Institutional Large Animal Care and Use Committee.

Cows were maintained in a single pasture for the duration of this experiment. Before the start of treatment

cows were stratified by calving date, calf BW, cow BW, cow BCS, and dystocia score. Cows were assigned randomly to one of two estrous synchronization protocols that included either CIDR for 14 d (CIDR14) or 7 d (CIDR7). At the start of the experiment, 31 d before the breeding season (D 0), cows were 74 d ± 18 d postpartum. Average calving date was February 14.

Nutrition

Cows had free access to good quality, chopped mixed-grass alfalfa hay, and any pasture grasses that were available during the experiment. Cows and calves were supplemented 0.5 kg•hd $^{-1}$•d $^{-1}$ cracked barley, water, and a trace mineral-salt supplement. The TDN of the diet exceeded the NRC requirement for lactating beef cows with a mature weight of 545 kg by approximately 18% (NRC, 1996).

Estrus Synchronization, AI, and Pregnancy Diagnosis

Thirty-one d (D -31) before the start of the breeding season (D 0) CIDR14 cows were given exogenous progesterone via a CIDR, 14 d later (D -17) CIDR were removed. CIDR7 cows received CIDR on D -7 before the start of the breeding season (D 0). Seven d later CIDR were removed from CIDR7 cows and CIDR14 and CIDR7 were given $PGF_{2\alpha}$ (D 0; 25 mg/cow; im). Cows that showed estrus within 60 h after $PGF_{2\alpha}$ were AI 12 h later. Cows that did not show estrus within 60 h were given GnRH (100 ug/cow; im) and were fixed-time AI (TAI) 72 h after $PGF_{2\alpha}$ (D 3).

Cows that did not exhibit estrus by 60 h after $PGF_{2\alpha}$ were assigned an interval to estrus of 72 h. Assigning an interval of 72 h for cows that did not exhibit estrus within 60 h after $PGF_{2\alpha}$ allows for a less biased estimation of the mean interval to estrus because heifers that did not exhibit estrus would not be included in the statistical analysis of the interval. If many cows from a given treatment are not included, the estimation of the mean would be biased to reflect an artificially low estimation for that treatment mean compared to a treatment in which only a few cows are not included. Assuming a fixed time for the maximum interval to estrus after $PGF_{2\alpha}$ accounts for and removes this innate bias. Pregnancy rates to AI were determined by transrectal ultrasonography of the uterine contents of each cow 35 d after TAI (D 38).

Statistical Analyses

Interval from $PGF_{2\alpha}$ to estrus was analyzed by ANOVA for a completely randomized design using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). The model included treatment and means were separated by the PDIFF procedure of SAS. Proportions of cows that showed estrus by 60 h after $PGF_{2\alpha}$ and AI pregnancy rates were analyzed by chi-square analyses using the PROC FREQ procedure of SAS.

Results

Interval to estrus after $PGF_{2\alpha}$ was shorter ($P < 0.05$) for CIDR7 cows than for CIDR14 cows, 68 and 54 h (SEM = 14.4 h), respectively. More ($P < 0.05$) CIDR7 cows (60%) exhibited estrus by 60 h after $PGF_{2\alpha}$ than CIDR14 cows (20%; Figure 1).

More ($P < 0.05$) CIDR7 cows were inseminated 12 h after estrus than CIDR14 cows (Table 1). AI pregnancy rates for cows inseminated 12 h after estrus, TAI pregnancy rates, and overall AI pregnancy rates did not differ ($P < 0.05$) between CIDR14 and CIDR7 cows (Table 1).

Discussion

The practical use of estrous synchronization protocols depends on ease of implementation. For this reason, many researchers strive to identify protocols that lower the number of times cows are handled and eliminate the necessity for detecting estrus, and at the same time yield acceptable AI pregnancy rates. One estrous synchronization protocol that uses a CIDR for seven d and $PGF_{2\alpha}$ given at CIDR removal usually yields an acceptable estrous synchronization response and AI pregnancy rates (Lucy et al., 2001). However eliminating detection of estrus through the use of TAI may result in less than desirable AI pregnancy rates (Larson et al., 2006). Preliminary evidence from our laboratory has indicated that the use of an estrous synchronization protocol that includes CIDR for 14 d followed by $PGF_{2\alpha}$ 17 d later produces an a highly synchronized estrous response and acceptable AI pregnancy rates in beef heifers.

The first objective of this study was to determine the estrous synchronization response of cows using a protocol that included a CIDR for 14 d followed by $PGF_{2\alpha}$ 17 d after CIDR removal. We found that fewer cows that were on the CIDR14 estrous synchronization protocol exhibited estrus by 60 h after $PGF_{2\alpha}$ than cows given the CIDR7 protocol. These results indicate either one of two possibilities CIDR14 cows did not respond to this estrous synchronization protocol or treating cows with the CIDR14 protocol resulted in a delay in the estrous synchization response of greater than the 60-h detection interval. We believe it is highly unlikely that CIDR14 cows did not respond to this protocol because overall AI pregnancy rates did not differ between CIDR14 and CIDR7 cows. One would expect that if CIDR14 cows did not respond to this estrous synchronization protocol then AI pregnancy rate would have been drastically lower. Thus, the detection interval used in the present study may not have been long enough to accurately measure estrous synchronization response in CIDR14 cows. The physiological basis for this observation is not clear.

AI pregnancy rates for cows synchronized using either CIDR protocol fixed-time AI and GnRH in this study were higher than what has been reported previously. Generally, AI pregnancy rates for cows inseminated using fixed-time AI are lower than AI pregnancy rates for cows

inseminated 12 h after estrus (Lucy et al., 2001; Larson et al., 2006). We expected to observe lower AI pregnancy rates for CIDR14 cows than CIDR7 cows since a greater proportion of CIDR14 cows were inseminated by TAI. However, AI pregnancy rates for cows inseminated 12 h after estrus, TAI pregnancy rates, and overall AI pregnancy rates were similar between treatments.

In conclusion using a CIDR for 7 d with $PGF_{2\alpha}$ given at the time of CIDR removal yields a superior estrous synchronization response compared to that of using a CIDR for 14 d followed by $PGF_{2\alpha}$ 17-d later. However, both CIDR protocols yield similar and acceptable AI pregnancy rates when combined with TAI and GnRH in primiparous, suckled beef cows. Further study is needed to determine if these results are reliable and repeatable due to the small number of animals in each protocol. Nevertheless, these data indicate that the requirement for estrous detection may be eliminated using an estrous synchronization protocol that includes CIDR for 14 d followed by $PGF_{2\alpha}$ 17-d later, fixed-time AI and GnRH in primiparous, suckled beef cows.

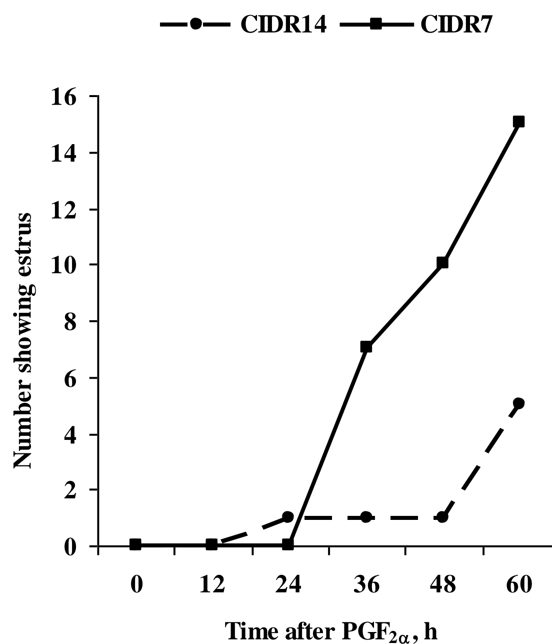

Figure 1. Cumulative distribution of number of cows exhibiting estrus at 12-h intervals after $PGF_{2\alpha}$. Proportions of cows that had exhibited estrus by 60 h differed (P < 0.05).

Table 1. Proportions of cows inseminated 12 h after estrus and pregnancy rates for cows bred 12 h after estrus, timed AI at 72 h (TAI) after $PGF_{2\alpha}$, and overall AI pregnancy rates using estrus synchronization protocols that included 14 d (CIDR14) or 7 d (CIDR7) of CIDR [1]

	Treatment			
Variable	CIDR14	CIDR7	X^2	*P* value
n	25	25		
% AI 12 h after estrus	20.0	60.0	8.3	<0.05
AI pregnancy rates 12 h after estrus	60.0	86.7	1.7	0.20
TAI pregnancy rates	75.0	70.0	0.1	0.77
Overall AI pregnancy rates	72.0	80.0	0.4	0.51

[1]Pregnancy rates determined by ultrasonography 35 d after TAI.

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Proceedings, Western Section, American Society of Animal Science
Vol. 58, 2007

Effects of fetal and uterine genotype on placentome morphology in sheep

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Abstract

A recent study evaluated uterine capacity in a prolific sheep breed [Romanov (R); litter size=3 to 5] compared to a common U.S. breed [Columbia (C); litter size=1 to 3] by transferring R or C embryos into either a R or a C ewe, resulting in R embryos in R recipients (RR, n = 9), R embryos in C recipients (RC, n = 4), C embryos in R recipients (CR, n = 7), and C embryos in C recipients (CC, n = 8). On d 130, individual placentome (PLAC) weight, diam., morphological types, and total number of PLAC were recorded. There was no difference among groups in the total number (70.3 ± 2.5) of PLAC. However, for the C breed, both fetuses and uteri had a greater ($P < 0.01$) percentage of type A compared to type B and C PLAC, whereas fetuses and uteri of the R breed had a greater percentage ($P < 0.01$) of type A PLAC than type B, C, or D PLAC. Further, compared with C, the R breed had an increased percentage ($P < 0.01$) of type A PLAC (45.5 vs. 74.3 ± 6.4%). Regardless of uterine or fetal breed, type D PLAC were heavier ($P < 0.01$) than all other types, type C were heavier than type A, and type B were similar in weight to types A and C. Although C fetuses had heavier ($P = 0.05$) individual PLAC compared to R fetuses (10.1 vs 7.7 ± 0.9 g), R uteri PLAC had heavier individual PLAC than the C uteri (10.4 vs. 7.3 ± 1.1 g). For C fetuses or uteri, there was no difference among PLAC types as a percentage of total PLAC weight. However, for both R fetuses and uteri, type A PLAC made up the majority [~70% of total PLAC weight ($P < 0.03$)]. PLAC diam. was larger ($P = 0.05$) in RC vs. CC, with RR and CR being intermediate [3.5 vs. 2.6, and 3.0 and 2.9 cm (SE = 0.3) for RC vs. CC, and RR and CR, respectively]. Types A and B PLAC were smaller ($P < 0.01$) than type C and D PLAC from C fetuses, whereas in R fetuses type A PLAC were smaller ($P = 0.04$) than types B, C, or D, which were similar. The overall reduction in fetal and placental weight in R recipients may be due to a greater proportion of smaller, type A PLAC comprising the majority of PLAC in the R uterus during pregnancy.

KEYWORDS: placentome, pregnancy, ewes

Introduction

The placentome is the site of nutrient, oxygen, and waste exchange between the dam and fetus; and is essential for fetal development during pregnancy. The placentome consists of interdigitated fetal and maternal villi formed from the growth of trophoblasts and caruncular endometrium (Stegemen, 1974; Reynolds et al., 2005; Ward et al., 2005). The numbers of placentomes range from 70 to 120 in the ovine species (Senger, 2003).

Vatnick et al. (1991) has classified the placentome into 4 morphological types. The first of these types is the type A placentome, which is concave in shape and consists of maternal tissue surrounding a small portion of fetal tissue. Types B and C placentomes are intermediate in shape, with type B consisting of fetal tissue that has begun to grow over the surrounding maternal tissue and type C consisting of equal portions of maternal and fetal tissue. The type D placentome is convex in shape and consists of fetal tissue surrounding a small portion of maternal tissue.

To determine how placental size may impact fetal development, Vatnick et al. (1991) demonstrated that increased placental mass, resulting in a shift to more advanced placentome types (from A to D), rescued the growth-restricted fetuses in a twinning model. Further evidence that placentome type may assist in fetal growth was reported by Vonnahme et al. (2006). When 2 groups of ewes were subjected to a 50% nutrient restriction during pregnancy, fetal weight of those ewes whose placentomes converted to more advanced types was not affected compared to the ewes whose placentomes did not change in morphology, in which fetal weight was reduced by ~30%.

In a reciprocal, embryo transfer study, in which Romanov (a prolific breed) and Columbia (a standard U.S. breed) ewes were utilized, Borowicz et al. (2006) reported that regardless of fetal breed, the Romanov uterine environment had reduced fetal and total placental weight. Romanov ewes typically have increased litter sizes compared to Columbia ewes; however, the exact mechanism behind their prolificacy is not known.

The objective of this study was to determine if fetal or ewe breed impacts placentome morphology during late pregnancy.

Materials and Methods

Animal Procedures

This study was approved by the North Dakota State University Institutional Animal Care and Use Committee. Donor ewes and recipients (n = 38) were synchronized with progestagen sponges (InterVet, Dublin, Ireland) for 14 d. Superovulation protocols were similar to methods previously used in our laboratory (Stenbak et al., 2001). After removal of the implants, donor ewes were detected for signs of estrus using a vascectomized ram. At first detection of estrus (d 0), the donors were bred to an intact ram. On d 2 after estrus, laparotomy was performed and embryos were collected by flushing the oviduct of the donor ewe. The oviduct was flushed from the uterine end towards the infundibulum into a Petri dish. Embryos were located using a stereoscope, and one

embryo was transferred into each recipient ewe into an oviduct ipsilateral to a corpus luteum. Embryos were transferred from Romanov and Columbia donors into Romanov and Columbia recipients, thereby creating 4 treatment groups: a Romanov embryo in a Romanov uterus (**RR**), a Romanov embryo in a Columbia uterus (**RC**), a Columbia embryo in a Romanov uterus (**CR**), and a Columbia embryo in a Columbia uterus (**CC**).

On d 130 of gestation, the recipient ewes were stunned via captive bolt and exsanguinated. The gravid uterus was obtained, and the fetus was removed and weighed. Individual placentomes were removed from the gravid uterus, counted, and placentome type was determined using the Vatnick et al. (1991) classification system. Further, individual placentome weights and diameters were recorded for all placentomes. Placentome diameters were measured by placing the placentomes on a flat surface and determining the length and width, both in cm.

Placentome Calculations

Calculations made included total and average placentome weight and average placentome diameter. Further, within each placentome type, total and average weight and average diameter were calculated. To determine how much each type was contributing to the total placentome weight, the percentage of total placentome weight for each type was also calculated.

Statistical Analysis

Data was analyzed using PROC GLM (SAS Inst. Inc., Cary, NC). The dependent variables included total placentome number, the percentage of each specific placentome type of the total placentome number, average placentome weight, average placentome diameter, total placentome weight, and the percentage weight of specific placentome type of the total placentome weight. The effects of ewe breed, fetal breed, and placentome type, and all interactions were evaluated. If there were no significant interactions, main effect means are reported. Means ± SE were considered to be significantly different if $P < 0.05$.

Results

Reciprocal transfer between Romanov and Columbia breeds resulted in no effect ($P = 0.06$) on the total number (70.3 ± 2.5) of placentomes. Columbia fetuses and uteri had greater ($P < 0.01$) percentages of type A placentomes compared to type C placentomes (Table 1). Romanov fetuses and uteri had greater ($P < 0.01$) percentages of type A placentomes compared to types B, C, and D placentomes, and the Romanov breed also had a greater percentage of type A placentomes than the Columbia breed. Overall, the average weight of type D placentomes was heavier ($P < 0.01$) than all other types [5.8, 6.6, 8.5 vs. 14.7 g (SE = 0.4) for types A, B, C, and D, respectively], with type C being heavier than type A placentomes, which were similar in weight to type B placentomes. Although Columbia fetuses had heavier ($P = 0.05$) individual placentomes compared to Romanov fetuses (10.1 vs. 7.7 ± 0.9 g), Romanov uteri had heavier ($P = 0.03$) individual placentomes than Columbia uteri (10.4 vs. 7.3 ± 1.1 g). The total weight of type A placentomes in Romanovs was greater ($P < 0.07$) than all other types within the Romanov or the Columbia breeds (Table 1). No difference was observed among types as a percentage of the total weight of placentomes for Columbia uteri and fetuses. However, for Romanov uteri and fetuses, type A placentomes made up the majority of the placental weight (~ 70%, $P < 0.03$, Table 1). Placentomes from Columbia fetuses had smaller ($P < 0.01$) types A and B placentomes compared to types C and D placentomes (Table 1). However, placentomes from Romanov fetuses had smaller type A placentomes compared to types B and D placentomes, with types B, C, and D placentomes being similar in size (Table 1).

Reciprocal transfer between Romanov and Columbia fetal and uterine breeds resulted in larger ($P = 0.05$) placentome diameters in the RC compared to the CC group, with both RR and CR groups being intermediate [3.5 vs. 2.6, and 3.0 and 2.9 cm (SE = 0.3) for RC vs. CC, and RR and CR, respectively].

Discussion

To our knowledge, this is the first attempt to determine fetal and uterine impacts on placentome type. Our study demonstrates that breed affects placentome weight and morphology. Romanov fetuses and uteri had an increase proportion of type A placentomes compared to Columbia fetuses and uteri. Similar findings are reported by Arndt et al. (2006) where, the placenta consisted of more type A placentomes compared to all other types. This data is also similar to findings reported by Clarke et al (1998) in pregnant ewes that were nutrient restricted. Thus, we can conclude that type A placentomes comprised the majority of the placenta within the fetus regardless of fetal or uterine breed.

In this study there was no effect of breed on placentome numbers. This data may imply that breed does not affect the number placentomes. In agreement with our study, Grazul-Bilska et al. (2006) has shown similar number of placentomes in single pregnancies in mixed breeds. In this study Romanov ewes had only one fetus, even though the Romanov breed is known to be prolific; had multiple fetuses been transferred to donor ewes the results may have been similar to data reported by Grazul-Bilska et al. (2006) for multiple pregnancies. Breed of the uterine environment may not affect placentome numbers; however fetus number may play a role in placentome numbers within the placenta. However, these data indicate that breed does not affect the number of placentomes.

Type A placentomes are known to be smaller than type D placentomes, which were absent from the Romanov breed in this study. Columbia fetuses had heavier placentomes compared to Romanov fetuses; however Romanov uteri had heavier placentomes compared to Columbia uteri. In a recent study Arndt et al. (2006) reported in white faced ewes, that type A placentomes are smaller than all other types. This indicates that more prolific uteri consist of heavier placentomes; and that uterine breed may affect the weight of the placentome types. However, placentome type does not seem to be important in Romanov fetal growth, as regardless of uterine type, there was no difference in weight. It appears that when only one embryo is

transferred into the Romanov, or if one Romanov embryo is transferred to a Columbia uterine environment, there is no reason to transform into a more advanced placentome type, as uterine competition is minimal. Moreover, the proportion of the total placentome weight from the weight of type A placentomes in Romanov ewes was greater than in the Columbia. Uterine and fetal factors driving fetal weight appear to differ between these two breed types. There was no difference in placentome diameters between the Columbia and Romanov breeds based on uterine environment. Columbia and Romanov uterine breed and fetal breeds did not affect placentome diameters. However, placentome type affected diameters; with type D placentomes being larger than type A placentomes. These findings are similar to those reported by Arndt et al. (2006), which showed type D placentomes being larger than type A placentomes.

In summary, ewe breed did not affect the number of placentomes. However, placentome weight was affected by both fetal and uterine breeds. Both maternal and fetal environments affect the morphology of the placentomes within the uterus.

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Table 1. Effects of fetal breed and uterine breed on placentome morphology and size

Item	Columbia				Romanov							
	Placentome type				Placentome type					*P*-values		
	Type A	Type B	Type C	Type D	Type A	Type B	Type C	Type D	Pooled SE	Breed	Type	Breed x Type
Fetal breed												
Percentage	45.5^{a}	24.7^{b}	22.3^{b}	26.4^{ab}	74.3^{c}	19.1^{b}	12.41^{b}	8.3^{b}	2.5	0.87	< 0.01	0.02
Weight												
Total, g	150.1^{a}	108.4^{a}	143.3^{a}	207.0	260.1^{b}	105.7^{a}	46.5^{a}	35.7^{a}	13.4	0.32	0.01	0.03
% of total	38.2^{a}	24.4^{a}	25.6^{a}	37.0^{ab}	70.6^{b}	23.3^{a}	12.4^{a}	10.0	2.7	0.78	0.01	0.01
Avg diameter, cm	2.2^{a}	2.3^{a}	3.3^{b}	4.3^{b}	2.4^{a}	3.0^{b}	2.7^{ab}	3.6^{b}	0.1	0.69	< 0.01	0.05
Uterine breed												
Percentage	42.2^{a}	26.3^{ab}	21.8^{b}	22.5^{abc}	77.5^{c}	17.5^{b}	12.8^{b}	12.2^{ab}	2.5	0.84	< 0.01	< 0.01
Weight												
Total, g	152.7^{a}	142.3^{a}	116.2^{a}	136.4^{a}	257.5^{b}	71.8^{a}	73.6^{a}	106.2^{ab}	13.4	0.84	0.01	0.07
% of total	35.9^{a}	27.2^{a}	22.2^{a}	27.5^{a}	73.0^{b}	20.5^{a}	15.8^{a}	19.5^{ab}	2.7	0.68	0.01	< 0.01
Avg diameter, cm	2.2	2.4	3.1	3.1	2.4	2.9	3.0	4.8	0.1	0.07	<0.01	0.34

[a,b,c,d] Means ± pooled SE within a row and breed differ ($P < 0.05$).

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECTS OF MATERNAL DIETARY RESTRICTION AND SELENIUM INTAKE ON PLACENTOME DEVELOPMENT AND PROLIFERATION IN THE EWE[1]

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ABSTRACT: To examine the effects of maternal nutrient restriction and dietary selenium (Se) on placentome development and cellular proliferation, pregnant Targhee-cross ewe lambs (n = 36; 53.8 ± 1.3 kg BW) were randomly allotted to 1 of 4 treatments in a 2 x 2 factorial arrangement. Factors were nutrition [control (CON) fed at requirements vs. restricted (RES) fed at 60% requirements] and dietary Se [adequate Se (ASe), 7.4 µg/kg BW vs. high Se (HSe) from Se-enriched yeast, 81.5 µg/kg BW]. Selenium treatments were initiated 21 d before breeding and nutritional restriction began on d 64 of gestation. At slaughter on d 135 ± 5 of gestation, the gravid uterus and its contents were removed, weighed, and frozen or perfusion-fixed with Carnoy's fixative. There was no effect of diet on placentome number or weight, caruncular (CAR) or cotyledonary (COT) tissue weight, CAR cellular proliferation, CAR DNA, CAR or COT RNA, CAR or COT RNA:DNA, CAR protein, or CAR protein:DNA. There were nutritional effects on gravid and empty uterine weight, with CON ewes having heavier gravid and empty uterine weights compared to RES ewes [9.89 vs. 8.70 ± 0.33 kg (*P* = 0.01); 0.80 vs. 0.68 ± 0.02 kg (*P* = 0.001)]. Control ewes also tended to have more COT protein compared with RES ewes [50.96 vs. 42.88 ± 3.20 mg/g (*P* = 0.08)]. There was an increase in the number of proliferating COT cells in HSe compared to ASe ewes [6.76 vs. 3.81 ± 0.93 (*P* = 0.03)] and in RES compared with CON ewes [6.49 vs. 4.08 ± 0.93 (*P* = 0.08)]. The COT protein:DNA tended to be reduced in RES compared with CON ewes [15.85 vs.12.41 ± 1.28 (*P* = 0.07)]. Cotyledonary DNA tended to be greater in HSe compared with ASe ewes [3.91 vs. 3.11 ± 0.31 mg/g (*P* = 0.08)]. Although there was no Se by nutrition interaction, fetuses from HSe ewes tended to be heavier than those of ASe ewes [4.03 vs. 3.64 ± 0.16 kg (*P* = 0.08)] and fetuses of CON ewes tended to be heavier than those of RES ewes [4.05 vs. 3.62 ± 0.16 kg (*P* = 0.06)]. Thus, it appears that sufficient intake and supplemental Se increases fetal weight in the absence of an increase in placental, CAR, or COT weights. However, these nutritional treatments during pregnancy also may influence the cellularity and function of the placenta.

Key Words: nutrition, placentome, sheep

Introduction

Selenium (Se) is a trace element that is essential for normal growth and development (Sunde, 1997; McDowell, 2003). Because of their effects on animal health, both Se deficiency and excess have resulted in economic liabilities for livestock producers (Underwood and Suttle, 2001; McDowell, 2003). Supranutritional levels (2- to 4-fold above normal requirements) of Se from yeast have been shown to reduce the combined incidence of lung, colorectal, and prostate cancers by as much as 50% in humans (Clark et al., 1996; Combs and Lu, 2001). However, the effects of supranutritional dietary Se on the growth and cellularity of normal, rapidly proliferating tissues have not been evaluated in detail.

Recent work has shown that nutrient restriction during pregnancy in the ewe reduces maternal jejunal and total small intestinal mass by 17 and 20% respectively (Scheaffer et al., 2004). It also has been reported that high dietary Se results in reduced placentome weight and number in pregnant ewes (Ward et al., 2004).

Sheep have a cotyledonary type of placenta, and a macroscopic classification system has been developed to reflect the relative amounts of maternal and fetal tissue comprising the individual placentomes (Vatnick, et al., 1991). There is limited data on the effects of nutrition on placentome type in the ewe, and to our knowledge, there are no data on the effects of Se on placentome type. However, advanced morphological types have been observed in several fetal growth restriction models in the ewe (Vatnick et al., 1991; McMullen et al., 2005; Vonnahme, et al., 2006).

Because there is limited data on the combined effects of high Se and nutrient restriction on growth and cellularity of the placenta, the objectives of this study were to investigate the influence of maternal supranutrtional Se intake and nutrient restriction on uterine weight, placentome number and weight, caruncular and cotyledonary weights, placentome types, and placental cellularity in pregnant ewe lambs.

[1]This research was supported in part by National Research Initiative Competitive Grants no. 2003-35206-13621 and 2005-35206-15281 from the USDA Cooperative State Research, Education, and Extension Service, by NIH Grand HL 64141, and partially by USDA-IFAFS Grant No. 00-52102-9636.

Materials and Methods

This study was approved by the North Dakota State University Institutional Animal Care and Use Committee. Thirty-six Targhee-cross ewe lambs were randomly assigned to individual pens (0.91 x 1.2 m) and allotted to 1 of 4 treatments in a 2 x 2 factorial arrangement. Dietary treatments were Se intake [adequate Se (ASe), 7.4 μg/kg BW vs. high Se (HSe) from Se-enriched yeast, 81.5 μg/kg BW] and nutrition level [control (CON), 100% of the NRC (1985) requirements vs. restricted (RES), 60% of the NRC requirements]. Se treatments were initiated 21 d before breeding (d 0) and continued until slaughter (d 135 of gestation). Nutritional level treatments were initiated on d 64 of gestation and continued until slaughter. Diets, consisting of alfalfa hay, were similar in CP (16.0%) and ME (2.12 Mcal /kg). Diets were fed once daily, with free access to water and Se-free salt (Table 1). HSe ewes received supplements containing Se-enriched yeast (Alltech Inc., Nicolasville, KY) whereas the ASe ewes received supplements without added Se.

Ewes were slaughtered on d 135 ± 5 of gestation. One h before slaughter, ewes were injected via jugular venipuncture with 5-Bromo-2'-deoxyuridine (BrdU; 5 mg/kg BW) which is incorporated into the DNA of proliferating cells during the S-phase of the cell cycle (Jablonka-Shariff et al., 1993). Ewes were stunned with a captive-bolt gun and exsanguinated. Immediately after exsanguination, the gravid uterus was removed and weighed. Further, the fetus was removed and weighed. A portion of the pregnant tract was fixed in Carnoy's solution. Individual placentomes were counted, removed from the uterus and classified according to Vatnick et al. (1991), and weighed. The caruncular (CAR; maternal placental) and cotyledonary (COT; fetal placental) tissues were separated, weighed, snap-frozen in isopentane, and stored at -80°C until further analysis. Empty uterine weight was recorded.

The CAR and COT samples were analyzed for concentrations of DNA and RNA using the diphenylamine and orcinol procedures (Reynolds et al., 1990) respectively. Protein concentration was determined using the Coomassie brilliant blue G (Bradford, 1976) with bovine serum albumin (Fraction V; Sigma Chemical) as the standard (Johnson et al., 1997). The RNA:DNA and protein:DNA ratios were used as indexes of potential cellular activity per cell and cell size respectively.

Data were analyzed as a 2 x 2 factorial arrangement of treatments using GLM procedures (SAS Inst. Inc., Cary, NC). Because ewe lambs carried both singles and twins, fetal number was used as a covariate. The model contained the effects of nutrition (CON vs. RES), Se (ASe vs. HSe), and the nutrition x Se interaction. When the interaction was significant ($P < 0.05$), the means were separated by the least significant difference test.

Results and Discussion

Although there was no interaction of Se and nutritional level on fetal weight, fetuses from HSe ewes tended ($P = 0.08$) to be heavier compared with those from ASe ewes. Further, fetuses from CON ewes also tended ($P = 0.06$) to be heavier compared with those from RES ewes (Table 2). However, there were no treatment effects on total placentome weight, average placentome weight, CAR weight, or COT weight (Table 2). Similarly, Vonnahme and others (2003) found that maternal nutrient restriction from d 28 to 78 of gestation resulted in heavier fetal weight in control ewes with no difference in total placentome weight, CAR weight, or COT weight. However, Clarke and others (1998) subjected ewes to dietary restriction from d 30 to 80 of gestation and found no differences in fetal weight at d 80 between the control and restricted groups. That same study found that restricted ewes had a lower average placentome weight compared with control ewes (Clarke, et al., 1998). In this current study, average placentome weight was not affected; however, the periods of restriction are different (d 30 to 80 in the Clarke et al. study vs. d 64 to 135 in current study). In addition, supranutritional Se was fed in the current study, and it is possible that Se may minimize the effects of nutrient restriction.

Borowicz and others (2005) found that feeding supranutritional levels of HSe wheat to pregnant ewes from d 50 to 135 of gestation did not affect fetal weight at d 135 of gestation. In the study by Borowicz and others (2005), HSe wheat was fed beginning on d 50 of gestation, whereas in the current study HSe yeast was fed beginning 21 d before breeding. The differences in source of selenium and length of treatment may be why different fetal weight results were observed between these two studies from our laboratories.

Although there was no difference in placentome weight, the placental 'efficiency' (the fetal weight : placentome weight ratio) tended to be lower ($P = 0.06$) in RES compared with CON ewes. This decrease in placental efficiency may be associated with decreases in placental vascularity, which could affect nutrient extraction by the placental and, therefore, warrants further investigation.

The number of proliferating cells in the CAR did not differ among treatments (Table 2), which is in contrast to the data of Borowicz et al. (2005), who reported decreased CAR cellular proliferation in HSe compared to control ewes. This conflicting evidence concerning Se's effect on placental cellular proliferation warrants further study. However, in the COT, HSe ewes had an increase ($P = 0.03$) in the number of proliferating cells compared with ASe ewes, and RES ewes tended to have an increase ($P = 0.08$) in the number of proliferating cells compared with CON ewes. In jejunum, which is another nutrient transferring tissue, Soto-Navarro and others (2004) found that the total number of proliferating cells was almost double in steers fed HSe wheat compared with control steers.

Dietary nutrient restriction and supranutritional levels of Se had no effect on the cellularity of the CAR (Table 3). In contrast, HSe tended to increase ($P = 0.08$) COT DNA concentrations and reduce ($P = 0.07$) the COT protein:DNA ratio compared with those of the ASe ewes (Table 3). Nutrient restriction had no effect on COT DNA or RNA concentrations (Table 3); however, compared with CON ewes, RES ewes tended to have a decrease ($P \leq 0.08$)

in the protein concentration and protein:DNA ratio in the COT compared to CON ewes (Table 3). The decrease in COT protein:DNA ratio due to both HSe intake and nutrient restriction indicates that cell size was likely reduced in the COT due to both HSe intake and nutrient restriction.

There was no effect of treatment on placentome number (Table 2). In the study by Vonnahme and others (2003), there was no effect of nutrient restriction on the total number of placentomes. The study by Borowicz and others (2005), showed decreased placentome number and weight due to high Se wheat intake in the ewe; however, our results do not match these findings. Again, the source of selenium and the length of treatment may be critical in determining Se's effects on the placentome.

There were more type A placentomes than any other placentome type (Table 4; $P < 0.001$). There were also more type B placentomes than type D placentomes ($P < 0.001$). In agreement with data from our laboratory (Arndt, et al., 2006), type D placentomes were heavier on average than any other placentome type (Table 4; $P < 0.001$), and type C placentomes were heavier than types A and B placentomes ($P < 0.001$), which did not differ (Table 4). Average diameters of the placentomes did not differ by type (Table 4).

There were no treatment effects on the number of type A, B, C, or D placentomes (Tables 5 and 6). There is evidence that the number of type D placentomes increases as a result of poor intrauterine conditions, such as maternal dietary restriction during early or mid-pregnancy (Heasman et al., 1998; Steyn et al., 2001; McMullen, et al., 2005); however, no such results were observed in the present study. There were no treatment effects on total or average weight for type A, C, or D placentomes (Table 5). There was a Se x nutritional level on total type B placentome weight ($P = 0.01$; Table 6). In CON ewes, those fed HSe had a greater total weight of type B placentomes (Table 6). Numerically, the HSe, CON ewes had the greatest number of type B placentomes, which may have contributed to the observed increase in total placentomal weight (Table 6). There was also a Se x nutritional level effect on the average weight of type B placentomes ($P = 0.02$). The ASe, RES ewes had a greater average weight of type B placentomes (Table 6). Moreover, type C placentomes had a lower ($P = 0.04$) average weight in HSe compared with ASe ewes. It appears that Se and nutrition may alter gross placentome morphology; specifically, that of type B and C placentomes. These placentomes are often considered "transitional types" between type A and type D placentomes (Vatnick, et al., 1991). Perhaps the nutritional treatments target these transitional placentomes.

Future studies are necessary to determine if maternal nutrition impacts placental vascularity or blood flow, which will impact transplacental exchange of nutrients and, ultimately, fetal growth and development.

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Table 1. Chemical composition of alfalfa hay and supplements (DM basis) fed to control [100% of the NRC (1985) requirements] or restricted (60% of control) ewes (DM basis)

		Supplement[1]	
Item, %	Alfalfa hay[2]	Control	Selenium
DM	87.4	85.6	86.1
Ash	9.9	1.95	2.3
CP	16.13	10.0	12.7
ADF	27.3	5.5	5.0
NDF	37.2	26.2	26.5
Se, ppm	-	0.32	43.2

1The supplement contained 0.32 ppm and 43.2 ppm Se for Control and Selenium, respectively, and was fed to provide 7.4 µg/kg BW or 81.5 µg/kg BW daily for control and high-Se ewes.

[2]The alfalfa hay was chopped, averaging 3.8 cm in length.

Table 2. The effect of level of nutrition and dietary Se on fetal BW, placentome number, placentome weight, average placentome weight, CAR weightt, COT weight, gravid uterine weight, empty uterine weight, CAR proliferation, and COT proliferation

	Nutrition [1]		Selenium [2]			*P*-value		
Item	CON	RES	ASe	HSe	SE	Nut	Se	Se X Nut
Fetal BW, kg	4.05	3.62	3.64	4.03	0.16	0.06	0.08	0.58
Placentome number	93.05	90.77	87.94	95.87	4.78	0.74	0.25	0.20
Total placentome weight, g	524.66	503.67	496.00	532.33	22.35	0.51	0.27	0.85
Avg placentome weight, g	5.76	5.91	6.08	5.59	0.40	0.80	0.40	0.17
CAR weight, g	99.55	89.73	91.65	97.63	6.45	0.46	0.33	0.33
COT weight, g	325.57	297.50	300.64	322.43	18.80	0.50	0.24	0.97
Gravid uterine weight, kg	9.89	8.70	8.92	9.67	0.33	0.01	0.11	0.19
Empty uterine weight, kg	0.80	0.68	0.72	0.76	0.02	0.001	0.26	0.20
CAR proliferating cells, number	0.18	0.12	0.17	0.13	0.03	0.24	0.39	0.54
COT proliferating cells, number	4.08	6.49	3.81[a]	6.76[b]	0.93	0.08	0.03	0.40
Placental efficiency	11.53	10.43	10.98	10.98	0.40	0.06	0.99	0.31

[ab]Means ± SE within a row having different superscripts differ ($P < 0.05$).
[1] M=non-restricted ewes fed at 100% of the NRC (1985) requirements; R=ewes fed to 60% of M.
[2] ASe = 7.4 µg of Se/kg BW (no added Se); HSe = 81.5 µg of Se/kg BW (HSe).

Table 3. Effect of level of nutrition and dietary Se on estimates of cellularity in maternal and fetal tissues of the placentome

	Nutrition [1]		Selenium [2]			*P*-value		
Item	CON	RES	ASe	HSe	SE	Nut	Se	Se X Nut
Cotyledon								
DNA, mg/g	3.61	3.41	3.11	3.91	0.31	0.65	0.08	0.38
RNA, mg/g	3.73	4.26	3.73	4.26	0.26	0.15	0.15	0.41
RNA:DNA	1.18	1.29	1.31	1.16	0.10	0.43	0.29	0.78
Protein, mg/g	50.96	42.88	48.70	45.13	3.20	0.08	0.44	0.73
Protein:DNA	15.85	12.41	15.84	12.42	1.28	0.06	0.07	0.45
Caruncle								
DNA, mg/g	1.62	1.89	1.76	1.74	0.21	0.36	0.94	0.54
RNA, mg/g	2.91	3.54	2.95	3.49	0.37	0.22	0.30	0.88
RNA:DNA	2.80	2.39	2.79	2.40	0.66	0.65	0.67	0.97
Protein, mg/g	41.62	38.44	36.82	43.24	5.06	0.65	0.38	0.33
Protein:DNA	35.17	24.30	30.49	28.98	7.06	0.27	0.88	0.86

[1] M=non-restricted ewes fed at 100% of the NRC (1985) requirements; R=ewes fed to 60% of M.
[2] ASe = 7.4 µg of Se/kg BW (no added Se); HSe = 81.5 µg of Se/kg BW (HSe).

Table 4. Count, percentage, total weight, average weight, and average diameter of types A, B, C, and D placentomes

Item	A	B	C	D	SE	*P*- value
Count	60.42[a]	21.03[b]	10.55[bc]	5.52[c]	4.63	< 0.001
Percentage	64.08[a]	23.45[b]	11.87[c]	8.22[c]	4.39	< 0.001
Total weight, g	304.99[a]	114.15[b]	81.89[b]	57.66[b]	24.63	< 0.001
Avg weight, g	5.10[a]	6.07[a]	8.20[b]	10.16[c]	0.62	< 0.001
Avg diameter, cm	6.07[a]	2.53[a]	3.48[a]	3.00[a]	2.82	0.69

[abc]Means ± SE within a row having different superscripts differ ($P < 0.05$).

Table 5. Effect of level of nutrition and dietary Se on placentome morphology[1]

Item	Nutrition [2]		Selenium [3]		SE	*P*-value		
	CON	RES	ASe	HSe		Nut	Se	Se x Nut
Type A								
Number	56.37	62.21	60.22	58.36	7.60	0.58	0.86	0.09
Total weight, g	293.09	291.68	277.77	307.00	38.50	0.98	0.59	0.12
Avg weight, g	5.14	5.09	5.26	4.97	0.35	0.91	0.55	0.28
Type B[4]								
Type C								
Number	11.23	10.20	10.31	11.13	3.33	0.82	0.85	0.78
Total weight, g	87.50	80.33	83.47	84.37	24.86	0.83	0.8	0.99
Avg weight, g	8.82	8.18	9.69[a]	7.31[b]	0.84	0.57	0.04	0.58
Type D								
Number	7.54	9.54	12.86	4.22	6.16	0.81	0.31	0.96
Total weight, g	83.83	41.72	92.53	33.02	40.04	0.44	0.28	0.35
Avg weight, g	8.70	15.90	14.66	9.99	6.39	0.41	0.59	0.40

[ab]Means ± SE within a row having different superscripts differ ($P < 0.05$).
[1]Means are present for main effects only when there was no Se x nutritional interaction.
[2]M=non-restricted ewes fed at 100% of the NRC (1985) requirements; R =ewes fed to 60% of M.
[3] ASe = 7.4 µg of Se/kg BW (no added Se); HSe = 81.5 µg of Se/kg BW (HSe).
[4]Selenium x nutritional level interaction present (see Table 6).

Table 6. Effect of level of nutrition and dietary Se on placentome morphology of type B placentomes[1]

Item	ASe, CON	ASe, RES	HSe, CON	HSe, RES	SE	*P*-value
Type B						
Number	14.28	17.13	30.37	19.59	6.87	0.27
Total weight, g	48.33[a]	116.32[ab]	174.38[b]	109.86[ab]	27.82	0.01
Avg weight, g	4.49[a]	8.08[b]	6.24[ab]	5.22[a]	1.00	0.02

[ab]Means ± SE within a row having different superscripts differ ($P < 0.05$).
[1]Means are presented for Se x nutritional level interactions.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

STUDY OF THE PORTASCC® MILK TEST TO ESTIMATE SOMATIC CELL COUNT (SCC) AND DETECT SUBCLINICAL MASTITIS IN SHEEP

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ABSTRACT: An on-farm test for determining udder health would benefit sheep producers. The PortaSCC® milk test is a rapid cow-side test which uses a test strip that requires a small drop of milk and produces a blue color proportional to the somatic cell count (SCC) in the milk. The strip color can be read by visual comparison to a color chart or quantitatively with a reflectometer. The objective of this study was to assess the effectiveness of the PortaSCC® milk test to estimate SCC and detect subclinical mastitis in sheep. Ninety-two Rambouillet-Merino ewes were milk sampled from each udder half at weaning (89 ± 16 d; mean ± SD) and 24 h post-weaning. Milk samples were analyzed for SCC by PortaSCC® test and compared to SCC obtained by flow cytomety (FC) and traditional methods (TM) using a Bentley 2000 component analyzer. The SCC corresponding to no color change (NC), light blue (LB), blue (B), and dark blue (DB) on the color chart were < 200 (< 5.3 log10), 200 (5.3 log10), 750 (5.9 log10) and 2,000 x 103 cells/mL (6.3 log10), respectively. Flow cytometry SCC values were 244 ± 153, 364 ± 516, 446 ± 516 and 9,097 ± 607 x 103 (mean ± SE) and 5.2 ± .02, 5.5 ± .07, 5.6 ± .06 and 6.2 ± .09 log10 for NC, LB, B and DB, respectively, with 204, 18, 18 and 13 udder sides reported for each color respectively. Traditional SCC values for NC, LB, B and DB were 131 ± 146, 351 ± 595, 991 ± 538 and 11,889 ± 652 x 103, respectively and 4.9 ± .03, 5.3 ± .10, 5.6 ± .09 and 6.5 ± .15 log10, respectively. Actual SCC values for NC, LB, and B did not differ and were lower ($P < .0001$) than DB; log10 transformation values, however, increased ($P < .02$) as color became darker. Cell counts positively correlated with the % reflectance for FC ($r = .77$, $P < .0001$) and TM ($r = .85$, $P < .0001$). Although no difference ($P > .7$) was noted between colors NC, LB and B for SCC, values in log10 transformation differed among colors. Our results suggest the PortaSCC® milk test is suitable for determining SCC and udder health status.

Key Words: Mastitis, Somatic Cell Count

Introduction

Mastitis, an inflammation of the mammary gland, is an infectious disease of lactating ewes that occurs in all sheep-producing countries (Jones and Watkins, 2000). Mastitis causes many losses for the sheep industry including premature culling, a decrease in milk quality and quantity, poor lamb growth, and in severe cases, death (Watson and Buswell, 1984; Torres-Hernandez and Hohenboken, 1979).

Acute mastitis can be readily diagnosed either visually or by palpation, but the only means of identifying subclinical mastitis is by measuring (SCC) in the milk (Keisler et al., 1992). A rapid cow-side milk test (PortaSCC®) that produces a color change on a test strip proportional to SCC in the milk, is now manufactured for use in dairy cattle. An on-farm milk test for determining udder health would provide the producer with a quick and cost-effective tool to make immediate decisions to keep and treat or cull a ewe. The objective of this study was to assess the effectiveness of the PortaSCC® milk test to estimate SCC and detect subclinical mastitis in sheep.

Materials and Methods

Animal Management. Ninety-two Rambouillet x Merino multiparous and primiparous lactating ewes (2-6 yr of age) were used in this study. All ewes were placed in a dry-lot and pen-fed alfalfa pellets a week prior to parturition and remained there through the first 60 d following parturition. Ewes were group fed 1.8 kg alfalfa pellets/head/day before parturition. All ewes were allowed free access to water and mineralized salt blocks. Following parturition, the ewes were pen fed 2.7 kg alfalfa and .23 kg of corn/head/day. Ewes were placed on pasture at 60 d postpartum and remained there until weaning (89 ± 16 d). Milk samples were

collected the day of weaning and 24 h postweaning.

Milk Sampling. Before sampling, udders were disinfected with isopropyl alcohol and the first ~3 mL of milk from each teat was stripped and discarded. A 40 mL sample was collected from each udder half and placed on ice for analysis of SCC. Upon arrival at the laboratory the milk samples were gently shaken and 33µL of milk was pipetted from each sample tube onto the sample window of the PortaSCC® milk test strips along with 100µL of activator solution. The strips were allowed to develop for 1 h, during which time a color reaction took place correlating to the SCC level in the milk. The blue color generated by the color reaction was read visually by comparison to the Quick Check Color Chart and quantitatively by a hand-held reflectometer produced by PortaSCC®. One of four colors was recorded for each test strip: no color change (NC), light blue (LB), blue (B), and dark blue (B). Test strip colors NC, LB, B and DB represent SCC for cow's milk of < 200 (< 5.3 log10), 200 (5.3 log10), 750 (5.9 log10) and 2,000 x 10^3 (6.3 log10) cells/mL respectively on the PortaSCC® Quick Check Color Chart.

SCC Analysis. Milk samples were analyzed for SCC by PortaSCC® milk test and compared to SCC obtained by flow cytometry (FC) and traditional methods (TM). For FC, each sample was gently shaken and a 50-µL sample of milk was pipetted from each tube into separate test tubes. Cells in the milk were counted by dilution in a detergent solution that also contained propidium iodide (PI) to label nuclei of lysed cells and beads at a known concentration. All samples were analyzed on a Beckman Coulter (Fullerton, CA) XL/MCL. The SCC were determined by traditional methods using a Bentley 2000-Somacount 500 Combi® (Bentley Instrumments, Chaska, MN). The upper SCC limit for the Bentley 2000 is 9,999 x 10^3 cells/mL, thus actual SCC for seven ewe sides that exceeded this limit were estimated using linear regression.

Statistical Procedure. SAS software version 9.1 (SAS Corporation, Cary, NC) was used to perform all statistical analyses. The SAS FXQTQT RSREG regression macro (Fernandez, 2007) was used to determine the relationship between the two methods (FC vs. TM) used to determine SCC. Linear regression between FC and TM was determined using actual SCC values and log10 transformed values. The SAS FXONEQL macro (Fernandez, 2007) was used to detect the effect of SCC of the four colors observed from the test strips and on percent reflectance given by the digital reflectometer. Effect of day of sampling was determined by the SAS macro FXQLQL (Fernandez, 2007).

Results and Discussion

Linear regression revealed that cell counts were positively correlated ($P < .84$) between FC and TM. No difference ($P > .50$) was detected between the two procedures for actual cell count (343 and 299 ± 48 x 10^3 cells/mL for TM and FC, respectively). However, when examined in log10, values were greater ($P < .03$) for TM than for FC (343 and 299 ± 48 x 10^3 cells/mL and 5.3 and 5.1 ± .02 log10 cells/mL, respectively). Actual SCC values for FC and TM did not differ ($P > .05$) by day, however, log10 values were less ($P < .03$) at weaning compared to 24 h postweaning (Table 1). Actual SCC values for NC, LB and B did not differ ($P < .05$) for FC or TM, but were lower ($P < .0001$) than values for DB; FC SCC log10 values for NC were less ($P < .05$) than LB, B and DB (Table 2). Values for LB and B did not differ ($P > .15$) but were lower ($P < .05$) for DB. Somatic cell count log10 values for TM differed among the four colors, with values increasing ($P < .05$) from NC to DB.

McFarland (2000) and Surian (2001) reported that ewes with SCC above 500 x 10^3 cells/mL (5.6 log10 cells/µL) were classified as likely having an infection and above one million cells/mL was suggested to indicate subclinical mastitis. By these standards, log10 SCC ranges by TM reported for colors NC and LB remain within normal SCC limits of a healthy udder. Values reported for color B were greater ($P > .05$) than log10 SCC observed for colors NC and LB, but less than ($P < .0001$) color DB. The actual SCC for color B was 991 ± 538 x 10^3 cells/mL which would be within the cell range reported for color B (750 x 10^3 to < 2 million cells/mL) reported for cow's milk on the PortaSCC® color chart. Any SCC values in this range would be considered to be near the value associated with subclinical mastitis. Color DB represented at least 2 million cells/mL and all SCC values reported for this range exceeded that threshold with the mean above 7 million cells/mL.

The PortaSCC® milk test reports SCC range values for each color but does not give range values for % reflectance. Therefore, reflectance values must be determined to be within or above normal values for a healthy

udder depending on its correlation to the SCC. In this study, SCC positively correlated with the % reflectance for FC (r = .77, P < .0001) and TM (r = .85, P <.0001). Reflectance values for NC and LB did not differ and were lower (P < .05) than those for B and DB. Values for color B were also lower (P < .05) than those for DB. In correlation to SCC, values below the .51 ± .06 % reflectance range reported for color B would be considered within normal limits of a healthy udder, whereas those above this range would indicate subclinical mastitis.

Implications

This study indicates that the SCC values for sheep milk tested by the PortaSCC® milk test were within the ranges reported for cattle and the test can detect SCC below 200 x 10^3 cells/mL and above 2 million cells/mL, making it a valuable tool to determine SCC and detect ewes with subclinical mastitis. Udder sides with SCC in the DB range are considered to have subclinical mastitis and the producer would be recommended to treat or cull the ewe. Therefore, the PortaSCC® milk test could be used as a rapid on-farm test for subclinical mastitis, giving the producer the opportunity to cull or treat the affected animal(s) and improve herd health and productivity.

Acknowledgements

The authors would like to thank PortaSCC® for their support, the PortaSCC® representative Darby McChesney for technical support and guidance and the following for their assistance with this study: Laura Millsap, Michelle Burrows, Elsbeth Grimmer, Erin Donaldson, Heather Mobley, Amber Long, Michelle Coker, Andy Miller, Cody Byrne, Kathryn Dyer and Matt Polak.

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Table 1. Effect of sampling day on somatic cell count (SCC) measured by flow cytometry (FC) and traditional methods (TM) in actual cell count and in log10 transformation.

Sampling Day	FC SCC	FC log10	TM SCC	TM Log_{10}
Day 1[a]	971 ± 70	5.6 ± .04[b]	1779 ± 86	5.5 ± .07[b]
Day 2[a]	995 ± 63	5.7 ± .04[c]	1870 ± 86	5.7 ± .06[c]

[a] Day 1 = day of weaning, Day 2 = 24 h postweaning.
[b,c] Column values with different superscripts differ ($P \leq .03$).

Table 2. Comparison of somatic cell count (SCC) values by flow cytometry (FC) and traditional methods (TM) to PortaSCC® test strip color and % reflectance detected by the PortaSCC® digital reflectometer.

Strip Color	FC SCC	FC log10	TM SCC	TM log10	Reflectance[b]
No Color[a]	244 ± 153[c]	5.2 ± 0.02[c]	131 ± 146[c]	4.9 ± 0.03[c]	0.01 ± 0.02[c]
Light Blue	364 ± 516[c]	5.5 ± 0.07[d]	351 ± 595[c]	5.3 ± 0.10[d]	0.09 ± 0.07[c]
Blue	446 ± 516[c]	5.6 ± 0.06[d]	991 ± 538[c]	5.6 ± 0.09[e]	0.51 ± 0.06[d]
Dark Blue	9097 ± 607[d]	6.2 ± 0.09[e]	11889 ± 652[d]	6.6 ± 0.15[f]	4.36 ± 0.08[e]

[a] Indicates no color change noted on the PortaSCC® test strip.
[b] Indicates the % reflectance measured by the PortaSCC® digital reflectometer.
[c,d,e,f] Column values with different superscripts differ ($P < 0.05$).

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECTS OF SUPPLEMENTATION OF TASCO-EX ON INFERTILITY IN YOUNG MALE GOATS EXPERIENCING HEAT-STRESS

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ABSTRACT: A study was conducted at the Angelo State University Research Center to determine the effects of supplementation of the kelp extract product, Tasco-EX, on physical and fertility traits in young male goats challenged by heat stress. Twenty genetically similar young Boer bucks were randomly divided into two equal experimental groups. The first group received tri-weekly dosage of Tasco-EX so that the total weekly supplementation for each goat was 35g. The second group received no Tasco-EX and served as the control. All goats were held under feedlot-type conditions and identical high-energy diets were offered *ad libitum.* Supplementation spanned an 84-d period during which weekly average high temperatures ranged from 32.1-38.2° C. Data were collected for scrotal growth, ADG, live-animal ribeye area (REA), and rectal temperature as well as sperm-cell concentration (both visual estimation and hemacytometer count) and sperm-motility grade (1-7 score). No effects were observed on scrotal circumference growth ($P = 0.22$) or final REA ($P = 0.75$). Average daily gain was not affected on a periodic ($P = 0.72$; $P = 0.32$) or total ($P = 0.75$) basis. Rectal temperatures were surprisingly higher ($P = 0.01$) for the supplemented group than the control by an average of almost 0.2° C. Although no differences were observed for sperm-motility grades ($P = 0.23$) or visual estimations of concentration ($P = 0.41$) in semen samples collected via electroejaculation, actual sperm-cell concentration data revealed a 1.2 billion cells/mL average increase in the supplemented group over the control ($P = 0.10$). These data suggest supplementation of Tasco-EX to young male Boer goats can maintain sperm cell concentrations despite increased body temperature. Future examination of the product's effects on morphology will provide a clearer picture of the effects on overall fertility.

Key Words: Tasco, kelp extract, heat stress, goats, fertility

Introduction

Heat stress is a major limiting factor of goat production in Texas and the Southwestern U.S., as goats respond to increased ambient temperatures with both reduced physical performance (McDaniel and Parker, 2004) and lowered reproductive capacity (Rockett et al., 2001). In males, fertility drops when outward stressors illicit necessary physiological changes intended to aid in internal cooling. Some of these, such as higher respiratory frequency (Allen et al., 2001a) and more active mitochondrial metabolic pathways (Valko et al., 2004) inadvertently increase the levels of dangerous free radical compounds. These compounds can destroy cell membranes (Long and Krammer, 2003), nuclear and mitochondrial DNA (Lopes et al., 1998), and can even initiate apoptosis (Marchetti et al., 2005). The result of free radical damage is almost always cellular demise, and immature sperm cells seem exceptionally vulnerable compared to somatic cells (Volger et al., 1991).

Intracellular levels of antioxidant compounds are crucial to protection against free radical damage (Gulec et al., 2006), and supplementation of certain antioxidants during periods of free-radical overproduction has been shown to reduce symptoms of heat-stress and the resulting infertility (Breezezinska-Slebodzinska et al., 1995; Evans et al., 2002; Leonard et al., 2001). Galipalli et al. (2004a) described kelp extract as an abundant source of two important antioxidants: α-tocopherol and glutathione. Thus, the objective of the present study was to determine any effects on heat-related infertility and performance of a kelp-extract supplementation regiment administered to young male Boer goats.

Materials and Methods

Pure-bred, intact male Boer goats (n = 20) of Angelo State University stock, 100 days of age and 21 days post-weaning, were gathered in late May. The goats were grouped according to size to eliminate social dominance and confined five per pen at the Angelo State University Management, Instruction, and Research Center on May 26, 2006. All goats used in the study were visually inspected to ensure proper health and physical soundness. After an 11-d adjustment period during which the goats were acclimated to the experimental environment, all goats began an identical 84-d *ad libitum* feeding regimen designed to simulate a high-energy feedlot-style diet (Table 1). This feeding regimen was initiated on June 6, 2006. At this time, 10 goats selected at random were designated to serve as the control group and received no experimental treatment. The remaining 10 goats received a dose of Tasco-EX seaweed (kelp) extract three times weekly for the duration of the 84-d feeding period. Ten-g doses of the extract were administered on Mondays and Wednesdays, and 15-g doses were given on Fridays to allow for the weekend.

The extract was in paste form and administered orally from a 100-g tube to the back of the throat. Effects of the treatment were defined by physical traits as well as semen quality traits. Physical considerations consisted of scrotal circumference growth, average daily gain, ribeye area and internal body temperature. Ribeye area measurements were conducted on the live animal using an ALOKA 500 (ALOKA, Inc., Wallingford, CT) ultrasound machine. Internal body temperatures were obtained rectally on a semi-weekly basis for eight weeks.

Table 1. Analysis of experimental diet[a, b]

Ingredient	
Crude protein	16.00%
Crude fat	2.50%
Crude fiber	17.00%
Ca	1.00%
P	0.30%
K	1.00%
NaCl	1.25%
Cu	25.00 ppm
Se	0.30 ppm
Vitamin A	4545.45 IU/kg
Vitamin E	9.09 IU/kg
Monensin	4.55 IU/kg

[a]analysis is as fed.
[b]*ad libitum.*

Semen quality was defined by visual estimate of semen concentration, actual sperm cell concentration determined by hemacytometer count, and comparative motility score. Initial measurements for all physical traits were recorded on day 0 (June 5, 2006), one day before the first oral administration, and final measurements were recorded on day 85 (August 29, 2006), one day after the conclusion of the experimental period. Periodic measurements for growth traits and body temperature were recorded throughout the 84-d period.

Each goat represented an individual experimental unit. All data were analyzed using the greatest linear means (GLM) function of SAS (SAS Institute, Cary, NC).

Results and Discussion

Periodic and overall average daily gains (Table 2) were not significantly different between goats administered Tasco-EX and the control group, suggesting that supplementation of Tasco-EX to goats under feedlot conditions does not improve weight gain. The findings contradict results reported for the meal form of the product (Allen et al., 2001b), as well as for seaweed extract administered under pasture conditions (Leupp et al., 2005). However, results of previous studies may be products of factors absent in the current study. Tasco-14, the meal version of the seaweed product, has been compared to medium quality alfalfa hay in its protein and energy content (Ventura and Castañón, 1997), and because much of this digestible organic material is removed during the extraction process, it is not available in the extracted product, and the result may be less growth.

Table 2. Average daily gain in kg/d of adolescent Boer goats administered Tasco-EX and their control counterparts

	Treatment			
Time period	Control	Tasco-EX	SE	*P*-value
Jun 6-Jul 5	0.34	0.28	+/-0.04	0.32
Jun 6-Aug 1	0.30	0.32	+/-0.33	0.72
Jun 6-Aug 29	0.29	0.28	+/-0.02	0.75

Two hypotheses can be offered for the success of seaweed extract under pasture conditions. Leupp et al. (2005) detailed the effects of Tasco-EX on increased digestibility of poor quality roughage, which would allow supplemented animals more efficient use of available forage and, consequently, higher rates of gain. This effect was not observed in the present study because no poor quality roughage was offered. The roughage contained within the high-energy diet was of good quality. The second element of increased pasture gains is the immunological activity of the extract and its interaction with microbial life present on grazed pastureland. Spiers et al. (2005) found that certain microbes commonly present on growing forage during warm-weather months can cause a decrease in intake and, thus, rate of gain. Specifically, Spiers used the model of endophyte toxicity in fescue to illustrate the medicinal effects of seaweed extract supplemented to livestock grazing in the presence of toxin-producing microbes. Because the goats in the present study were given a processed feed, microbial toxicity was not a factor. No evidence was found in the study to indicate weight gain can be improved in feedlot goats through the supplementation of Tasco-EX.

Average values for ribeye area (Table 3) were similar between treatment and control groups. Although Ventura and Castañón (1997) found that protein availability was improved by supplementing seaweed extract, the goats in the current study received adequate amounts of dietary protein, and any additional protein was most likely lost through excretion.

Goats administered Tasco-EX averaged a 9.90 cm scrotal circumference (Table 3), while their control counterparts averaged 7.65 cm. Due to high variance, these values were not significant ($P = 0.22$). Replication of the trial on a larger scale is needed to verify the tendencies of the values for scrotal growth recorded in this study.

Average rectal temperatures (Table 4) were unexpectedly higher for the experimental group than the control ($P = 0.01$) in the presence of elevated ambient temperatures. This contradicts findings by Saker et al. (1998), Leonard et al. (2001), and Evans et al. (2002), but supports those of Allen et al. (2001a). As with weight gain, the contrast in rectal temperature values of the

current and previous studies may be explained by immunological function, rather than direct stress reduction.

Table 3. Average measurements for scrotal circumference growth in cm and ribeye area in in^2 for adolescent Boer goats administered Tasco-EX and their control counterparts

	Treatment			
	Control	Tasco-EX	SE	*P*-value
Scrotal circumference growth	7.65	9.90	+/-1.27	0.22
Initial scrotal circumference	21.60	20.60		
Final scrotal circumference	29.20	30.50		
Ribeye area	1.98	2.01	+/-0.08	0.75

Table 4. Average rectal temperature readings in °C for adolescent Boer goats administered Tasco-EX and their control counterparts

	Treatment			
Date of reading[a]	Control	Tasco-EX	SE	*P*-value
Jun 16	39.13	39.29	+/-0.14	0.44
Jun30	39.48	39.71	+/-0.12	0.20
Jul 14	39.22	39.46	+/-0.08	0.04
Jul 28	39.16	39.26	+/-0.12	0.54
Overall average	39.25	39.43	+/-0.04	0.01

[a]readings taken between 0800 and 0930.

Average comparative motility score (Table 5) for goats administered Tasco-EX was not different from the control group ($P = 0.23$). The experimental group did exhibit more consistency, with 80% of the animals scoring in the top quarter of the scale. Only 50% of the control group scored as well. Heat stress has been reported to effect semen motility by damaging germ cell DNA during early spermatogenesis (Rockett et al., 2001), but the results of this study fail to support previous accounts that suggest high levels of antioxidants such as Vitamin E and glutathione found in seaweed extract reduce detriment to immature sperm cells and testicular tissue (Brezezinsha-Slebodzinska et al., 1995; Galipalli et al. 2004a; Galipalli et al. 2004b; Allen et al., 2001b).

Average concentration of spermatozoa (Table 5) was 24% higher for the experimental group than for the control, a significant difference ($P = 0.10$). Average concentrations for both groups were above the 2 to 3 billion cells per ml average reported by Coffey et al. (2004), although some individual goats in the control group fell well below the minimal value for proper breeding soundness. This data indicates that supplementation of seaweed extract hinders the detrimental effect heat stress exhibits on fertility (Cameron and Blackshaw, 1980; Rocket et al., 2001).

Table 5. Average measurements for semen quality traits of adolescent Boer goats administered Tasco-Ex and their control counterparts

	Treatment			
Semen trait	Control	Tasco-EX	SE	*P*-value
Motility[a]	2.70	1.80	+/-0.51	0.23
Visual concentration[b]	1.90	1.70	+/-0.17	0.41
Actual concentration[c]	3.01	4.22	+/-0.50	0.10

[a]1 = extreme movement; 7 = no movement.
[b]1 = cloudy; 3 = clear.
[c]actual concentration is value x 10^9 sperm cells/ml.

Based on the data collected in this and other studies, the hypothesized mechanism for the improvement of fertility under heat stress conditions is a disruption in the stepwise apoptotic pathway described by Vera et al. (2004) rather than a physiological reduction in internal temperature, as suggested in some reports (Allen et al., 2001b; Leonard et al., 2001). Certain studies of the apoptotic process have shown it to be increased by heat stress (Rockett et al., 2001), specifically through the increased production of dangerous free radicals during elevated respiration rates (Saker et al., 2001; Evans et al., 2002). Administering Tasco-EX provides a boost in available antioxidants in the system, which neutralizes a higher number of free radicals, thus protecting vulnerable immature spermatocytes and testicular tissue.

Implications

Supplementation of Tasco-EX improved sperm cell concentration in semen samples from adolescent Boer goats facing heat stress. Ignoring female fertility and embryonic survival, supplementation of seaweed extract during high ambient temperatures may provide summer breeding opportunities in some production scenarios. Cellular protection appears to be the mechanism behind reduction of heat stress-induced infertility by seaweed extract and not decreased body temperature, as internal temperature was actually increased by the product. Supplementation of seaweed extract does not improve average daily weight gain or size of ribeye area in goats under feedlot conditions.

Acknowledgments

The authors gratefully acknowledge Lane Murphy for literary assistance.

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Proceedings, Western Section, American Society of Animal Sciences

Vol. 58, 2007

LIFESPAN OF SPRAGUE DAWLEY RATS IMMUNIZED WITH AN LHRH FUSION PROTEIN

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ABSTRACT: The first objective of this study was to evaluate the effectiveness of an LHRH fusion protein in suppressing reproductive function in male Sprague Dawley rats (n=90). Even though this procedure is producing an autoimmune disease, we hypothesize that immunocastration would not have a negative impact on the lifespan of rats. To test this hypothesis male rats were immunized with a recombinant ovalbumin-luteinizing hormone-releasing hormone (oval-LHRH) fusion protein to examine the effects of immuno-castration on lifespan. The immunized rats were given a primary injection of oval-LHRH with complete Freund's adjuvant followed by two boosters of oval-LHRH and incomplete Freund's 4 and 8 wk later. The intact rats in the control group were injected with recombinant ovalbumin alone using the same adjuvant. Blood samples and testicular measurements were collected on 28-day intervals. Efficacy of the vaccine was determined by concentrations of LHRH antibodies associated with testicular atrophy. Of the LHRH immunized rats 97.8% responded generating antibodies against LHRH coupled with testicular size reduction. The effect of LHRH immunization on lifespan was examined by comparing the age at which time 50% of each treatment group died. This 50% survival time for the intact control rats was 22.5 with a SE of 1.5 and the immunized 26 months with a SE of 0.5. Based on the Wilcoxon test for comparison of survival curves, there was a notable, but non-significant difference in survival between immunized and control groups (P=0.074). This is an important observation in the consideration of using such vaccines in controlling reproduction in wild, feral or pet animals.

Key Words: LHRH, Immunocastration, Lifespan

Introduction

Luteinizing hormone – releasing hormone (LHRH) is a decapeptide synthesized by the hypothalamus and is vital for control of gonadotropins, which stimulate gametogenesis in both male and female animals. Inhibiting reproduction in many species has been accomplished through a series of immunizations containing oval-LHRH fusion protein. This stimulates the immune system to produce antibodies that bind endogenous LHRH and inhibit the secretion of gonadotropins resulting in decreased androgen and production of estrodiol, thus preventing gametogenesis, and resulting in immunocastration. This has been very effective in rams, pigs, cattle, and mice (Ülker et. al., 2005; Jaros et. al., 2005; Ribiero et. al., 2004; Stevens et. al., 2005; Quesmell et. at., 2000). Since this creates a form of an autoimmune disease there is a concern that it may have an adverse effect on the lifespan of immunized animals. Surgical castration has been shown to have a positive effect on longevity in rats and cats, where the mean lifespan was significantly higher in castrated animals than the intact population (Asdell et. al., 1967; Hamilton et. al., 1965) . The objectives of the present research were to examine the efficacy of the vaccine and to compare the lifespan of oval-LHRH immunized male Sprague Dawley rats to intact control rats.

Materials and Methods

The antigen was produced by techniques previously described (Zhang et. al., 1999; Quesnell et. al., 2000). The *E. coli* strains BL21 (Novagen Inc., Madison, WI) and MV1190 were used to produce and clone recombinant oval-LHRH fusion protein. Seven segments of the LHRH decapeptide were inserted into the carrier protein ovalbumin with a 6-histidine sequence at the carboxyl terminus. Purification of the protein was accomplished with His-Bind affinity chromatography using a Ni^{2+} column. The protein was then emulsified in modified complete Freund's adjuvant for the primary immunization and modified incomplete Freund's adjuvant for the subsequent two boosters.

One month old male Sprague Dawley rats were divided into two groups, one containing those immunized with oval-LHRH fusion protein (n=90), and the other of intact controls immunized with only ovalbumin and adjuvant (n=10). Rats were given the primary immunization at 2 months of age and two booster injections at 4 and 6 months. Blood was collected monthly by saphenous or tail venipuncture. Rats from which blood was collected via the tail vein were first anesthesized with isofluorane. Testicular width was measured with calipers at ever blood draw starting 9 months after primary injection. Rats were kept for the entirety of their lives and were caged in pairs until a cage mate died at which time they were housed singularly. Terminally ill animals were humanely euthanized with carbondioxide followed by decapitation.

Serum was diluted 1:1000 then evaluated for percentage of ^{125}I-LHRH bound by using a radioactive binding assay previously described (Johnson, et. al., 1988). Serum testosterone concentration was determined by RIA (DSL-4000, Diagnostic Systems Laboratories Inc., Webster, TX). Antibody concentrations were compared to testicular size.

The lifespan of the immunized rats was compared to that of the control group with the Wilcozon test for

comparison of survival curves. It compares the age at which 50% of each treatment group has died.

Results and Discussion

The three injections of oval-LHRH fusion protein caused a reduced testicular size in 88 of the treated rats (97.8%). This indicates that immune cells produced antibodies with affinity to endogenous LHRH causing the decreased size of the testes. Similar results occurred in a study where testes of treated mice had a significant lower weight compared to those of intact mice (Quesmell et. at., 2000). In the present study 31 of the immunized rats experience regrowth of the testes, the rest had a persisting testicular width of ≤ 0.5 cm that through out their lifespan (Fig.1)

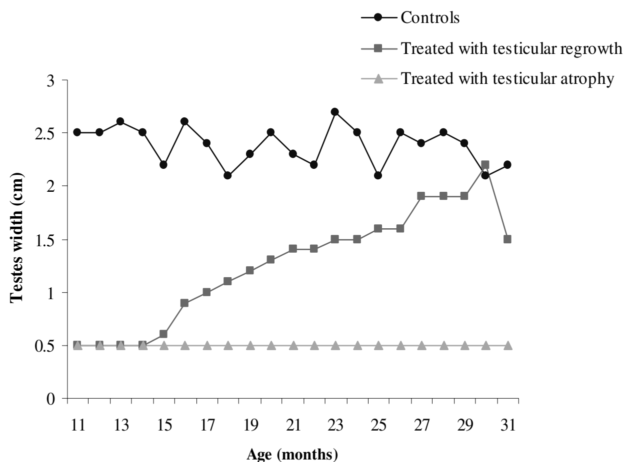

Fig.1. Mean testicular width of immunized and control rats.

The mean percentage of ^{125}I-LHRH bound in serum at a dilution of 1:1000 was slightly lower in the treated rats with a regrowth of testes than the treated rats with prolonged atrophy (Fig. 2). No LHRH antibodies were detected in the serum of the intact control rats.

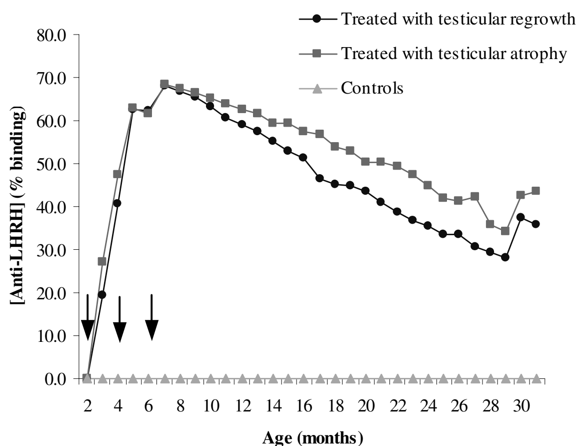

Fig. 2. Mean percent of bound ^{125}I-LHRH in control and immunized rats. Arrows represent times at which animals were immunized.

Immunization with LHRH induced an autoimmune disease by stimulating immune cells to produce antibodies against the endogenous LHRH hormone, without adversely affecting the lifespan of the treated animals. The results indicate that immunocastrating with oval-LHRH fusion protein may increase the lifespan. This was examined by comparing the age at which 50% of the rats in each treatment group died. The immunized rats had a 50% survival time of 26 months with a SE of 0.5, and the controls had a 50% survival time of 22.5 months with a SE of 1.5. This provides evidence that there was a notable, but non-significant increase in lifespan for the immunized rats when compared to the controls (Fig. 3).

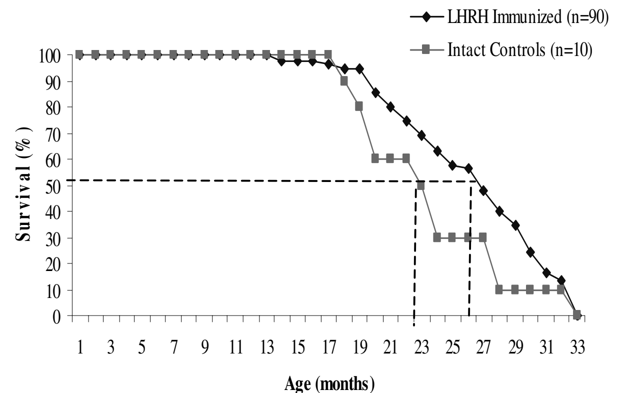

Fig. 3. The lifespan of immunized and control rats showing the 50% survival time for each treatment group.

Implications

Although the results from this study may need further verification, immunizing with oval-LHRH fusion protein has been shown to be an affective form of immunocastration in rats. It has also indicated a possible increased lifespan in immunized animals. These are important findings when considering using such vaccines in controlling reproduction in wild, feral or pet animals, where surgical castration can be expensive, not feasible, or can cause mortality.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

BIOPRYN, A BLOOD-BASED PREGNANCY TEST FOR MANAGING BREEDING AND PREGNANCY IN CATTLE

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ABSTRACT: BioPRYN is an enzyme-linked immunosorbent assay for detection of pregnancy-specific protein B (PSPB) in the blood of cows. Testing is available in 14 laboratories in the U.S., Canada and Hungary. The objectives of this study were to demonstrate the efficacy, practicality and rate of use of BioPRYN for cattle; to determine test sensitivity (SN, true pregnant [P] that tested P) and specificity (SP, true not P [NP] that tested NP); and to test the pregnancy rate in market dairy cows. Whole blood samples were collected and sera were analyzed by BioPRYN. In experiment 1, 336 dairy cows were pregnancy tested by BioPRYN at 30 to 36 days and pregnancy status was confirmed by ultrasound at 37 to 43 days after artificial insemination (AI). There were 172 P and 164 NP cows. Sensitivity was 100% and SP was 87.8%. In experiment 2, 191 beef heifers were palpated to assure they were NP before estrous synchronization. Blood were taken at time of palpation. One heifer was detected NP by palpation but tested P by BioPRYN. This heifer aborted after estrous synchronization. All other cows were detected NP by palpation; however, BioPRYN revealed two P with no evidence of abortion. BioPRYN SN and SP were 100 and 98.9%. In experiment 3, 208 Holstein cows were sold, primarily due to NP status on the farm, at sale yards in West TX and Eastern NM. Only cows judged reproductively sound and NP by rectal palpation were purchased. BioPRYN showed that 51.9% were P. Number of BioPRYN tests sold to laboratories and/or producers by BioTracking LLC was 52,830, 85,034, and 207,199 in 2004, 2005, and 2006, respectively. These results indicated that BioPRYN is a sensitive, specific and reliable test for pregnancy in beef and dairy cattle and rate of use is increasing and that many of the reproductively sound dairy cows sold at sale yards are pregnant.

Key Words: BioPRYN, PSPB, cattle, pregnancy test

Introduction

BioPRYN is an enzyme-linked immunosorbent assay (ELISA) for pregnancy in ruminant animals by detecting the presence of pregnancy-specific protein B (PSPB) in the serum or plasma. The PSPB was found by our laboratory (Butler et al., 1982 and Sasser et al., 1986) in the fetal placenta of cows.

A radioimmunoassay (RIA; Sasser et al., 1986) was developed for measuring PSPB in blood and tissues of cattle. The PSPB was in the blood of some pregnant heifers as early as 15 days after AI and in an increasing proportion of heifers for the next 13 days thereafter. Humblot et al., (1988) showed, on a herd basis, that application of the RIA at 30 days after conception was appropriate for detection of pregnancy. The protein remained in blood throughout pregnancy. There was a low concentration of serum PSPB (2 to 5 ng/mL) from about 24 days until 70 days after conception. Then there was a linear increase in concentrations to a 20 ng/mL by 150 days and 73 ng/mL by 262 days. During the last three weeks of gestation, concentrations increased to 500 ng/mL (Sasser et al., 1986). The concentration of PSPB declined after parturition with a half-life of approximately 7 days and was non-detectable by RIA by 60 to 90 days postpartum (Kirakofe et al., 1993).

The BioPRYN ELISA was developed to provide a convenient and inexpensive test for pregnancy and has been commercially available for three years. The objectives of this study were to demonstrate the efficacy, practicality and frequency of use of BioPRYN for cattle; to determine test sensitivity and specificity; and to test the pregnancy rate in marketed dairy cows.

Materials and Methods

Antibodies and protein: The PSPB was isolated (Butler et al., 1982) from placenta of cows that were less than 100 days in gestation and was used to immunize a New Zealand White rabbit (Sasser et al., 1986). Rabbit anti-bovine PSPB serum (B5) was adsorbed to 96-well micro-titer plates (Maxisorp, Nunc Inc.). Horse radish peroxidase (HRP) was conjugated to immunoglobulin G (IgG) from rabbit anti-PSPB serum by maleimide activation to form B-5, IgG HRP (B5-HRP).

BioPRYN assay: The BioPRYN assay is a typical sandwich ELISA. Rabbit anti-PSPB serum was coated to 96-well micro-titer plates and was used to capture PSPB if it was in a serum sample. The B5-HRP was used to

bind to the PSPB that was captured. The development of color occurred with the addition of 3,3',5,5',-Tetramethylbenzidine, the substrate for HRP. Sulfuric acid was added to stop the reaction and optical density for each well was obtained from a plate reader (VersaMax, Molecular Devices, Inc). The cutoff OD for the assay plate was determined from the mean OD (triplicate wells) of each of two PSPB standards. The cutoff for determining pregnancy or non-pregnancy status was equivalent to that of the RIA for testing pregnancy in cattle (Sasser et al., 1986; Humblot et al., 1988).

Evaluation of BioPRYN testing: Whole blood samples were collected in vacuum tubes containing no additives and sera were analyzed by BioPRYN.

BioPRYN sensitivity and specificity: The results of the ultrasound or palpation tests were assigned the "true value" and those of BioPRYN were assigned the "test value." Sensitivity was true pregnant that tested pregnant x 100 and specificity was true not pregnant that tested not pregnant x 100.

Experiment 1: Three hundred thirty-six dairy cows were tested for pregnancy using BioPRYN from 30 to 36 days after AI. One week later (from 37 to 43 days) a follow up test was done by ultrasound examination.

Experiment 2: One hundred ninety-one blood samples were obtained from beef replacement heifers in two commercial herds in Northern Idaho and Eastern Washington. The body weight (mean $\pm$ SD) was 414 $\pm$ 47 kg and body condition score (scale: 1 to 10 with 10 as obese) was 6.74 $\pm$ 0.44. All animals were scheduled for an experiment, unrelated to this study, which included synchronization of estrus using CIDR and prostaglandin F2 alpha (Howard, 2005). To assure that heifers were not pregnant and were ready for the synchronization of estrus program, they were tested for pregnancy by rectal palpation and BioPRYN. A prostaglandin F2 alpha treatment was given to all heifers based upon rectal palpation results.

Pregnancy rate in marketed dairy cows:

Experiment 3: Two hundred eight Holstein cows were sold, primarily due to non pregnant status on the farm, at sale yards in West TX and Eastern NM. At time of purchase, only cows that were judged reproductively sound and tested non-pregnant by rectal palpation were purchased and examined by BioPRYN.

BioPRYN rate of use: BioPRYN tests were sold directly to producers or to affiliate laboratories by BioTracking LLC. Sales records over the past three years were used to show change in use of this technology.

Results and Discussion

BioPRYN assay performance: Figure 1 shows the optical density (OD) readings for samples in a typical plate. The solid line is the cutoff that was calculated from the two PSPB standards on each assay plate. The pregnancy status of cows for the samples with OD's falling between the solid and dotted lines are designated "pregnant but repeat" (above solid line, two samples) and "open but repeat" (below solid line, two samples). There is a distinct difference in values for OD readings for the open and pregnant cows respectively (outside the dotted lines). This difference helps assure proper classification of pregnancy status, especially for accurate designation of non-pregnant cows that would then be safe for re-synchronizing the estrous cycle; and, pregnant animals would not be aborted. The cutoff point has purposefully been set at a low level to avoid treatment of pregnant cows with re-synchronizing hormones. The "repeat" ranges add margins of safety. Normally, cows at 30 days of gestation have OD's distinctly in the "pregnant" range and those that are not pregnant are normally distinctly in the "open" range. If the OD falls within the range of the dotted lines, the pregnancy status cannot be determined. This can occur if a) an embryo has died and is being resorbed leaving residual PSPB that was detected, b) the sample was taken too early (before 30 days after conception), or c) embryo development was delayed or inadequate for delivery of sufficient PSPB to the circulation of the dam. A second blood sample collected four days or more after the first one can result in definitive detection. More than 80% of samples submitted a second time, collected over a one-year period from one dairy tested non-pregnant (unpublished).

Experiment 1: Table 1 shows the results of BioPRYN and ultrasound tests in commercial dairy herds in Hungary. There were 172 pregnant and 164 non-pregnant cows from the ultrasound test. The BioPRYN assay correctly categorized all pregnant cows for a sensitivity of 100%. Twenty non-pregnant cows were classified pregnant using BioPRYN; giving a specificity of 87.8%. None of the animals were placed in the "repeat" category. A sensitivity of 100% is highly desirable because non-pregnant animals can reliably be synchronized and without fear mistakenly treating a pregnant cow. The value for specificity of 87.8% is much lower than sensitivity. This lower specificity is due to, in part, the time interval between BioPRYN blood sampling and the time of the follow up ultrasound test. Embryos could potentially die during this time interval and, hence, obtaining a 100% specificity value would be rare. The value for specificity will inherently be lower than the value for sensitivity and depends upon variable embryo loss rates among herds and time after breeding. If cows are tested at 30 days after AI, embryo loss has a greater affect on specificity of a test than after 40 days. This is merely due to a lesser rate of embryo loss as time in pregnancy progresses (Alexander et al., 1995). Embryo loss can be high. Alexander et al. (1995) showed that the RIA for PSPB in detecting pregnancy had a 5.3% rate of embryo loss when tested first at 30 to 45 days and again at 60 days.

Experiment 2: All heifers in this experiment were expected to be non-pregnant. They were used to

demonstrate the effectiveness of the BioPRYN test in detecting non-pregnant animals. Rectal palpation detected that all 191 heifers were non-pregnant. The BioPRYN test detected 188 as non-pregnant and three as pregnant. One of the pregnant animals aborted after prostagladin F2 alpha treatment. The remaining two were not observed to have abortion and one heifer OD reading fell in the range of "pregnant but repeat" suggesting that the quantity of PSPB in the circulation was low. A repeat BioPRYN test after four days or more could have confirmed the status of that heifer. The other one had an OD typical of animals that are early in pregnancy. Abortion signs are less likely to be observed this early in pregnancy. For data analysis, these two animals were assigned as detected incorrectly by BioPRYN; this may be an incorrect assignment.

With the above assumptions, rectal palpation erred on one pregnant heifer. The two other heifers that BioPRYN detected as pregnant may have been too early in pregnancy to be detected by palpation. The OD readings suggest they were in and early stage of pregnancy. Calculation of sensitivity and specificity for BioPRYN using palpation as a "true value" may not be valid. However, for BioPRYN, these values were 100 and 98.9% in the group of heifer that were mostly not pregnant.

Experiment 3: Marketed dairy cows were purchased on three different occasions during January and February of 2007. One hundred-eight of the 208 tested pregnant (51.9%) by BioPRYN. The reason for sale of each cow is unknown but many were likely sold because of presumed reproductive failure on the dairy. In addition, palpation at time of purchase showed they were non-pregnant, although reproductively sound. The BioPRYN test found a high rate of pregnancy and data suggest that either minimal or inaccurate testing for pregnancy on the dairy could lead to unintended sale of pregnant cows. These data show that there was a high rate of pregnancy in marketed dairy cows at sale barns during the above two months.

BioPRYN rate of use: Table 2 shows the number of cattle tests sold by BioTracking LLC directly to producers or affiliate laboratories over a three-year period. There was a large increase in use of BioPRYN during this time. Increase was 61% from 2004 to 2005 and 144% from 2005 through 2006 or a 1.6 and 2.4 fold increase by year respectively. The affiliate laboratories sold more than the home laboratory, BioTracking LLC, in 2006. The affiliate laboratories are located at 10 sites in the US, two in Canada and one in Hungry.

These data show that the BioPRYN test has high sensitivity and specificity for testing cattle early during pregnancy or for testing non-pregnancy. Breeders used the test in increasing numbers over the past three years. It was shown that many reproductively sound dairy cows, marketed at sale yards, are pregnant. Several cow may not have been sold had accurate pre-sale testing been performed.

Implications

The BioPRYN test is equivalent to ultrasound and more accurate than palpation for detection of pregnancy. The test offers producers and veterinarians a convenient option for pregnancy testing in cattle without the variability in training and expertise of the one performing the test. A significant number of dairy cows that were marketed were pregnant and may not have been sold from the dairy had this been known. More accurate testing on the farm would save considerably in replacement costs if this marketing did not occur. The significant increase in number of cows tested shows that the BioPRYN test can become widely accepted. Testing is available in the US, Canada and Hungary.

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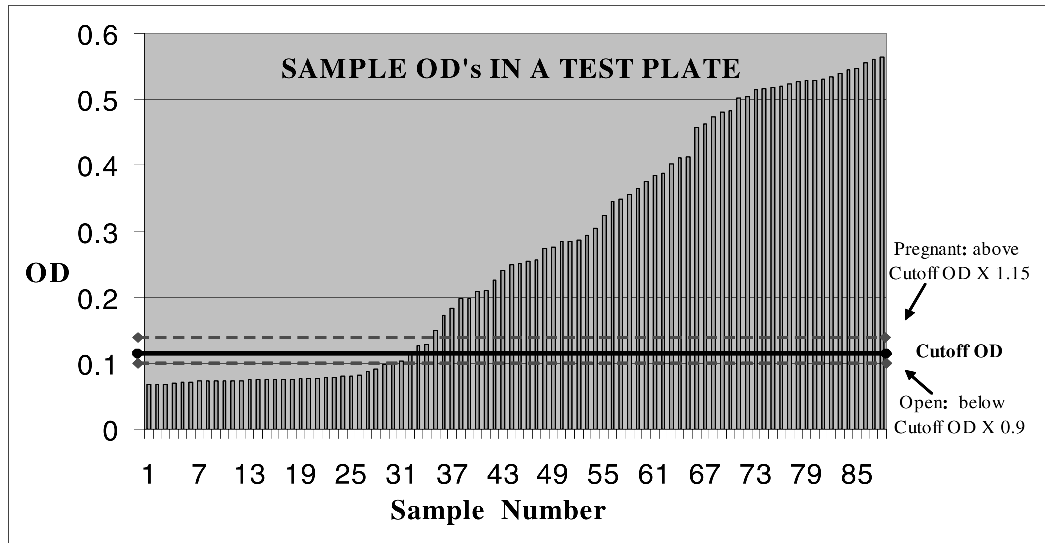

Figure 1. Optical density (OD) readings arranged in order from low to high values for 88 samples in a test plate. The cutoff OD is depicted as a solid line while an undecided range is shown between the dotted lines. Values outside the dotted lines are designated pregnant or non-pregnant (open). Owners were asked to send a new sample from the undecided as soon as a report is received.

Table 1. Agreement between the BioPRYN test for pregnancy at 30 to 37 days after artificial insemination (AI) and the ultrasound (US) test for pregnancy at 38 to 43 days after AI in commercial dairy cows.

		BioPRYN		Total by US
		Pregnant	Not Pregnant	
Ultrasound Test	Pregnant	172	0	172
	Not Pregnant	20	144	164
Total by BioPYRN		192	144	Over all Total 336

Table 2. Number of test wells sold by BioTracking and affiliate laboratories over three years as a demonstration of increased acceptance of the BioPRYN test for pregnancy in cattle.

	Number of Sample Wells Sold Each Year		
	2004	2005	2006
BioTracking Laboratory	29,334	45,786	86,815
Affiliate Laboratories	23,496	39,248	120,384
Total	52,830	85,034	207,199

Proceedings, Western Section, American Society of American Science
Vol. 58, 2007

EFFECTS OF OVERNUTRITION AND UNDERNUTRITION ON SERUM METABOLIC HORMONES AND ESTRADIOL-17β CONCENTRATION IN SHEEP

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ABSTRACT: To determine the effects of energy in diet on serum concentrations of insulin, triiodothyronine (T_3), thyroxine (T_4) and estradiol-17β (E_2), ewes (n = 48; 59.4 ± 1.3 kg initial BW; 2.3 ± 0.1 initial BCS) were divided into control (C; n = 14), overfed (OF; 2 x C; n = 17) and underfed (UF; 0.6 x C; n = 17) nutritional planes for 8 wk before blood samples and ovary collection. Ewes were individually fed once daily with pelleted diets containing 2.4 Mcal of ME/kg and 13% crude protein (DM basis). Control ewes were fed 760 g/50 kg BW daily, OF ewes were fed ad libitum (at least 200% of control), and UF ewes received 456 g/50 kg BW daily (60%) of the control diet. Every two wk during nutritional treatment BW and BCS were determined. Follicular development was induced by twice daily injections of FSH on d 13 and 14 of the estrous cycle. Blood samples from jugular vein and ovaries were collected on d 15 of the estrous cycle. For each ewe, number of ovarian follicles was determined. During 8 wk, C did not change BW or BCS, OF gained ($P < 0.001$) 13.2 ± 0.9 kg but UF ewes lost ($P < 0.001$) 14.8 ± 0.8 kg, and BCS increased ($P < 0.001$) by 1.6 ± 0.1 for OF, decreased ($P < 0.001$) by 0.8±0.1 for UF and did not change for C ewes compared with initial BW and BCS. Serum insulin concentration tended ($P < 0.15$) to be greater in OF than UF ewes, but E_2 concentration tended ($P < 0.15$) to be greater in UF than OF ewes. Nutritional treatment did not affect serum T_3, T_4, and ratio of T_4:T_3, and number of visible small and large follicles. These data show that: 1) overfeeding and underfeeding result in altered BW and BCS; 2) overfeeding tended to increase serum insulin and underfeeding tended to increase serum E_2 but not T_3 or T_4; and 3) nutritional treatment does not affect number of ovarian follicles in FSH-treated ewes. Thus, high energy diets may enhance serum insulin but not E_2 and metabolic hormones, while low energy diets may enhance serum E_2 but not metabolic hormones in sheep. Furthermore, the mechanism through which enhanced energy in diet may affect insulin levels or decreased energy in diet may affect E_2 levels, and potentially metabolic and reproductive function including embryonic survival, remains to be elucidated.

Key words: overnutrition, undernutrition, hormones, ovarian follicles, sheep

Introduction

Nutritional status is a major factor influencing an animal's ability to maintain health and reproduce (Robinson, 1990; Webb et al., 1999; O'Callaghan et al., 2000). Nutrition has a significant impact on numerous reproductive and metabolic functions including hormone production, fertilization, and early embryonic development (Boland et al., 2001; Armstrong et al., 2003; Boland and Lonergan, 2005). The effect of diet on hormones regulating metabolic functions (e.g., insulin, triiodothyronine [T_3], thyroxine [T_4]) or reproduction (e.g., GnRH, FSH, LH, estradiol-17β [E_2]) in domestic ruminants have been demonstrated in several studies (Webb et al., 2004; Forcada and Abecia, 2006; Scaramuzzi et al., 2006). For example, enhanced levels of insulin were frequently observed in sheep or cows fed high energy diet (Vinoles et al., 2005; Armstrong et al., 2001, 2003). However, the existing data concerning the effects of diet on peripheral hormones concentration are frequently contradictory and relatively limited.

We hypothesized that overfeeding or underfeeding of non-pregnant ewes will alter peripheral metabolic hormones including insulin, T_3, T_4, and E_2. Therefore, the aim of the present study was to evaluate the effects of nutritional plane (control vs. overfeeding or underfeeding) on plasma concentration of insulin, T_3, T_4 and E_2, and on ovarian follicle number in FSH-treated ewes.

Materials and Methods

Treatment of animals

Western range (predominantly Targhee and Rambouillet) 2 to 3 years old ewes were standardized for live weight and BCS. Ewes were housed and fed in individual pens (0.86 x 1.47 m) at the Animal Nutrition and Physiology Center with 14 h of darkness and 10 h of light at 12°C with free access to water and mineral supplements. Ewes were divided into three groups: control (n = 13) received a maintenance diet (see below), overfed (n = 17) were fed ad libitum, and underfed (n = 17) received 60% of controls for 8 wks before blood sampling and ovaries collection. Every two wk, during the duration of the experiment ewes were weighed and BCS was evaluated. Estrus was synchronized by insertion of chrono-gest sponges (Intervet, UK) to the vagina for 14 d. By using vasectomized rams, estrus was detected 40 to 48 h after sponge withdrawal. Ewes received twice daily (morning and evening) injections with FSH-P (Sioux Biochemical, Sioux Center, IA, USA) on days 13 (5 mg/injection) and 14 (4 mg/injection) following estrus (d 0) as described before (Stenbak et al., 2001; Borowczyk et al., 2006). On d 15 of the estrous cycle blood samples were collected and ewes were ovariectomized (Luther et al. 2005). The study was initiated during the normal breeding season in August and finished in November. All procedures were approved by the IACUC of NDSU.

Nutritional management

After arrival and a 3-d adaptation to individual pens and pelleted diets, ewes were allocated randomly to three

nutritional groups. The diet contained: dehydrated beet pulp, 36.5%; dehydrated alfalfa, 20.3%; corn, 24.2%; soy hulls, 16%; soybean meal, 3.0% (% of dietary DM). The pelleted (0.48 cm diameter) diet, which was prepared and analyzed on site, supplied 2.4 Mcal/ME and 130 g crude protein (13%) per kg of diet DM basis and was offered in one portion daily. Dietary management procedures for both groups were similar to those described by Borowczyk et al. (2006). Control ewes received 760 g/50 kg BW daily (100%), overfed ewes were fed ad libitum (at least 200% of control), and the underfed ewes received 456 g/50 kg BW daily (60%) of controls (DM basis).

Samples collection and hormone assays

Blood samples (10 ml) were collected at ovariectomy from the jugular vein using vacutainers (Becton Dickinson, Franlin Lakes, NJ) and centrifuged (20 min at 1,500 x g), followed by collection of serum, freezing and storing at -70 C before hormone analysis. Concentration of insulin, T_3, T_4, and E_2 was determined using competitive chemiluminescent enzyme immunoassay (Immulite 1000, Siemens, Los Angeles, CA).

Following ovariectomy, the number of visible small ($\leq$ 3 mm) and large (> 3 mm) follicles on each ovary was determined.

Statistical analysis

Data were analyzed statistically by using the GLM program of SAS (SAS Inst., Inc., Cary, NC). When the overall F-test for treatment tended to be significant ($P \leq 0.15$), means were separated using the method of least significant difference.

Results

At the time treatment was initiated, BW was similar for control, overfed and underfed ewes (58.2 ± 2.9, 60.9 ± 1.6 and 59.2 ± 2.2 kg, respectively; Figure 1). Body weight of overfed was greater ($P < 0.0001$) than control or underfed ewes, and BW of underfed ewes was lower ($P < 0.0001$) than control ewes at 2 to 8 wk of experiment (Figure 1). Changes in BW were different ($P < 0.001$) for nutrition groups at wk 8 (Table 1). Average daily gains were greater or lower ($P < 0.001$) for overfed or underfed compared with control ewes, respectively (Table 1). When compared to initial BW, control ewes lost 0.05 ± 0.8 kg, overfed ewes gained 13.2 ± 0.9 kg but underfed ewes lost 14.8 ± 0.8 kg over the 8 wk experiment.

At the beginning of treatment, BCS was similar for control, overfed and underfed ewes (2.3 ± 0.2, 2.3 ± 0.1 and 2.4 ± 0.1 respectively; Figure 1). Body condition score of overfed ewes was greater ($P < 0.0001$) than control or underfed ewes at wk 2 to 8, but BCS of underfed was lower ($P < 0.0001$) than control or overfed ewes at wk 4 to 8 of the experiment (Figure 1). Changes in BCS were different ($P < 0.0001$) for nutrition groups at wk 8 (Table 1). During the 8 wk experiment, BCS increased by 0.4 ± 0.2 and by 1.6 ± 0.1 for control and overfed ewes, respectively, but decreased by 0.8 ± 0.1 for underfed ewes to compare with initial BCS.

Serum insulin concentration tended ($P < 0.15$) to be greater in overfed than underfed ewes, but E_2 concentration tended ($P < 0.15$) to be greater in underfed than overfed ewes (Figure 2). Serum concentration of T_3, T_4 and T_4:T_3, ratio were similar for control, overfed and underfed ewes (T_3: 118 ± 12, 117 ± 15 and 141 ± 14 ng/dL; T_4: 10 ± 1, 10 ± 1 and 11 ± 1 μg/dL; and T4:T3: 90 ± 8, 91 ± 8 and 81 ± 7, for control, overfed and underfed ewes, respectively). Plasma E_2 concentration was positively correlated ($P < 0.002$) with the total number of follicles ($r^2 = 0.475$), and number of large follicles ($r^2 = 0.491$).

Mean number of visible, small, and large follicles per ewe were similar for control, overfed, and underfed ewes (Table 1).

Discussion

The present study demonstrated that overfeeding resulted in greater BW and BCS, but underfeeding resulted in lower BW and BCS when compared to control ewes. Furthermore, peripheral insulin levels tended to be higher in overfed than underfed ewes, but peripheral E_2 levels tended to be lower in overfed than underfed ewes. Nutritional treatment did not affect peripheral T_3 or T_4 levels, or number of ovarian follicles.

In previous studies, for mature ewes fed for several wk a low or high energy diet, decreased or increased BW and BCS, respectively, were observed (Abecia et al., 1999; Lozano et al., 2003; Borowczyk et al., 2006). Thus, several weeks of overfeeding or underfeeding profoundly affects both BW and BSC in sheep.

In the present experiment, enhanced peripheral insulin concentration was observed in overfed ewes. Similarly, increased peripheral insulin level has been demonstrated for sheep fed high compared with maintenance diets for 3 to 12 mo with high BCS to compare with sheep fed maintenance diet with lower BCS (Caldeira et al., 2007a,b), and for sheep fed high energy diet for 6 d (Vinoles et al., 2005). On the other hand, Scaramuzzi et al. (2006) reported decreased insulin level in sheep managed in negative energy balance. Furthermore, serum insulin concentration was not affected by underfeeding or overfeeding for 14 d in sheep (Lozano et al., 2003). Such contradictory results may be due to different nutritional treatments, duration of treatment (e.g., from 6 d to 12 mo), and possibly other conditions like location or breed.

In the current study enhanced serum E_2 levels were observed in underfed compared to overfed ewes. Similarly, enhanced peripheral E_2 levels were observed in cows fed restricted diets for 2 to 8 wk (Jorritsma et al., 2003; Freret et al., 2006). Levels of E2 in peripheral blood may be related to E_2 clearance, e.g., in ewes fed restricted diet, a lower hepatic clearance may lead to greater E_2 concentration. However, this subject requires further investigation. In our study, levels of E_2 in serum were positively correlated with the number of follicles. This result is not surprising since ovarian follicles are the major source of estrogens (Sanger, 2003).

Nutritional treatment did not affect serum T_3 and T_4 concentration or T_4:T_3 ratio in our experiment. However, feeding restrictions of pregnant ewes from d 62 to 132 of pregnancy resulted in lower serum T3 and T_4, and enhanced T_4:T_3 ratio (Wart et al., 2005). Furthermore, plasma T_3 but not T_4 concentrations were decreased in ewes fed low or high energy diet for about 100 d with low

and high BCS compared with normal BCS (Caldeira et al., 2007a). Moreover, ewes fed low or high energy diets for 54 to 74 wk with low or high BCS, respectively, had decreased plasma T_3 and T_4 compare to ewes fed maintenance diets with BCS maintained at similar level during nutritional treatment (Caldeira et al., 2007b). Different effects of diet on peripheral T_3 and T_4 concentrations in above studies are likely due to differences in duration of nutritional treatment, reproductive stage, and breed.

In the present experiment, nutritional plane had no effect on the number of ovarian follicles. Peura et al. (2003) reported for adult ewes that low (0.7 x) or high (1.3 x) maintenance diets for 3 to 5 months before FSH-induced superovulation did not affect ovulation rates. Moreover, superovulatory responses after FSH-treatment were not affected by feeding diets at 0.5, 1.0, or 1.5 x maintenance during peri-conception period in adult ewes (Kakar et al., 2005). In contrast, O'Callaghan et al. (2000) reported increased number of larger follicles in ewes fed 2 x maintenance diet to compare with 0.5 or 1 x maintenance in non-stimulated ewes, and decreased number of follicles in ewes fed a 0.5 x maintenance diet to compare with 1 or 2 x maintenance diet in FSH-treated ewes. These discrepancies may be due to breed, location and hormonal treatment protocols, which differed in these studies. Thus, these results show that nutritional treatments may affect the number of visible follicles in sheep. Moreover, number of follicles in the present study was similar to that previously reported for FSH-treated mature ewes fed a maintenance diet during the normal breeding season and seasonal anestrus (Stenbak et al., 2001; Grazul-Bilska et al., 2003; Luther et al., 2005).

Numerous experiments indicate that nutrition has direct effect on some reproductive function by affecting hormonal production (O'Callaghan and Boland, 1999; Lucy, 2003; Hunter et al., 2004). For example, for underfed or overfed mature ewes with enhanced or decreased blood progesterone concentrations, respectively, altered oocyte and embryo quality was observed (McEvoy et al., 1995; Lozano et al., 2003). Furthermore, for cows fed high energy diets with enhanced insulin levels, impaired early embryonic development was reported (Adamiak et al., 2005). This indicates that effects of nutrition on oocyte and embryonic development may be indirectly linked through regulation of hormone secretion. In the present study, we have not evaluated the oocyte quality. Therefore, future studies should be undertaken to define the association between nutrition, hormone levels, and oocyte quality and early embryonic development.

Effects of nutrition on peripheral hormone levels may reflect the general energy balance (e.g., maintenance diet vs. low or high energy diets) but also can be attributed to the specific nutrients in diets, such as vitamins, minerals, and other supplements (Wrenzycki et al., 2000). Because, several studies provided contradictory and inconsistent results of dietary effects on serum metabolic hormones and E_2 concentrations, a detailed study using a large number of animals should be undertaken to determine the effects of nutrition on metabolic and reproductive hormone secretion in domestic ruminants.

In summary, this experiment demonstrated that: 1) overfeeding and underfeeding resulted in altered BW and BCS; 2) overfeeding tended to increase serum insulin but underfeeding tended to increase E_2 but not T_3 or T_4; and 3) nutritional treatment did not affect follicular development in FSH-treated ewes. Thus, high energy consumption may enhance serum insulin, but not E_2 or other metabolic hormone levels, while low energy intake may enhance E_2 but not metabolic hormones in sheep. Furthermore, the mechanism through which enhanced or reduced energy in diet may affect insulin and E_2 levels, and potentially metabolic and reproductive function including embryonic survival, remains to be elucidated. We manipulated total dietary intake in the present study, but future investigations that address specific dietary nutrient composition should provide insight into the underlying mechanisms associated with nutrition effects on metabolic and reproductive function.

Acknowledgements

The authors would like to Mr. Kim Kraft, Tim Johnson, Wes Limesand and Terry Skunberg, and other members of our laboratory for their technical assistance.

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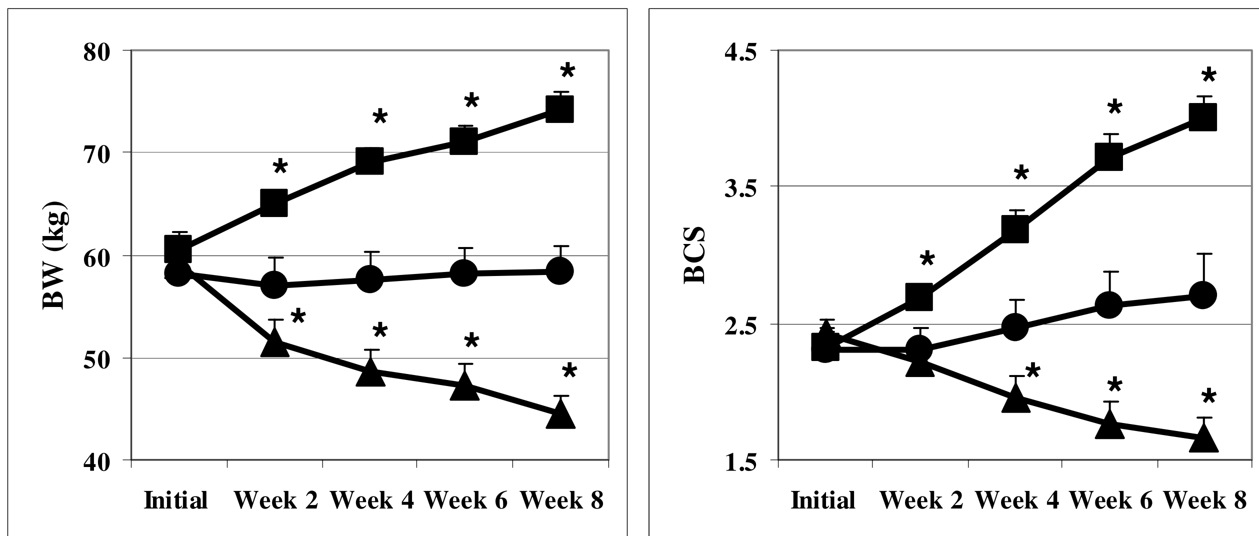

Figure 1. Body weight (left) and BCS (right) in control (circles), overfed (squares) and underfed (triangles) ewes. *$P < 0.0001$; Means ± SEM differ from control within a specific week.

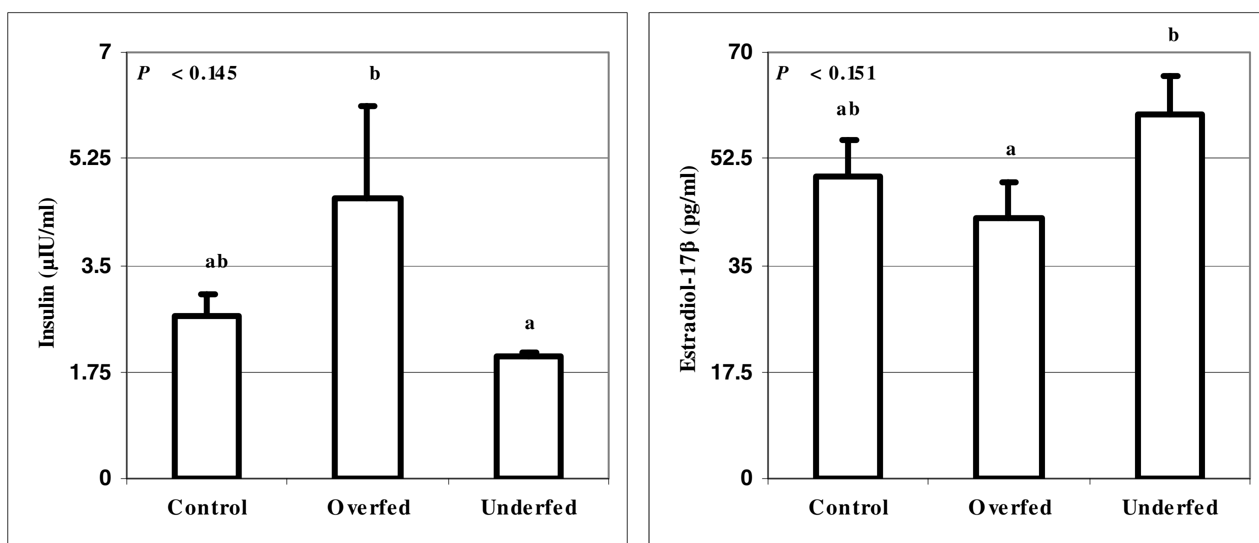

Figure 2. Insulin (left) and E_2 (right) serum concentration in control (n = 13) overfed (n = 16) and underfed (n = 15) ewes. [a,b] $P < 0.05$; Means ± SEM with different superscripts differ, unprotected F-test.

Table 1. Effects of nutrition on BW, ADG, BCS, and the number of ovarian follicles in control, overfed, and underfed ewes.

Item	Control	Overfed	Underfed	*P* value
Number of ewes	13	17	17	
Initial BW, kg	58.2 ± 2.9	60.9 ± 1.6	59.2 ± 2.2	0.693
Final BW, kg	58.3 ± 2.6	74.1 ± 1.8	44.4 ± 1.9	0.0001
Difference in BW, kg	0.05 ± 0.8	13.2 ± 0.9	-14.8 ± 0.8	0.0001
ADG, kg	0.00 ± 0.01	0.24 ± 0.02	-0.26 ± 0.01	0.0001
Initial BCS	2.3 ± 0.2	2.3 ± 0.1	2.4 ± 0.1	0.775
Final BCS	2.7 ± 0.3	3.9 ± 0.1	1.7 ± 0.2	0.0001
Difference in BCS	0.4 ± 0.2	1.6 ± 0.1	-0.8 ± 0.1	0.0001
Total follicles (n)	27.2 ± 2.9	27.2 ± 2.8	29.8 ± 2.8	0.748
Large follicles (n)	17.0 ± 1.8	16.7 ± 2.5	16.7 ± 2.1	0.995
Small follicles (n)	10.4 ±1.5	10.5 ± 1.2	13.1 ± 1.6	0.334

*All values (mean ± SEM) are expressed per ewe.

Proceedings, Western Section, American Society of American Science
Vol. 58, 2007

SUPEROVULATION IN SHEEP: NUMBER AND WEIGHT OF THE CORPORA LUTEA AND SERUM PROGESTERONE

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ABSTRACT. To determine similarities and differences between non-superovulated and superovulated ewe models, data collected from several experiments (1988-2005) were analyzed. Mature non-pregnant non-superovulated (n = 91) or superovulated (n = 299) ewes of mixed breeds were used for evaluation of luteal function. To induce superovulation, ewes were injected twice daily (morning and evening) with FSH-P purchased from Schering (Kenilworth, NJ; 1988 - 1994; n = 128) or Sioux Biochemical (Sioux Center, IA; 1996 - 2005; n = 171) on days 13 to 15 of the estrous cycle. At CL collection on d 5 or 10 of the estrous cycle, number of CL was determined, and for selected ewes the CL were weighed and blood samples were collected for determination of serum progesterone (P4). Number of CL after FSH treatment from two sources was similar, therefore data are combined. Superovulated ewes had greater ($P < 0.001$) number of CL than non-superovulated ewes (16.2 ± 0.5 vs. 1.9 ± 0.1). Weight of CL on day 5 of the estrous cycle was similar for superovulated and non-superovulated ewes (252.2 ± 4.1 vs. 224.7 ± 15.6 mg), but on day 10, weight of CL from superovulated ewes was less ($P < 0.05$) than from non-superovulated ewes (379.9 ± 4.0 vs. 598.7 ± 18.5 mg). Luteal tissue mass per ewe was greater ($P < 0.001$) for superovulated than non-superovulated ewes on d 5 and 10 of the estrous cycle. Serum P4 concentration on d 5 of the estrous cycle was similar for superovulated and non-superovulated ewes (2.3 ± 1.1 vs. 1.3 ± 0.1 ng/ml), but on d 10 tended to be greater ($P < 0.06$) in superovulated than non-superovulated ewes (5.7 ± 1.3 vs. 3.8 ± 0.3 ng/ml). When P4 concentration in serum was expressed per g of luteal tissue mass, values were similar for non-superovulated and superovulated ewes on d 5 and 10 of the estrous cycle. Moreover, all P4 values were greater ($P < 0.05$) on d 10 than on d 5 of the estrous cycle. Thus, despite of some differences in CL number and CL weight, the major function of the CL, P4 production seems to be not altered in superovulated ewes. Therefore, these data indicate that our superovulated ewe model may be used for studies of luteal function. *Supported by USDA, NIH and NSF grants for ATGB, DAR and LPR, and NIH grant P20 RR016741 from the INBRE program of the NCRR (1988-2007).*

Introduction

Assisted reproduction technologies (ART) have been used in agriculture for many decades to increase reproductive potential of domestic farm animals (Gordon, 1997, 2005; Grazul-Bilska, 2004). In sheep, use of these techniques can help enhance reproductive efficiency (Cognie et al., 2003). Superovulation protocols allow to take advantage of the relatively short gestation length of sheep and utilize the ewe to her fullest potential (Gordon, 1997; Gonzales-Bulnes et al., 2004).

Superovulation was developed approximately 55 years ago and has been implemented in sheep research and production (Driancourt and Fry, 1992; Gordon, 1997, 2005). Treatment with FSH causes multiple follicles to develop followed by ovulation and creation of multiple corpora lutea (CL; Gordon, 1997, 2005). Thus, the superovulated ewe has 5 to 15 CL (Hild-Petito et al., 1987; Jablonka-Shariff et al., 1993; Gonzales-Bulnes et al., 2004). When CL from superovulated and non-superovulated ewes were compared, morphology and function were similar (McClellan et al., 1975; Hild-Petito et al., 1987). Since CL obtained from superovulated versus non-superovulated ewes have not been compared in detail, our study was designed to further determine similarities and differences of CL development and function in two ewe models.

The aims of this study were to: 1) compare the effects of two separate FSH preparations on the number and weight of CL; 2) determine the number and weight of CL in superovulated vs. non-superovulated ewes; and 3) determine serum progesterone (P4) concentration in superovulated vs. non-superovulated ewes.

Materials and Methods

Animal Treatment and Tissue Collection

The Institutional Animal Care and Use Committee at NDSU approved all animal procedures in this study. From 1988 to 2005, mature non-pregnant ewes (n = 390) of mixed breeds (predominantly Targhee x Rambouillet) were used for several experiments to evaluate luteal function. A portion of ewes was non-superovulated (n = 91), and a portion of ewes was superovulated (n = 299). To induce superovulation, ewes were injected twice daily (morning and evening) with FSH-P (FSH with 10% luteinizing hormone) purchased from Schering (Kenilworth, NJ; 1988 - 1994; n = 128 ewes) or Sioux Biochemical (Sioux Center, IA; 1996 - 2005; n = 171 ewes) on days 13 (5 units/injection, day 0 = estrus), 14 (4 units/injection) and 15 (3 units/injection) of the estrous cycle (total dose = 24 units) to induce superovulation (Grazul-Bilska et al., 1991, 2001). Standing estrus (day 0 of the estrous cycle) was determined by using vasectomized rams.

At CL collection on d 5 or 10 of the estrous cycle, number of CL was determined for all non-superovulated (n = 91) and superovulated (n =299) ewes. CL were

weighed for a portion of non-superovulated (n = 86) and superovulated (n = 87) ewes. Blood samples were collected for selected non-superovulated (n = 24) and superovulated (n = 15) ewes on day 5 and 10 of the estrous cycle to determine serum P4 concentration.

Progesterone RIA

Progesterone concentrations in extracted serum were measured as previously reported (Jablonka-Shariff et al., 1993; Vonnahme et al., 2006). Sensitivity of the assay was 12.5 pg/tube. The intra- and inter-assay coefficients of variation ranged from 3.4 to 6.4% and from 7.1 to 12.6%, respectively.

Statistical Analysis

Data was analyzed using the general linear model (GLM) procedure of SAS and presented as means ± SEM. When the F-test was significant ($P < 0.05$), differences between specific means were evaluated by using least significant differences test (Kirk, 1982).

Results

Source of FSH did not affect number and weight of CL. A similar number of ovulations was achieved when sheep were treated with FSH-P from Schering and Sioux Biochemical (15.3 ± 0.7 and 16.9 ± 0.8 CL per ewe, respectively; $P > 0.05$). Therefore, data for these two FSH preparations were combined for further analysis.

The number of CL and the weight of each CL for non-superovulated and superovulated ewes on d 5 and 10 of the estrous cycle are presented in Table 1. The number of CL per ewe was greater ($P < 0.001$) in superovulated ewes than non-superovulated ewes (Table 1). The percentage of non-superovulated ewes with 1, 2, 3 or 4 CL was 30 % (n = 27), 56 % (n = 51), 9 % (n = 8), and 5.5 % (n = 5), respectively. The number of CL for superovulated ewes which responded to FSH-treatment (n = 245), ranged from 5 to 52/ewe; 89% of ewes had 5-25 CL, and 11% had 26-52 CL (Figure 1). On d 5 of the estrous cycle, the weight of the individual CL was similar for non-superovulated and superovulated ewes. But on d 10, individual CL weight from the non-superovulated ewes was greater ($P < 0.05$) than from the superovulated ewes (Table 1). Total luteal tissue weight per ewe was greater ($P < 0.001$) for superovulated than non-superovulated ewes on d 5 and 10 of the estrous cycle (Table 1). The individual CL weight and total luteal tissue weight per ewe were greater on d 10 than on d 5 of the estrous cycle for both non-superovulated and superovulated ewes (Table 1).

Serum P4 concentrations, and P4 secretion expressed per g of luteal tissue mass in non-superovulated and superovulated ewes is presented in Table 2. Serum P4 concentrations were similar for non-superovulated and superovulated ewes on d 5 of the estrous cycle. However, on d 10 of the estrous cycle, serum P4 concentration tended to be greater ($P < 0.06$) in superovulated than non-superovulated ewes (Table 2). When P4 secretion was expressed per g of total luteal tissue mass per ewe, P4 values were similar for non-superovulated and superovulated ewes. In addition, all P4 values were greater on d 10 than on d 5 of the estrous cycle for both non-superovulated and superovulated ewes.

Number of CL was positively correlated ($P < 0.001$) with serum P4 concentration ($r^2 = 0.578$) and luteal tissue mass ($r^2 = 0.940$), and serum P4 concentration was positively correlated ($P < 0.01$) with luteal tissue mass ($r^2 = 0.640$) and P4 secretion expressed per g of luteal tissue mass ($r^2 = 0.434$).

A proportion of ewes (overall 16.3%; n = 54) did not respond to FSH induction of superovulation, which was manifested by the presence of 1 to 4 CL after FSH treatment. During years 1988 to 1994 (when FSH-P from Schering was used) and during years 1995 to 2005 (when FSH-P from Sioux Biochemical was used) lack of superovulatory response to FSH treatment was similar between these two FSH preparations (13.2 ± 2.8 % and 19.5 ± 3.1 % of ewes, respectively).

Discussion

Our study demonstrated no differences in number of CL in response to superovulation treatment with FSH from two different sources/preparations. This indicates that these FSH preparations were equally active in induction of superovulation. The ratio of FSH:LH is critical for the development of the ovum (Donaldson, 1991; Senger, 2003; D'Alessandro et al., 2005). The purified FSH preparation is optimal when it includes less than 10 % LH (Donaldson, 1991; Boscos et a., 2002). Therefore, in this study we used the FSH preparations containing less than 10 % LH.

Differences in CL number were observed for superovulated vs. non-superovulated ewes in this study. The number of CL ranged from 1 to 4 (average 1.9) and from to 5 to 52 (average 16.2) for non-superovulated and superovulated ewes, respectively. Similar average numbers of CL per ewe were reported in other studies using a multiple FSH-treatment protocol (Amiridis et al., 2002; Ammoun et al., 2006; Mossa et al., 2006; Veiga-Lopez et al., 2006). However, when lower doses of FSH, or one injection of FSH, or PMSG were used, number of CL varied from 1 to 6 (Boscos et al., 2002; Riesenberg et al., 2001; Hild-Petito et al., 1987). Thus, number of CL after induced superovulation depends on dose and type of hormone used (e.g., FSH or PMSG), frequency of treatment, and also time of treatment related to the stage of the estrous cycle.

In the present study, the weight of individual CL and serum P4 concentration in superovulated ewes were similar to non-superovulated ewes on d 5 of the estrous cycle. However, on d 10 of the estrous cycle, CL weight was less in superovulated than non-superovulated ewes, but serum P4 concentration was greater in superovulated than non-superovulated ewes. Hild-Petito et al. (1987) reported similar CL weight for non-superovulated and superovulated ewes on d 10 of the estrous cycle, but greater serum P4 concentrations in superovulated than non-superovulated ewes. These discrepancies in CL

weight are likely due to the different superovulation protocol used in these studies. Thus, superovulation protocols may affect not only the number of CL, but also weight of fully differentiated CL.

On d 5 of the estrous cycle, the CL is still rapidly growing and differentiating, therefore, differences in CL weight or serum P4 concentrations could not be observed during the early luteal phase for non-superovulated and superovulated ewe models. By d 10, when the CL reaches its fully functional and differentiated stage, differences in weight and serum P4 concentrations for non-superovulated and superovulated ewes were observed. Thus, as compared to non-superovulated ewes, when multiple CL are developing in superovulated ewes, growth seems to be limited and they fail to achieve their typical weight. Furthermore, it seems that because there are more CL on the superovulated ovary that they had less room to grow and consequently grew smaller to approximately 0.6x of the size of individual CL found on non-superovulated ovaries. Furthermore, reduced luteal weights on d 10 of the estrous cycle in superovulated ewes is likely associated with control of luteal function by LH (Niswender and Nett, 1994). Since greater luteal tissue mass can produce more P4, as observed in our and other studies (Amiridis et al., 2002) in superovulated ewes, P4 through negative feedback may inhibit LH secretion which in turn may limit growth of the CL. However, this concept requires further investigation.

When P4 secretion was expressed per g of luteal tissue mass, P4 values were similar for superovulated and non-superovulated ewes on d 5 and 10 of the estrous cycle. Similar observations were reported by Hild-Petito et al. (1987). Thus, luteal tissues in superovulated ewes secrete amounts of P4 similar to non-superovulated ewes which is likely due to tight control by LH. Furthermore, it has been demonstrated that CL structure and function measured by P4 secretion and in vitro responsiveness of luteal cells to LH and dbcAMP treatment were similar for non-superovulated and superovulated ewes (McClellan et al., 1975; Hild-Petito et al., 1987). We have also demonstrated in several studies, that luteal cells from superovulated ewes responded to LH or dbcAMP stimulation by increasing P4 secretion in vitro (Grazul-Bilska et al., 1991; 1995, 1996). Therefore, these data indicate that function of CL in superovulated ewes is similar to function of CL in non-superovulated ewes.

Several other studies demonstrated that serum or plasma P4 concentrations were enhanced in superovulated ewes during the estrous cycle (Hild-Petito et al., 1987; Amiridis et al., 2002). Furthermore, Amiridis et al., (2002) showed a positive relationship between number of CL and serum P4 levels on d 5 of the estrous cycle. In our study, positive correlations were also observed between P4 secretion and CL number and luteal tissue mass. This clearly demonstrates that the amount of P4 circulating in the blood is relative to the total luteal tissue mass. However, as discussed above, secretion of P4 seems to be limited by LH and possibly other factors in superovulated ewes.

In this study, approximately 16% of ewes did not respond to the FSH treatment as indicated by presence of only 1 to 4 CL. In agreement with our data, Cognie (1999) reported that about 20% of ewes do not respond to superovulatory treatment. It has been hypothesized that a lack of superovulatory response to FSH by some ewes is due to a heterogeneity in the morphological features of the ovulatory follicles or to the number of small antral follicles present in the ovaries when FSH treatment was initiated (Draincourt, 2001; Cognie et al., 2003). Also season, genetics and nutritional treatments may all contribute to the variability of responsiveness to FSH treatments (Cognie, 1999). Future study should be undertaken to determine why a relatively large proportion of ewes does not respond to the FSH-treatment.

In summary, this study demonstrated that 1) two FSH preparations had similar effects on the number and weight of the CL in superovulated ewes; 2) number of CL was greater in superovulated than non-superovulated ewes; 3) weight of individual CL was similar on d 5 but less on d 10 in superovulated than non-superovulated ewes; 4) serum P4 concentration was similar on d 5 but greater on d 10 in superovulated than non-superovulated ewes; 5) P4 secretion expressed per g of luteal tissue mass was similar on d 5 and 10 for superovulated and non-superovulated ewes, and 6) 16% of ewes did not respond to FSH treatment.

Implications

Preparations of FSH used in this study were effective in inducing superovulation in ewes. Variation in number of CL and weights of luteal tissue between non-superovulated and superovulated ewes did not significantly affect P4 secretion when expressed per g of luteal tissue mass. Therefore, this superovulated ewe model is a reasonable model for the study of luteal function. This model is also helpful for generating larger amounts of luteal tissue per animal for use in complex studies of the CL function.

Acknowledgments

The authors would like to thank Mr. Robert Weigl, Mr. Wes Limesand, Mr. Tim Johnson, Mr. Terry Skunberg and other members of our laboratory for their technical assistance, and Ms. Julie Berg for clerical assistance.

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Table 1. The effects of superovulation on number and weight of the CL on d 5 and 10 of the estrous cycle.

	Non-superovulated	Superovulated
Number of CL	1.9 ± 0.1^a (n = 91 ewes)	16.2 ± 0.5^b (n = 245 ewes)
Weight of CL (mg)		
Day 5	224.7 ± 15.6 (n = 39 CL)	252.2 ± 4.1 (n = 443 CL)
Day 10*	598.7 ± 18.5^A (n = 123 CL)	379.9 ± 4.0^B (n = 936 CL)
Luteal tissue mass/ewe (g)		
Day 5	0.46 ± 0.06^a (n = 24 ewes)	3.74 ± 0.37^b (n = 29 ewes)
Day 10*	1.20 ± 0.05^a (n = 62 ewes)	6.12 ± 0.49^b (n = 58 ewes)

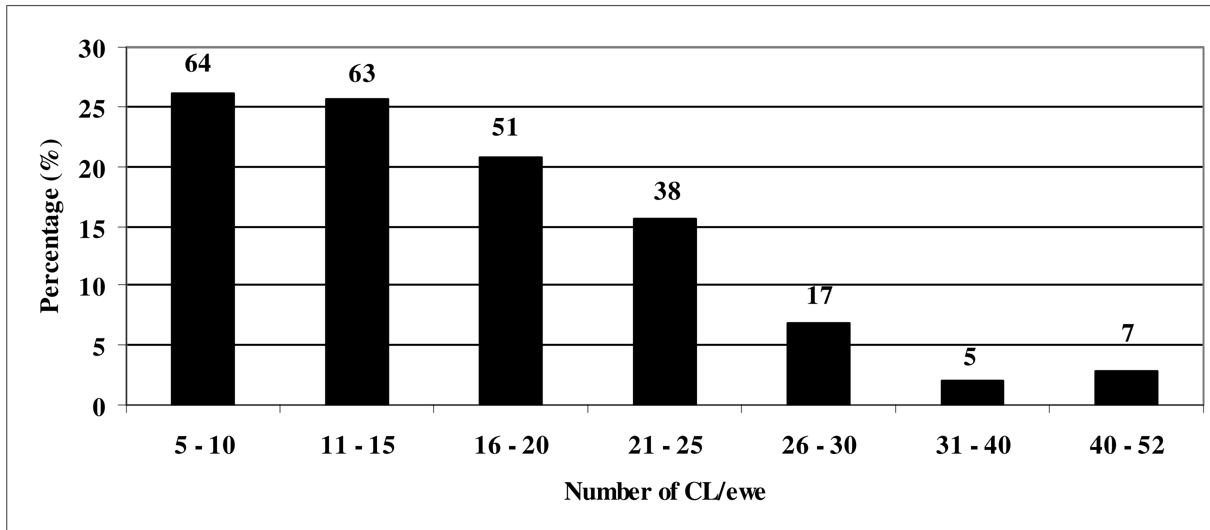
^{a,b} $P < 0.001$; ^{A,B} $P < 0.05$; means ± SEM with different superscripts differ within a row;
*$P < 0.05$; means ± SEM for CL weight and luteal tissue mass on d 10 are greater than on d 5 of the estrous cycle within a column.

Table 2. The effects of superovulation on progesterone (P4) concentration in serum (ng/ml) on d 5 (n = 12 for non-superovulated and n = 5 for superovulated) and 10 (n = 12 for non-superovulated and n = 10 for superovulated) of the estrous cycle.

		Non-superovulated	Superovulated
P4 (ng/ml)	Day 5	1.28 ± 0.13	2.32 ± 1.06
	Day10**	3.82 ± 0.33^a	5.75 ± 1.26^b
P4 (per g of luteal tissue)*	Day 5	0.22 ± 0.02	0.27 ± 0.05
	Day 10**	0.68 ± 0.04	0.87 ± 0.26

*calculated by dividing P4 concentration in serum by luteal tissue mass per ewe.
**$P < 0.001$; means ± SEM for P4 values on d 10 are greater than on d 5 of the estrous cycle within a column.
^{a,b} $P < 0.06$; means ± SEM with different superscripts differ within a row;

Fig. 1. Percentage of superovulated ewes (n = 245) with multiple CL (number above bar indicates number of sheep).



Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECT OF FEED PEAS ON RUMINAL FERMENTATION, DIGESTIBILITY, AND NITROGEN LOSSES IN DAIRY COWS

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ABSTRACT: The objective of this experiment was to investigate the effect of partial substitution of soybean meal and corn grain with feed peas on ruminal fermentation, digestibility, and urinary N losses in a replicated 3 × 3 Latin square design with 6 ruminally-cannulated dairy cows. Treatments were: (1) control diet; (2) rolled peas diet (RP) – 15% dry rolled peas replacing 45% of the corn grain and 78% of the SBM; and (3) ground peas (GP) – as RP, but peas were coarsely ground through a hammer mill. Diet had no effect ($P = 0.151$ to 0.881) on ruminal pH and total and individual VFA. Acetate to propionate ratio was increased ($P = 0.001$) with the RP diet compared with the control and GP diets. Ruminal ammonia concentration was greater ($P = 0.010$) for the pea diets compared with the control. Total tract apparent digestibility of DM, OM, NDF, N, and starch were not different ($P > 0.05$) between the control and GP diets. The RP diet had lower ($P = 0.004$ to 0.036) total tract digestibility of DM, OM, N, and starch than the control and GP diets. Peas had greater ($P < 0.001$) N solubility *in situ* than SBM (32 vs. 16%, respectively). Urinary N losses, as proportion of N intake, tended to be greater ($P = 0.062$) for GP than the control and RP diets. In conclusion, these data suggest that pea protein is more soluble in the rumen than SBM protein and inclusion of 15% peas in the diet of dairy cows resulted in elevated ruminal ammonia concentration. Peas have to be coarsely ground for dairy cow diets to avoid depression in total tract digestibility of nutrients.

Key Words: Feed Pea, Dairy Cow, Ruminal Fermentation, Digestibility

Introduction

The growing dairy industry in the Pacific Northwest, and particularly Idaho, offers an opportunity for expanding the markets for non-traditional, locally-grown feedstuffs such as dry peas. Peas have relatively high crude protein (**CP**) content and contain a significant amount of energy in the form of starch, which makes them a unique feed that can be substituted for higher priced protein and energy commodities like soybean meal (**SBM**), corn, and barley in dairy cow diets. Studies on feeding peas to dairy cows are very limited, particularly in N. America. Several studies investigated the effect of peas on milk yield and composition and concluded that when peas replace SBM and corn or barley, there was no effect on milk production or composition (Petit et al., 1997; Khorasani et al., 2001). In a related experiment with high-producing dairy cows, inclusion of feed peas at 15% of dietary DM, replacing SBM and corn grain had no effect on DM intake, milk yield, and milk composition (Vander Pol and Hristov, 2006). There is very limited research on the effects of inclusion of peas in dairy cow diets on ruminal fermentation and nutrient digestibility. Khorasani et al. (2001) reported a linear decrease in ruminal pH and a linear increase in ammonia concentration when peas replaced from 33 to 100% SBM in the diet of lactating dairy cows. Digestibility of dietary nutrients was not affected by inclusion of peas (Petit et al., 1997; Froidmont and Bartiaux-Thill, 2004). Thus, there is a need for more research, particularly in the U.S., on the effects of inclusion of dry peas in the diet of lactating dairy cows on ruminal fermentation, digestibility, and N losses. Therefore, the objectives of this study were to investigate the effect of isonitrogenous and isoenergetic substitution of 15% of dietary DM (as solvent-extracted SBM and steam-rolled corn grain) with U.S. No. 1 feed peas in the diet of lactating dairy cows on ruminal fermentation, total tract digestibility of nutrients, and urinary N losses.

Materials and Methods

Four multiparous and two primiparous Holstein cows fitted with 10-cm ruminal cannulae (Bar Diamond, Parma, ID) were used in this experiment. The cows (BW 680 ± 60 kg; days in milk 127 ± 29 d at the beginning of the trial) were cared for according to the guidelines of the University of Idaho Animal Care and Use Committee and were subjected to the experimental treatments in a replicated 3 × 3 Latin square design. Treatments were (Table 1): (1) control diet; (2) rolled peas diet (**RP**) – 15% (DM basis) dry rolled peas replacing 45% of the corn grain and 78% of the SBM; and (3) ground peas diet (**GP**) – as RP, but peas were coarsely ground through a hammer mill. Each experimental period consisted of 12 d for adaptation to the diet and 7 d for sampling. Cows did not receive rBST during the trial.

The diets were formulated (NRC, 2001) to provide similar amounts of NE_L, CP, RDP (68% of CP), and RUP (32% of CP) and meet the nutrient requirements of a Holstein cow yielding 35 kg milk/d with 3.5% milk fat and 3.0% true protein. Approximately 45% of the corn grain and 78% of the SBM in the control diet were replaced with 15% (DM basis) feed peas in the RP and GP diets. Peas were rolled or coarsely ground through a

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hammer mill prior to feeding. The Geometrical Mean Diameter of the ground peas was 1.44 mm (sieve shaker AS200, Retsch GmbH, Haan, Germany). Diets were fed as TMR at 0600 and 1800, *ad libitum* to approximately 5 to 10% orts. Cows had free access to fresh water and salt blocks through the duration of the trial.

Table 1. Ingredient and chemical composition of the diets fed in the trial.

	Diets	
Ingredient	Control	Pea[1]
Alfalfa hay[2]	28.0	28.0
Corn silage[3]	17.7	17.7
Whole cottonseed	7.0	7.0
Corn grain, steam-rolled	19.8	10.8
SSBM[4], 48% CP	7.4	1.6
Peas[5]	-	15.0
Dry distiller grain with solubles	6.0	6.0
Barley grain, steam-rolled	12.0	11.8
Mineral/vitamin mix	2.1	2.1
Composition[6] (% of DM)		
CP	15.7	16.2
NDF	36.9	35.4
NE_L, Mcal/kg DM	1.56	1.58
Starch	21.2	21.3
NFC	36.6	37.9

[1] Peas were rolled (RP diet) or coarse ground (GP diet).
[2] Alfalfa hay was 84% DM and (DM basis): 18.5% CP, and 43.4% NDF.
[3] Corn silage was 30% DM and (DM basis): 6.0% CP, and 48.2% NDF.
[4] Solvent-extracted SBM.
[5] Peas were U.S. No. 1 Feed peas (Federal Grain Inspection Service, Moscow, ID) and contained (DM basis): CP, 25.3% (83% soluble); starch, 48.0%; NDF, 9.2% and estimated NE_L, 1.98 Mcal /kg; TDN 86%; and NFC, 67% (DairyOne, Ithaca, NY).
[6] CP, NE_L, NDF, NFC, and starch and forage composition were analyzed/estimated by Cumberland Valley Analytical Services, Inc. (Maugansville, MD).

Whole ruminal contents samples were collected at 0, 2, 4, 6, 8, 10, 14, 18, 24, and 30 h following the morning feeding on the first day of each sampling period. Ruminal samples were collected from four locations in the rumen and the reticulum (approximately 250 g each) and composited. Aliquots were filtered through two layers of cheesecloth, immediately analyzed for pH, and processed for analyses of ammonia, total free amino acids (**TFAA**), and VFA (Foley et al., 2006).

Fecal samples (400 ml per sampling) were collected from the rectum or the ground, when fresh, during the first 3 d of each sampling period: at 9:00, 15:00, and 21:00 h (d 1), and at 3:00, 6:00, 12:00, 18:00 (d 2), and 0:00 h (d 3). Samples were stored frozen (-40°C) and later dried (65°C), ground through a 1-mm sieve, and composited per animal and period on a DM basis. Composite samples were analyzed (Foley et al., 2006) for ash/OM, N, NDF, starch, and acid insoluble ash (**AIA**). Apparent total tract digestibility was estimated using AIA as an intrinsic digestibility marker (Foley et al., 2006).

Total urine was collected during the last four days of each period. Urinary catheters (22 French, 75 cc, C. R. Bard Inc., Covington, GA) were positioned in the cows 24 h prior to initiation of the urine collection. Urine samples were acidified during collection to a pH < 3.0 by addition of 2 M H_2SO_4. Aliquots were diluted with distilled water, stored frozen at -20°C, and later analyzed (1:20 dilution) for creatinine and urea (Broderick et al., 2007), allantoin (Foley et al., 2006), and uric acid (commercial kit; Teco Diagnostics, Anaheim, CA) and N (1:10 dilution; Foley et al., 2006).

On the last 2 d of each period, blood samples were collected from the tail artery/vein before (0 h) and 6 h after the morning feeding. Plasma was collected after centrifugation at 1,500 × g for 40 min, frozen at -40°C, and later analyzed for urea N (**PUN**, Urea N kit, Ct. No. 640-8; Sigma Diagnostics, St. Louis, MO).

Two cows fed the GP and one fed the RP diets were used to determine *in situ* degradability of ground peas and SBM N. Samples for this trial were sieved through a 4.75 mm sieve to remove fines. Bags (Ankom Technology, Fairport, NY), containing 5-g air-dry sample were incubated in the rumen for 0 (water-washed, but not incubated in the rumen), 2, 4, 6, and 16 h. Bag processing following incubation and estimation of N degradability parameters were as described (Hristov et al., 2004), except a linear model was used to fit the data. The model used was: $y_0 + a*x$, where y_0 is the soluble fraction of N (%), and a is the degradation rate (slope, %/h).

Intake, digestibility, and urine data were analyzed by analysis of variance Latin square. Square and cow within square were random effects while all else were fixed. Ruminal fermentation data were analyzed as Latin square repeated measures, assuming ar(1) covariance structure. All data were analyzed using the PROC MIXED procedure of SAS (2003; SAS Inst., Inc., Cary NC). In all analyses, treatment comparisons were done using pair-wise *t*-test. Statistical difference was declared at $P \leq 0.05$. Statistical analysis of the *in situ* data was as described (Hristov et al., 2004).

Results and Discussion

Diet had no effect on ruminal pH and total and individual VFA concentration (Table 2). The two pea diets increased average ammonia ($P = 0.010$) and TFAA ($P = 0.006$) concentrations in ruminal fluid, compared with the control. There was no difference ($P > 0.05$) between the two pea diets. Similarly, Khorasani et al. (2001) reported a linear increase in ruminal ammonia concentration with increasing the replacement rate of SBM with peas from 0 to 100%. In beef steers fed forage-based diets, Reed et al. (2004) also reported a linear increase in ruminal ammonia concentration with increasing pea supplementation rate from 0 to 2.4 kg/d. Increased ammonia concentration in the rumen usually reflects increased proteolysis and deamination of dietary

protein and amino acids. As the ingredient (and chemical) composition of the diets in this trial were similar, except the inclusion of peas in GP and RP, it is clear that the greater concentration of ammonia in the rumen of the cows consuming the pea diets is an indication of greater degradability of pea compared with SBM/corn protein. These data are supported by the greater concentration of TFAA with the pea diets.

Table 2. Effect of inclusion of feed peas in the diet on ruminal fermentation in dairy cows (least squares means; n = 180).

Item	Control	Diet RP	GP	SE	*P*
pH	6.27	6.29	6.29	0.072	0.881
Ammonia, m*M*	6.1[b]	7.0[a]	7.3[a]	0.53	0.010
TFAA, m*M*	3.1[b]	3.5[a]	3.7[a]	0.30	0.006
Total VFA, m*M*	102.3	104.6	102.5	3.84	0.815
Acetate	67.5	71.6	68.4	2.07	0.289
Propionate	19.9	19.1	19.2	1.32	0.471
Iso-butyrate	0.75	0.77	0.82	0.026	0.207
Butyrate	10.9	9.9	10.6	0.38	0.213
Iso-valerate	1.45	1.48	1.55	0.146	0.354
Valerate	1.84	1.69	1.84	0.153	0.151
Acetate/ Propionate	3.48[b]	3.92[a]	3.66[b]	0.198	0.001

[a,b]Means without a common superscript differ at $P < 0.05$.

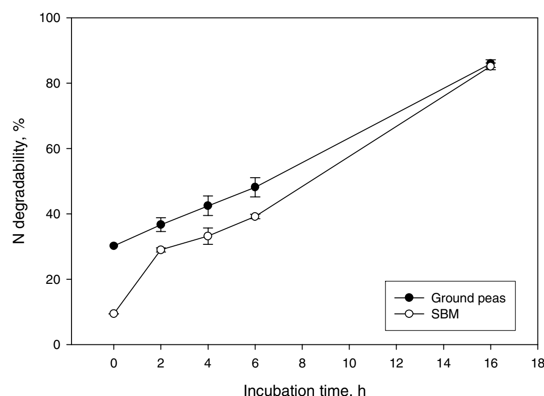

Figure 1. *In situ* degradability of pea and SBM N.

The *in situ* degradability data also support the conclusion that pea protein is more soluble/degradable in the rumen than SBM protein (Fig. 1). Within the 16 h incubation time employed in this experiment, degradation curves fitted well a linear model ($r^2 = 0.99$). It is noted that in 16 h, approximately 85% of the feed N disappeared from the bags. This *in situ* experiment demonstrated that solubility of pea N was greater ($P < 0.001$) that that of SBM (31.8 vs. 16.1%, respectively). The rate of degradation (the regression slope) was greater ($P = 0.003$) for SBM than for the peas (11.5 vs. 6.3 %/h, respectively). Similarly, Khorasani et al. (2001) reported approximately 3-times greater solubility of pea protein compared with SBM protein. Effective degradability of pea protein was also significantly greater (by 26%) than that of SBM protein. High ruminal solubility of pea protein (49%) was also reported by Walhain et al. (1992).

Diet had no effect on total and individual VFA concentrations in this study. Acetate to propionate ratio was increased ($P = 0.001$) with the RP diet compared with the control and GP diets. Froidmont and Bartiaux-Thill (2004) also did not report effect of peas, replacing barley grain, on ruminal VFA concentrations. Concentration of acetate linearly decreased and those of butyrate, valerate, and the branched-chain fatty acids increased with the inclusion of peas in the diet of beef cattle (Reed et al., 2004). Similarly, Khorasani et al. (2001) reported a linear decrease in ruminal acetate and linear increase in butyrate, isovalerate, and valerate concentrations when peas replaced SBM in the diet of lactating dairy cows.

Dry matter and starch intakes were greater (by about 1.6 and 0.5 kg/d; P = 0.046 and 0.012, respectively), for the control, compared with the two pea diets (Table 3). Intake of NDF was lower ($P = 0.011$) for RP, compared with the control and the GP diets. Nitrogen intake was lower ($P = 0.037$) for GP compared with the control diet. Intake of OM tended ($P = 0.052$) to be greater for the control diet. Total tract apparent digestibility of DM, OM, N, and starch were decreased (P = 0.004 to 0.036) by the RP diet compared with the control and the GP diets. Digestibility of NDF was similar ($P > 0.05$) between the control and the GP diet, but was lower ($P = 0.020$) for the RP diet. Petit et al. (1997) did not report any effects of inclusion of 20% cracked peas (replacing SBM and corn grain) on total tract nutrient digestibilities in dairy cows. Similarly, (Froidmont and Bartiaux-Thill, 2004) did not observe effects on digestibility with the inclusion of 14% peas in the diet, except CP digestibility was slightly reduced (by 6%) and NDF was increased (by 8%), compared with SBM. Our results clearly indicated reduced digestibility of the RP diet suggesting that peas have to be coarsely ground for dairy cow diets to avoid depression in total tract digestibility of nutrients.

Secretion of N with milk was greater ($P = 0.012$) for the control than for the pea diets (Table 4). As proportion of N intake, however, diet had no effect ($P = 0.208$) on milk N secretion. Absolute urinary N losses were not affected ($P = 0.201$) by diet, although there was a trend ($P = 0.062$) for increased relative N losses with the GP diet compared with the control or the RP diets. Relative urinary urea excretion also tended (P = 0.054 to 0.091) to be greater with the pea diets compared with the control. Absolute and relative fecal N losses were increased (P = 0.007 to 0.014) by the RP diet compared with the control and the GP diets. Concentration of PUN was not affected ($P = 0.099$) by diet, but tended to be greater ($P = 0.061$) with the GP compared with the control diet. Urinary allantoin and consequently total purine derivative excretions were greater (P = 0.017 and 0.014, respectively) for the control than for the pea diets, suggesting increased outflow of microbial protein from the rumen with the former diet. In a related experiment,

we did not find effects of peas on urinary N losses, or purine derivatives excretion (Vander Pol and Hristov 2006). We are not aware of other published research on urinary N losses in dairy cows fed diets with feed peas. Khorasani et al. (2001) did not find effect of graded inclusion of peas (0 to 100% SBM replacement rate) on duodenal microbial N flow or efficiency of microbial protein synthesis in the rumen of lactating dairy cows.

Table 3. Effect of inclusion of feed peas in the diet on intake and total tract apparent digestibility of nutrients in dairy cows (least squares means; n = 18).

	Diet				
Item	Control	RP	GP	SE	*P*
Intake, kg/d					
DM	23.9[a]	22.4[b]	22.2[b]	1.15	0.046
OM	22.0	20.6	20.6	1.06	0.052[1]
N	0.60[a]	0.58[ab]	0.55[b]	0.029	0.037
NDF	8.8[a]	7.9[b]	8.5[a]	0.42	0.011
Starch	5.1[a]	4.8[b]	4.5[b]	0.24	0.012
Digestibility, %					
DM	74.9[a]	68.8[b]	77.3[a]	1.29	0.005
OM	76.7[a]	70.6[b]	79.2[a]	1.28	0.004
N	74.1[a]	68.4[b]	76.3[a]	1.49	0.007
NDF	60.4[ab]	55.2[b]	65.7[a]	1.78	0.020
Starch	93.9[a]	87.8[b]	93.9[a]	1.70	0.036

[1] Control vs. RP/GP, $P < 0.05$.
[a,b] Means without a common superscript differ at $P < 0.05$.

Table 4. Effect of inclusion of feed peas in the diet on N utilization in dairy cows (least squares means; n = 18).

	Diet				
Item	Control	RP	GP	SE	*P*
Milk					
N, g/d	156[a]	138[b]	137[b]	7.3	0.012
% intake	26.3	23.7	25.2	0.75	0.208
Urine					
N, g/d	233	212	232	11.7	0.201
% intake	39.0	37.0	42.6	1.23	0.062[1]
Urea N, g/d	160	163	167	9.8	0.663
Prop. urinary N	0.68	0.76	0.71	0.018	0.054[2]
Prop. N intake	0.27	0.28	0.30	0.009	0.091[3]
Feces					
N, g/d	156[b]	191[a]	136[b]	18.4	0.014
% intake	25.9[b]	31.6[a]	23.7[b]	1.49	0.007
Urinary excretion, m*M*/d					
Allantoin	505[a]	415[b]	430[b]	40.6	0.017
Uric acid	48	48	48	3.9	0.997
Total PD[4]	553[a]	463[b]	478[b]	39.7	0.014
PUN[5]	16.3	18.7	16.3	1.37	0.099

[1] GP vs. RP, $P < 0.05$.
[2] Control vs. RP, $P < 0.05$.
[3] Control vs. GP, $P < 0.05$.
[4] Purine derivatives, allantoin and uric acid.
[5] Plasma urea N, mg/dl; GP vs. Control/RP, $P = 0.061$.
[a,b] Means without a common superscript differ at $P < 0.05$.

Implications

Inclusion of 15% feed peas in the diet of lactating dairy cows, replacing on an isonitrogenous and isoenergetic basis soybean meal and corn grain, resulted in elevated ruminal ammonia and total free amino acid concentration, but had no effect on pH and VFA concentration. The increased ammonia concentration in the rumen suggested greater degradability of pea protein than soybean meal protein, which was confirmed *in situ*. The greater ruminal degradability of pea protein may lead to increased urinary N losses. Our results suggest that peas have to be coarsely ground for dairy cow diets to avoid depression in total tract digestibility of nutrients.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

INFLUENCE OF SLICE BALING ALFALFA HAY ON DIGESTIVE FUNCTION OF STEERS CONSUMING A FEEDLOT FINISHING DIET

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ABSTRACT: A modification of the traditional alfalfa hay baling system has been developed. The system is referred to as slice baling and consists of slice chopping the hay after suncuring and before baling. This method chops the length of alfalfa stems to 7.6 cm. Slicing is proposed to cause less damage to leaves compared to grinding after baling. Leaves should be more consistent and less leaf material potentially is lost with slice baling. Four ruminally cannulated mixed-breed steers were used in a 4 × 4 Latin square design to evaluate effects of slice baled alfalfa hay in feedlot finishing diets on digestive function. Treatments were arranged in a 2 × 2 factorial. Factors were baling method (traditional or slice baling) and forage level (8 or 14%). Total tract digestibilities were estimated from intake and total fecal output. Total fecal output was collected and measure using fecal bags. There were no baling method effects (P > 0.15) on DM, OM, CP, or NDF intakes or DM, OM, and NDF digestibility. Neutral detergent fiber intake and OM digestibility were greater ($P \leq 0.08$) for 14 than for 8% forage. A baling method × forage level interaction (P = 0.01) was detected for CP digestibility. At 8% forage, CP digestibility was greater (P = 0.03) for slice than traditional alfalfa (75.6 vs. 72.6 ± 2.0%, respectively). However, at 14% forage, CP digestibility was similar (P = 0.23) for the 2 baling methods. Rumen volume and turnover time were greater ($P \leq 0.07$) for slice than traditional baled alfalfa, but fluid and particle passage rates were greater ($P \leq 0.07$) for traditional than slice alfalfa. Ruminal pH was not altered (P = 0.72) by baling method (5.49 and 5.44 ± 0.08, for traditional and slice baling, respectively). Ruminal molar proportion of acetate was greater (P = 0.03) for 14 than for 8% forage. The magnitude of ruminal function changes, due to slice bale feeding, were not great enough to expect improved digestive function

Key Words: feedlot cattle, forage, slice alfalfa

Introduction

A modification of the traditional alfalfa hay baling system is available. The system is referred to as slice baling and consists of slice chopping the hay after suncuring and before baling. This system chops the length of alfalfa stems to 7.6 cm. Slicing is proposed to cause less damage to the leaves compared to grinding after baling. Leaves should be more consistent. Also, less leaf material is lost with slice baling. Anecdotal information suggests that slice baling alfalfa results in improved quality (greater proportion of leaves), improves rumen function in feedlot cattle because of less formation of fines from leaves, results in better uniformity of the stem length, and saves cost associated with grinding.

Roughages are included in feedlot finishing diets to reduce digestive and metabolic problems (Galyean and Defoor, 2003). Most finishing diets generally contain 4.5 to 13.5% (DM basis) roughage with alfalfa hay and corn silage being the most common source (Galyean and Gleghorn, 2001). Forage is added to high-concentrate diets to stimulate chewing which is associated with increased saliva output (Balch, 1958), which plays a role in buffering acids produced during rumination. Both roughage concentration and physical form contribute to normal rumen function (Woodford et al., 1986). Dry matter intake increases with increasing roughage level but feed efficiency decreases because energy density of diets decreases with increasing roughage level (Bartle et al., 1994). With respect to physical form, forage particle size had no effect on finishing cattle performance (Shain et al., 1999). However, slice alfalfa has not been evaluated. Therefore, the objectives of this study were to evaluate the influence of baling method and roughage level on digestive function of cattle fed a steam-flaked corn-based finishing diet.

Materials and Methods

Four mixed-breed steers fitted with ruminal cannulas were used in a 4 x 4 Latin square design. Treatments were arranged in a 2 x 2 factorial. Factors were baling method (traditional or slice baling) and forage level (8 or 14%). Experimental periods were 15 d in length with 10 d for adaptation to the diets and 5 d for collection. Diets were offered ad-libitum once daily. All procedures and experimental protocols were approved by the New Mexico State University Institutional Animal Care and Use Committee.

Fecal output was collected using fecal bags on d 1 through 5 of each collection period. Fecal bags were emptied and weighed once daily. A 10% (wet basis) subsample of feces was collected from each steer daily during collections.

On d 12, CoEDTA (200 mL; Uden et al., 1980) was dosed intraruminally at 0600 as a marker of fluid passage rate. Ruminal fluid samples were collected at 0 (before dosing), 3, 6, 9, 12, 18, 24, 36, and 48, h after dosing. Ruminal fluid pH was determined immediately after collection and then samples were acidified with 7.2 N H_2SO_4

at 1 mL/100 mL ruminal fluid and frozen (-10ºC) for later analysis of Co, ammonia, and VFA. Also on d 12, Yb-labeled alfalfa (100 g; Sindt et al., 1993) was intraruminally dosed at 0600 for a marker of particulate passage rate. Ruminal content samples were collected at 0 (before dosing), 3, 6, 9, 12, 18, 24, 36, 48 and 72 h after dosing.

***Laboratory Analyses*.** Fecal samples were thawed, mixed, and subsampled, dried in a forced-air oven (50ºC) for 48 h, and ground in a Wiley mill (2-mm screen). Feed, orts, and fecal samples were analyzed for DM, OM, and CP (Methods 930.15, 942.05, and 990.02, respectively; AOAC, 1997). The NDF analyses was conducted according to Robertson and Van Soest (1991) using an Ankom 200 fiber analyzer (Ankom Co., Fairport, NY). Ruminal fluid samples were centrifuged at 20,000 × g for 20 min and analyzed for NH_3-N (Broderick and Kang, 1980), and VFA (Goetsch and Galyean, 1983), and cobalt was determined using an air-plus-acetylene flame using atomic absorption spectroscopy as described by Uden et al. (1980). Ytterbium was extracted as outlined by Hart and Poland (1984), and marker concentration was determined by atomic absorption spectroscopy using a nitrous oxide-plus-acetylene flame.

***Calculations*.** Dry matter intake was calculated by subtracting orts DM from feed DM offered. Liquid dilution rate was calculated by regressing the natural log of Co concentration on sampling time, and particle dilution rate by regressing the natural log of Yb concentration on sampling time.

***Statistical Analysis*.** The Mixed procedures of SAS (SAS Inst., Inc., Cary, NC) were used for all statistical computations. Data were analyzed as a Latin square design. The model included source, level, and period as fix effects, and steers as random effects. Ruminal data over time was analyzed as repeated measurements design using the Mixed procedures of SAS. The model included source, level, period, and time and the interaction of source × level × time as the fixed effects and steer nested with in period × source × level as random effects. If significant ($P < 0.10$) F-statistics were detected, means were separated using the method of least significant difference.

Results and Discussion

Effects of alfalfa baling method and level on intake and digestibility of steers consuming feedlot concentrate diets are shown in Table 2.There were no baling method effects ($P > 0.15$) on DM, OM, CP, or NDF intakes or DM, OM, and NDF digestibility. Neutral detergent fiber intake and OM digestibility were greater ($P \leq 0.08$) for 14 than for 8% forage. A baling method x forage level interaction ($P = 0.01$) was detected for CP digestibility. At 8% forage, CP digestibility was greater ($P = 0.03$) for slice than traditional alfalfa (75.6 vs. 72.6 ± 2.0%, respectively). However, at 14% forage, CP digestibility was similar ($P = 0.23$) for the 2 baling methods. Rumen volume and turnover time were greater ($P \leq 0.07$) for slice than traditional baled alfalfa, but, fluid and particle passage rate were greater ($P \leq 0.07$) for traditional than slice alfalfa. Ruminal pH was not altered ($P = 0.72$) by baling method (5.49 and 5.44 ± 0.08, for traditional and slice baling, respectively). Ruminal molar proportion of acetate was greater ($P = 0.03$) for 14 than for 8% forage.

It is thought that alfalfa hay dehydrates during storage resulting in less flexible leaves that pulverize during grinding. Because slice alfalfa does not require grinding, smaller forage particles end up in the diet from traditionally baled alfalfa than from slice alfalfa hay. Smaller particles escape from the rumen at a faster rate and they are exposed to microbial activity for a shorter period of time resulting in lower digestibility (Welch 1982). Even though fluid and passage rates in the present study were greater for the traditional baled alfalfa hay, intake and digestibility of DM, OM, and NDF were not affected by baling method. Our results agree with those of Shain et al. (1999) who reported no effects of forage particle size on growth performance when alfalfa or wheat straw where ground using 0.95, 7.6, or 12.7 cm screens. Also, Calderon-Cortes and Zinn (1996) observed that inclusion of sudan hay ground using 2.5 or 7.6 cm screens did not affect growth performance of finishing cattle, even though OM digestibility increased 2.3%. When an ingredient is used at a low level, the changes need to be large in order to have a significant impact on the total diet

In conclusion, inclusion of slice alfalfa hay at 8 or 14% of feedlot finishing diets does not affect ruminal function enough to expect improved digestive function

Implications

The magnitude of ruminal function changes, due to slice bale feeding, were not great enough to expect improved digestive function. Therefore, improvement in growth performance of cattle consuming finishing diets that include slice alfalfa is not expected.

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Table 1. Ingredient composition (% of DM) of experimental diets fed to steers

	Treatments[1]			
	8%		14%	
Ingredients	Traditional	Slice	Traditional	Slice
Ground alfalfa	8.00		14.00	
Slice alfalfa		8.00		14.00
Steam-flaked corn	75.88	75.88	69.22	69.22
Cottonseed meal	5.69	5.69	6.63	6.63
Urea	0.93	0.93	0.67	0.67
Tallow	3.00	3.00	3.00	3.00
Molasses	4.00	4.00	4.00	4.00
CLRC 2.5	2.50	2.50	2.50	2.50

[1]Treatments were a) Traditional 8% = ground alfalfa included at 8% (DM basis), b) Slice 8% = slice alfalfa included at 8% (DM basis), c) Traditional 14% = ground alfalfa included at 14% (DM basis), and d) Slice 14% = slice alfalfa included at 14% (DM basis).

Table 2. Effect of alfalfa baling method and level on total tract digestion of DM, OM, CP, and NDF in beef steers consuming concentrate diets

	Treatments[1]							
	8%		14%			P-values[2]		
Items	Traditional	Slice	Traditional	Slice	SE	Method	Level	M x L
Intake, kg/d								
DM	12.73	13.00	12.75	13.72	0.75	0.31	0.58	0.60
OM	12.11	12.31	12.08	12.83	0.71	0.40	0.71	0.68
CP	1.56	1.62	1.69	1.73	0.11	0.51	0.24	0.94
NDF	2.65	2.80	3.27	3.37	0.24	0.51	0.03	0.91
Fecal output, kg/d								
DM	2.45	2.46	2.67	2.76	0.25	0.77	0.29	0.86
OM	2.06	2.01	2.16	2.37	0.18	0.55	0.18	0.44
CP	0.43	0.38	0.42	0.47	0.04	0.96	0.12	0.09
NDF	0.98	1.00	1.16	1.22	0.12	0.69	0.11	0.85
Digestibility, %								
DM	80.81	80.95	78.84	79.41	1.77	0.72	0.21	0.87
OM	83.14	83.66	81.88	81.21	1.55	0.91	0.08	0.52
CP	72.58	75.79	74.27	72.53	1.97	0.16	0.27	0.01
NDF	62.23	62.60	63.93	63.31	3.80	0.93	0.54	0.80

[1]Treatments were a) Traditional 8% = ground alfalfa included at 8% (DM basis), b) Slice 8% = slice alfalfa included at 8% (DM basis), c) Traditional 14% = ground alfalfa included at 14% (DM basis), and d) Slice 14% = slice alfalfa included at 14% (DM basis).

[2]P-values: Method = effect of baling method (traditional or slice); Level = effect of forage level (8 or 14%); M x L = method x level interaction.

Table 3. Effects of alfalfa baling method and level on digesta kinetics, and ruminal pH, ammonia, and VFA molar proportion in beef steers consuming concentrate diets

	Treatments[1]					P-values[2]		
	8%		14%					
Items	Traditional	Slice	Traditional	Slice	SE	Method	Level	M x L
Ruminal volume, L	58.3	66.0	55.4	55.9	4.43	0.07	0.73	0.76
Fluid dilution rate, %/h	5.83	4.87	6.26	4.95	0.70	0.05	0.67	0.77
Turnover time, h	18.54	21.08	17.04	21.06	1.97	0.01	0.46	0.47
Outflow, L/h	3.19	3.17	3.45	3.32	0.25	0.66	0.35	0.80
Particle flow rate, %/h	3.96	3.18	5.59	4.35	0.58	0.08	0.03	0.68
pH	5.33	5.41	5.64	5.48	0.12	0.72	0.14	0.34
Ammonia, m*M*	3.30	3.37	3.92	4.25	1.00	0.72	0.57	0.97
VFA								
Total, m*M*	149.0	138.6	135.5	139.3	6.92	0.65	0.38	0.33
Acetate, mol/ 100 mol	57.05	56.71	64.33	66.51	3.21	0.78	0.03	0.70
Propionate, mol/100 mol	23.95	24.65	25.53	22.82	2.07	0.64	0.95	0.43
Acetate:Propionate ratio	2.50	2.34	2.59	3.04	0.27	0.66	0.19	0.29

[1]Treatments were a) Traditional 8% = ground alfalfa included at 8% (DM basis), b) Slice 8% = slice alfalfa included at 8% (DM basis), c) Traditional 14% = ground alfalfa included at 14% (DM basis), and d) Slice 14% = slice alfalfa included at 14% (DM basis).

[2]P-values: Method = effect of baling method (traditional or slice); Level = effect of forage level (8 or 14%); M x L = method x level interaction.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

LONG-CHAIN FATTY ACID FLOW TO THE DUODENUM OF CATTLE FED LIMITED AMOUNTS OF FORAGE PLUS SUPPLEMENTARY RUMINALLY UNDEGRADABLE PROTEIN CONTAINING FISHMEAL

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ABSTRACT: Twelve Angus crossbred cattle (8 heifers and 4 steers; average initial BW = 594 ± 44.4 kg) fitted with ruminal and duodenal cannulas and fed restricted amounts of forage plus a ruminally undegradable protein (RUP) supplement were used in a triplicated 4 X 4 Latin square design experiment to determine intestinal supply of long-chain fatty acids. Cattle were fed 4 different levels of chopped (2.54 cm) bromegrass hay (11.4% CP, 57% NDF; OM basis): 30, 55, 80, or 105% of the forage intake required for maintenance. Cattle fed below maintenance were given specified quantities of a RUP supplement (6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fishmeal; DM basis) designed to provide duodenal essential AA flow equal to that of cattle fed forage at 105% of maintenance. Experimental periods lasted 19 d (17 d of adaptation and 2 d of sampling). Although fatty acid intake from hay increased linearly ($P <$ 0.001) as cattle consumed more forage, total fatty acid intake increased (cubic, $P < 0.001$) as total OM intake decreased because fatty acid consumption increased (cubic, $P < 0.001$) as cattle consumed more supplement. As a result, total fatty acid flow to the duodenum increased linearly ($P < 0.001$) as intake of supplement increased. Duodenal flow of 14:1, 15:0, 15:1, and 18:0 increased linearly ($P < 0.05$) with increased forage consumption. A quadratic response ($P < 0.05$) was noted for duodenal flow of myristic, oleic, linoleic, and linolenic acids largely because duodenal flow of these fatty acids was least for cattle consuming forage at 105% of maintenance. The biohydrogenation intermediates 16:1*c*+*t*11, 18:1*t*11, 18:1*t*10, 18:1*t*12, and 18:1*t*13 responded to dietary treatment in a quadratic fashion ($P < 0.09$), but duodenal flow of CLA was not affected ($P \geq 0.149$) by dietary treatment. We conclude that a supplement consisting of 6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fishmeal (DM basis) can be fed to maintain or improve intestinal supply of fatty acids in cattle consuming limited amounts of forage.

Key Words: beef cattle, supplementation, fatty acids

Introduction

Studies evaluating nutritional effects on reproduction in ruminants often use restricted feed intake to limit the supply of dietary energy (Schillo, 1992). As an initial step toward separating physiological effects of protein from energy in ruminants, Scholljegerdes et al. (2005a) used an RUP supplement consisting of 6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fishmeal (DM basis) to balance intestinal supply of essential AA in cattle consuming restricted quantities of forage. Menhaden fishmeal contains 10.5% crude fat (NRC, 1982) and 8% of DM as oil with relatively high concentrations of ≥ 20C PUFA (Mattos et al., 2002). Ashes et al. (1992) reported that in vitro ruminal biohydrogenation was reduced in the presence of PUFA from fish oil. Biohydrogenation rates determined in vitro, however, may be much less than the actual in vivo rates (Moate et al., 2004). Because ruminal disappearance of long-chain fatty acids is minimal (Jenkins, 1993), we hypothesized that the RUP supplement used by Scholljegerdes et al. (2005a) would increase intestinal supply of total fatty acids in cattle fed forage at levels below NRC (2000) recommendations for maintenance. Our objective was to determine long-chain fatty acid flow to the duodenum of beef cattle fed limited amounts of forage plus supplementary RUP from a combination of porcine blood meal, hydrolyzed feather meal, and menhaden fishmeal.

Materials and Methods

General

Twelve ruminally and duodenally cannulated Angus cross cattle were used in a triplicated 4 × 4 Latin square experiment (8 heifers in a duplicate 4 × 4 Latin square and 4 steers in a single 4 × 4 Latin square conducted simultaneously; average initial BW = 594 ± 44.4 kg) in accordance with an approved University of Wyoming Animal Care and Use Committee protocol. Cattle were fed chopped (2.5 cm) bromegrass hay (9.4% ash, 11.4% CP, and 57% NDF on an OM basis) at 30, 55, 80, and 105% of the forage intake required for maintenance (NRC, 2000). Cattle fed below maintenance were given specified quantities of an RUP supplement (6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fishmeal; DM basis) designed to provide duodenal essential AA flow equal to that of cattle fed forage at 105% of maintenance (Scholljegerdes et al., 2005a). Basal flows of total essential AA were predicted for each animal at each experimental forage intake level using the equation reported by Scholljegerdes et al. (2004b; total essential AA flow to the small intestine, g/d = [0.055 × g of OM intake] + 1.546). The quantity of RUP supplement delivered was adjusted for anticipated RUP values at 30, 55, and 80% intake as reported by Scholljegerdes et al. (2005b). Amount of feed delivered was provided in equal portions at 0600 and 1800 daily. Each period of the Latin square lasted 19 d, with 17 d for diet adaptation to allow for adjustment of the digestive system to forage intake level and RUP supplement. Cattle had ad libitum access to water and trace-mineral salt (Champions Choice; Akzo Nobel Salt Inc.,

Clarks Summit, PA; guaranteed analysis [% of DM]: NaCl, 95 to 99; Co, Cu, I, Mn, Zn, and Fe, <1%) until d 14 of each sampling period. On d 14 of each sampling period, to avoid any confounding effects of salt intake on water intake and fluid passage rate, trace mineral salt was no longer provided. Feed refusals were not observed from d 10 through 19 of the experimental periods.

Sampling

As a marker for digesta flow, boluses of 5.0 g of Cr_2O_3 were dosed intraruminally at each feeding (total = 10 g of Cr_2O_3/d) from d 8 to 19 of each sampling period. Duodenal samples were collected for 2 d after the adaptation period. To account for feed composition throughout the trial, feeds were sampled every day and composited within each period. Beginning at 0400 on d 18 of each sampling period, duodenal (200 mL) samples were taken every 4 h. On d 19, collection times were advanced 2 h, so that samples were collected to represent every 2-h segment of a 24-h period. Duodenal digesta samples were frozen immediately before being lyophilized (Genesis SQ 25 Super ES Freeze Dryer; The VirTis Co., Gardiner, NY), ground to pass a 1-mm screen, composited within animal for each period, and then stored for subsequent analyses.

Laboratory Analyses

Feed and duodenal samples were analyzed for DM and ash (AOAC, 1990). Nitrogen content of feed was determined using a LECO FP-528 N analyzer (LECO Corp., Henderson, NV), and NDF content of feed was determined using an ANKOM 200 fiber analyzer (ANKOM Technology, Fairport, NY). Chromium concentration of duodenal digesta was determined (Hill and Anderson, 1958) by atomic absorption spectrophotometry (Model 210 VGP AA Spectrophotometer; Buck Scientific, Norwalk, CT) with an air-plus-acetylene flame. Feed (Table 1) and duodenal digesta samples were subjected to direct transesterification and analyzed for fatty acids as described by Scholljegerdes et al. (2004a) except H_2 replaced He as the carrier gas.

Calculations and Statistical Analyses

Flow of digesta was calculated by dividing the amount of Cr dosed by the concentration of Cr in the respective duodenal sample. Duodenal flow of fatty acids was calculated by multiplying the fatty acid concentration in duodenal digesta by duodenal digesta flow. Data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) for a Latin square in a randomized complete block design experiment. The effect of gender was included in the model as a block, and animal was used as the random effect. Orthogonal polynomial contrasts were used to compare linear, quadratic, and cubic responses to level of forage intake (Steel and Torrie, 1980).

Results and Discussion

Total Fatty Acid Intake

As anticipated, total fatty acid intake from hay increased linearly ($P < 0.001$) as cattle consumed more forage (Table 2). Total fatty acid intake, however, increased (cubic, $P < 0.001$) as total OM intake decreased because fatty acid consumption increased (cubic, $P < 0.001$) as cattle were offered more supplement.

Fatty Acid Flow

Total fatty acid flow to the duodenum increased linearly ($P < 0.001$) as intake of supplement increased (Table 3). In a review of the literature on factors affecting lipid balance in the rumen, Jenkins (1993) concluded that flow of fatty acids to the duodenum is generally closely related to intake of dietary lipids. In the same review, the author determined that 8% of dietary lipids disappeared from the rumen and microbial lipid synthesis was 15 g/kg of lipid-free OM digested in the rumen. Using those estimates in addition to values for OM truly digested in the rumen reported by Scholljegerdes et al. (2005a) cattle fed forage at 105% of maintenance should have had 173.6 g of fatty acids flowing to the duodenum daily, which was remarkably close to the 171.3 g/d that was observed. Duodenal flow of fatty acids in cattle fed the lowest amount of forage plus supplemental RUP was predicted to be 255.0 g/d, but actual flow was 221.3 g/d. This discrepancy between predicted and observed flow of total fatty acids was > 2 times the SEM for duodenal flow total fatty acids. The difference may be partially explained by reduced de novo synthesis as a result of enhanced uptake of exogenous lipid by microbial cells (Jenkins, 1993; Moate et al., 2004). Lipid disappearance from the rumen also was more common for diets with added fat than for control diets in the 15 studies reviewed by Jenkins (1993).

Duodenal flow of 14:1, 15:0, 15:1, and 18:0 increased linearly ($P < 0.001$) as forage intake increased, whereas flow of 16:0 increased linearly ($P < 0.001$) as supplement intake increased (Table 3). Greater duodenal flow of 16:0 and lesser flow of 18:0 as intake of supplement increased was consistent with studies in which sheep were fed diets containing fish oil (Wachira et al., 2000; Kitessa et al., 2001). An increase in duodenal flow of 16:0 in cattle fed supplement was expected considering that the concentration of 16:0 in the 2 major supplemental ingredients was 4.3 (feather meal) to 9.0 (fishmeal) times greater than that of the hay (Table 1). The hay was devoid of 14:0 (data not shown), thus the quadratic ($P = 0.005$) decrease in duodenal flow of 14:0 as forage intake decreased was also expected. In agreement with our results, Klusmeyer and Clark (1991) observed less flow of 18:0 to the duodenum in dairy cows fed fishmeal. Those authors also reported that ruminal biohydrogenation of C18 unsaturated fatty acids was reduced in cows fed fishmeal. Stearic acid is the final product of ruminal biohydrogenation of C18 unsaturated fatty acids (Jenkins, 1993). Therefore, the decrease in duodenal flow of 18:0 for cattle consuming supplement in the present study may be attributable to less extensive ruminal biohydrogenation of C18 unsaturated fatty acids.

Further support for less extensive biohydrogenation of C16 and C18 unsaturated fatty acids in cattle fed supplement is provided by the quadratic response ($P < 0.05$) for duodenal flow of 16:1*c*9, 18:1*c*9, 18:2n-6, and 18:3n-3 (Table 3). Intake of 18:3n-3 was slightly greater whereas intake of 18:2n-6 was nearly twice as great for cattle fed forage at 105 vs. 30% of maintenance. Intake

of 16:1*c*9 and 18:1*c*9 was greater in cattle fed supplement. Biohydrogenation of C16 and C18 MUFA occurs at a much slower rate than biohydrogenation of C18 PUFA (Moate et al., 2004). Thus, greater flow of 16:1*c*9 and 18:1*c*9 for cattle fed supplement was to be expected. Biohydrogenation of dietary 16:1*c*9 may have also contributed to the previously mentioned increase in duodenal flow of 16:0 in cattle fed supplement; however, this process was not going to completion as evidenced by the increase in duodenal flow of 16:1*t*9 (linear, $P = 0.045$) and 16:1*ct*11 (quadratic, $P = 0.01$). Quadratic ($P < 0.085$) responses to dietary treatment for duodenal flow of the biohydrogenation intermediates 18:1*t*11, 18:1*t*10, 18:1*t*12, and 18:1*t*13 again supports the notion that ruminal biohydrogenation of C18 unsaturated fatty acids was less complete in cattle fed supplement. Duodenal flow of 18:2*c*9*t*11 CLA, however, was not affected ($P \geq 0.149$) by dietary treatment. It has been proposed that the longer chain PUFA from fish oil inhibit complete ruminal biohydrogenation of 18:2n-6 by inhibiting growth of bacteria responsible for hydrogenating 18:1*t*11 or through inhibition of their hydrogenases (Griinari and Bauman, 1999). In their review of the literature, Khanal and Olson (2004) noted that fish oil supplementation increases concentration of milk fat CLA. Those authors also concluded that the highest concentration of milk fat CLA with fish oil supplementation was achieved when it was included at 2% of the diet DM. Fatty acids from supplement in the current experiment were included at 1.7% of OM for the treatment in which hay was fed at 55% of maintenance. The quadratic response ($P = 0.068$) for flow of 18:1*t*11 occurred because of the increase observed in cattle fed forage at 55% of maintenance. Our results are consistent with the suggestion that, because intestinal supply of 18:1*t*11 far exceeds that of 18:2*c*9*t*11 CLA, 18:1*t*11 serves as a precursor for conversion to 18:2*c*9*t*11 CLA via Δ-9 desaturase in mammary tissue (Bauman et al., 2003). Our laboratory documented that milk of beef cows increased from 0.3 to 0.97 g of 18:2*c*9*t*11 CLA/100 g of freeze-dried milk (Lake et al., 2007) by feeding cracked high-linoleate safflower seeds. In a companion study in which beef cattle were fed the same diets as those fed by Lake et al. (2007), Scholljegerdes et al. (2004a) reported that duodenal flow of 18:2*c*9*t*11 CLA was only increased from 0.2 to 0.3 g/d whereas duodenal flow of 18:1*t*11 increased from 12.7 to 72.4 g/d in cattle fed cracked high-linoleate safflower seeds. Fievez et al. (2003) illustrated that altered milk fat 18:2*c*9*t*11 CLA content was mainly dependent on supply of 18:1*t*11 and to a lesser extent on the activity of Δ-9 desaturase.

As opposed to evidence for less extensive biohydrogenation of C18 unsaturated fatty acids, biohydrogenation of ≥ 20-C PUFA seemed quite extensive for cattle fed supplement. Slightly greater flow of 20:5n-3 and 22:5n-3 to the duodenum in cattle fed forage at 80% of maintenance requirements coupled with a steady decline for the 55 and 30% of maintenance treatments resulted in detection of quadratic responses ($P = 0.079$ and 0.102, respectively) to dietary treatment (Table 3). These responses occurred despite increased intake of ≥ 20-C PUFA as forage intake decreased and supplement intake increased. Our results were not consistent with observations from in vitro experiments conducted by Ashes et al. (1992) and Gulati et al. (1999) in which ≥ 20-C PUFA from fish oil were not hydrogenated to any significant extent. Literature reports on in vivo experiments indicate that ruminal biohydrogenation of 20:5n-3 ranged from 76 (Wachira et al., 2000) to 92.6% (Chikunya et al, 2004) in sheep and 89.8 to 92.4% (Scollan et al., 2001) in steers fed fish oil. Ruminal biohydrogenation of 22:6n-3 ranged from 72.2 (Wachira et al., 2000) to 91.5% (Chikunya et al., 2004) in sheep and 86.6 to 90.4% (Scollan et al., 2000) in steers fed fish oil. Duodenal flow of 20:5n-3 was approximately 11% of 20:5n-3 intake in cattle fed the greatest quantity of supplement in the present experiment. The inability to detect 22:6n-3 in duodenal contents suggests that this fatty acid was completely hydrogenated in the rumen. This latter response may explain why Burns et al. (2003) did not detect an increase in endometrial content of 22:6n-3 in beef cows fed fishmeal.

Implications

Supplementation with a combination of 6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fishmeal (DM basis) should improve fatty acid status when beef cattle consume limited amounts of forage.

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Table 1. Concentration of predominant long-chain fatty acids in feed ingredients offered to beef cattle consuming restricted amounts forage plus supplemental ruminally undegradable protein

	Ingredient			
Fatty acid, mg/g	Bromegrass hay	Menhaden fishmeal	Hydrolyzed feather meal	Porcine blood meal
C16:0	2.27	20.38	9.74	1.94
C18:0	0.35	4.28	4.67	0.93
C18:1n-9	0.80	5.69	11.13	2.02
C18:2n-6	1.55	1.12	5.29	1.78
C18:3n-3	0.43	1.08	0.23	-
C20:5n-3	-	9.43	0.36	-
C22:6n-3	-	9.02	-	-
Total	6.01	84.00	37.64	6.26

Table 2. Intake by beef cattle consuming restricted amounts of bromegrass hay plus supplemental ruminally undegradable protein[1]

Intake	Forage intake level, % of maintenance				SEM[3]	Contrast *P*-value[2]		
	30	55	80	105		L	Q	C
OM, g/d								
Forage	3,178	5,827	8,475	11,124	104.5	0.001	1.00	1.00
Supplement	2,556	1,491	762	0	20.5	0.001	0.001	0.001
Total	5,733	7,318	9,237	11,124	115.6	0.001	0.001	0.01
Fatty acids, g/d								
Forage	20.8	38.0	55.3	72.6	0.3	0.001	0.912	0.918
Supplement	210.7	122.9	62.8	0.0	1.3	0.001	0.001	0.001
Total	231.5	160.9	118.1	72.6	1.0	0.001	0.001	0.001

[1]Cattle were fed 30, 55, 80, or 105% of the forage intake required for maintenance (NRC, 2000) plus a rumen undegradable protein supplement comprised of 6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fishmeal (DM basis).
[2]Orthogonal contrasts included linear (L), quadratic (Q), and cubic (C) effects among forage intake levels.
[3]n = 12.

Table 3. Long-chain fatty acid flow to the duodenum of beef cattle consuming restricted amounts of bromegrass hay and supplemental ruminally undegradable protein containing fishmeal[1]

Fatty acid, g/d	Forage intake level, % of maintenance				SEM[3]	Contrast *P*-value[2]		
	30	55	80	105		L	Q	C
C14:0	13.7	7.8	5.9	4.2	0.7	<0.001	0.005	0.217
C14:1	1.6	2.6	2.8	3.4	0.3	<0.001	0.443	0.384
C15:0	3.5	4.5	5.6	6.0	0.3	<0.001	0.292	0.637
C15:1	0.6	1.2	1.9	2.7	0.2	<0.001	0.853	0.985
C16:0	78.7	59.8	50.0	34.5	4.3	<0.001	0.697	0.457
C16:1*t*9	1.8	1.7	1.4	1.3	0.2	0.045	0.904	0.715
C16:1*c*9	5.4	5.9	4.2	2.8	0.4	<0.001	0.037	0.175
C16:1*ct*11	2.1	2.3	2.6	1.8	0.2	0.600	0.010	0.200
C18:0	43.8	46.7	58.9	64.7	4.2	<0.001	0.719	0.413
C18:1*t*9	1.2	1.6	2.2	1.7	0.4	0.247	0.245	0.480
C18:1*t*10	1.1	1.2	1.0	0.6	0.1	0.009	0.085	0.716
C18:1*t*11	7.9	10.1	8.8	8.0	0.8	0.804	0.068	0.296
C18:1*t*12	1.6	2.0	1.5	0.8	0.2	0.005	0.008	0.508
C18:1*t*13	3.6	4.5	2.4	2.0	0.3	<0.001	0.036	0.003
C18:1*c*9	7.6	8.0	7.4	5.8	0.5	0.013	0.050	0.916
C18:1*c*11	2.0	2.2	1.9	1.8	0.2	0.194	0.418	0.420
C18:1*c*13	0.5	0.9	0.7	0.8	0.1	0.181	0.386	0.200
C18:2*c*9*t*11	1.6	1.5	1.5	1.1	0.2	0.149	0.424	0.543
C18:2n-6	5.2	6.4	6.0	2.6	0.7	0.011	<0.001	0.486
C18:3n-3	3.9	2.8	1.5	2.1	0.3	<0.001	0.003	0.088
C20:5n-3	2.7	3.1	3.4	3.0	0.2	0.113	0.079	0.492
C22:5n-3	1.0	1.3	1.4	1.2	0.2	0.358	0.102	0.931
Unidentified	30.4	22.0	20.1	18.5	1.8	<0.001	0.076	0.451
Total	221.3	200.0	193.3	171.3	13.4	0.014	0.979	0.624

[1]Cattle were fed either 30, 55, 80, or 105% of the forage intake required for maintenance (NRC, 2000) plus 2,556, 1,491, 762, or 0 g/d, respectively, of a high ruminally undegradable protein supplement that contained 6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fish meal (DM basis).
[2]Orthogonal contrasts included linear (L), quadratic (Q), and cubic (C) effects among forage intake levels.
[3]n = 12.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

IN VITRO RUMINAL PROTEIN DEGRADABILITY OF BARLEY VARIETIES

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ABSTRACT: The objective of this study was to investigate the variability of *in vitro* ruminal protein degradability among barley varieties grown in Idaho. Two hundred forty seven barley samples from 12 locations (2004 cropping season) were analyzed for chemical composition and ruminal protein degradability utilizing an *in vitro* procedure, in which inhibitors were used to prevent microbial uptake of ammonia and amino acids released during breakdown of sample proteins. Barley samples included malting, feed, and two-, and six-row varieties. Samples were ground through a 1-mm sieve and analyzed for crude protein and *in vitro* protein degradability. Data were analyzed using the MEANS and GLM procedures of SAS. Concentration of crude protein in the samples averaged 10.4 ± 0.06% (minimum = 6.9% and maximum = 15.7%). Rate of ruminal protein degradability varied from 3.4 - 4.6%/h (Millennium, Idagold, Nebula, B5057) to 7.6 - 8.4%/h (Excel, Burton, CEB0149, WA1070149 and WA850197) and was not significantly different among barley varieties ($P = 0.501$). Estimated ruminal escape of barley protein varied from 43 - 46% (Excel, Burton, CEB0149, WA1070149 and WA850197) to 58 - 60% (Millennium, Idagold, Nebula, B5057) and was also not different among varieties ($P = 0.101$). The interaction between location and variety was investigated for a sub-group of samples including 12 barley varieties grown at 6 locations. Location and variety had no effect on rate of ruminal degradability ($P = 0.450$ and 0.574, respectively), or ruminal escape of barley protein ($P = 0.701$ and 0.118, respectively), but there was a significant interaction between the main effects ($P = 0.003$ and 0.009, respectively). Results from this study suggest that, depending on environmental factors, some barley varieties may have decreased protein degradability in the rumen.

Key Words: Barley, Protein, Ruminal Degradability

Introduction

Barley grain is often fed to lactating dairy cows in the western United States as an alternate energy source to corn grain. Barley is more fermentable in the rumen than corn (Herrera-Saldana et al., 1990; McAllister and Cheng, 1996) and diets based on barley grain produced greater ruminal dietary starch degradability (Feng et al., 1995; Tothi et al., 2003) and in some cases increased ruminal concentration of VFA (Feng et al., 1995; Yang et al., 1997) than corn-based diets. The rate of ruminal degradation of barley starch is controlled by the properties of the protein matrix, which surrounds the starch granules (McAllister and Cheng, 1996). Rumen microorganisms must colonize and digest the protein matrix before they can gain access to the sheltered starch granules. Thus, the extent and rate of colonization and digestion of the protein matrix may be the primary factor that dictates the feeding value of barley for ruminants. Yu et al. (2003), for example, reported that a barley variety with greater ruminal protein degradability (Harrington) also had greater effective degradability of DM and greater starch degradability in the rumen than a variety with lower protein degradability (Valier). Studies have indicated that digestive characteristics of feed barley are genetically related and selection of barley varieties for enhanced feeding value for ruminants may be possible (Ramsey et al., 2001; Kaiser et al., 2004). Khorasani et al. (2000) studied ruminal degradation characteristics of 60 barley cultivars and reported a large variability in barley DM solubility (33 to 56%) and effective degradability (74 to 89%). The authors concluded that genetic selection is a promising tool for enhancing the nutritional value of barley for ruminants. *In vitro*, ruminal responses to barley supplementation depended on its variety and starch composition (Hristov et al., 2002). WestBred Gustoe, for example, stimulated greater incorporation of ammonia N into bacterial protein than corn, or the other barley varieties tested. Another barley variety, normal or waxy Baronesse, was less efficient than corn in promoting ruminal ammonia utilization in lactating dairy cows (Foley et al., 2004). These results suggest that a significant variability in protein degradability exists among barley varieties, which may eventually determine the rate and extent of barley starch digestion in the rumen. Identifying barleys with slower rate of ruminal protein and starch degradation may alleviate concerns associated with feeding barley to dairy cows. Feed intake is the main factor determining milk and milk protein yields (Hristov et al., 2004) and reducing rates of ruminal starch digestion will significantly reduce the risk of digestive disturbances associated with high grain intake. Thus, the objective of this study was to investigate the variability in ruminal protein degradability among barley varieties grown in Idaho, *in vitro*.

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Materials and Methods

Two hundred forty seven barley samples from various locations in Idaho (2004 cropping season) were collected for this study. Overall, barleys from 12 locations and 50 varieties were used in the study. Barley samples included malting, feed, and two-, and six-row varieties. The samples were analyzed for crude protein (**CP**, N × 5.7) on a Costech ECS 4010 C/N/S elemental analyzer (Costech Analytical Technol., Inc., Valencia, CA) Samples were pulverized (Retsch MM200 micro mill; F. Kurt Retsch GmbH & Co. K. G., Haan, Germany) prior to the analysis. Ruminal protein degradability was assayed utilizing an *in vitro* procedure, in which inhibitors were used to prevent microbial uptake of ammonia and amino acids released during breakdown of sample proteins (Broderick, 1987). Incubations were replicated three times (n = 3). Standard feeds (casein and solvent-extracted and expeller soybean meals) were also incubated. Rumen inoculum was obtained from 2 lactating dairy cows before feeding. The diet fed to the donor cows contained alfalfa silage, corn silage, ground corn grain, solvent-extracted soybean meal, and a mineral/vitamin premix (CP, 16.5%; net energy of lactation, 1.60 Mcal/kg). Ammonia and free amino acids released from protein degradation were analyzed according to Broderick and Kang (1980). Ruminal escape of barley protein was estimated as: ruminal escape (%) = protein potentially degradable in the rumen, % × [rate of passage, %/h ÷ (rate of protein degradation, %/h + rate of passage)]. Passage rate was assumed to be 6%/h (Hristov and Broderick, 1996).

Crude protein and degradability data were analyzed sing the MEANS and GLM procedures of SAS (2003; SAS Inst., Inc., Cary NC). The interaction between location and variety was investigated for a sub-group of samples including 12 barley varieties (ACMetcalfe, Baronesse, Bob, CDCStratus, Colter, Creel, Criton, Harrington, Morex, Radiant, Steptoe, and Xena) grown at 6 locations (Ashton, Bonners Ferry, Genesee, Green creek, Moscow, and Soda Springs). Data were analyzed using the GLM procedure of SAS. The model included region, replication (region), variety, and region × variety interaction.

Results and Discussion

Descriptive statistics for CP and ruminal protein escape data for selected barley varieties (with more than 6 observations/variety) are presented in Tables 1 and 2, respectively. Crude protein content of the barley samples investigated was on average 10.4% and varied significantly (P < 0.001) among varieties (from 6.9, minimum to 15.7%, maximum). There was a significant variability within samples from the same variety. For example, CP content of CDCStratus varied from 9.1 (minimum) to 15.2% (maximum). Other varieties commonly used as animal feed also varied significantly in their CP content (Baronesse, for example). Rate of ruminal protein degradability varied from 3.4 - 4.6%/h (Millennium, Idagold, Nebula, B5057) to 7.6 - 8.4%/h (Excel, Burton, CEB0149, WA1070149 and WA850197) and was not significantly different among barley varieties (P = 0.501). Ruminal protein escape was also not significantly different (P = 0.101) among the barley varieties used in this study.

Table 1. Variability in crude protein content (%) among selected barley varieties.

Variety	n	Average	SD	Min	Max
Overall[1]	741	10.4	1.72	6.9	15.7
Selected varieties[2]					
00ID1550	15	9.5	1.01	8.9	11.4
2B96-5057	12	9.4	1.13	8.2	11.1
85B-2323	12	9.6	1.04	8.2	10.9
ACMetcalfe	18	10.9	1.79	8.7	13.7
B5057	12	11.1	1.35	9.6	12.7
Baronesse	33	11.4	2.20	8.5	15.7
Bear	12	11.2	1.55	9.5	13.5
Bob	18	10.6	1.76	9.0	13.3
Burton	12	10.1	1.42	8.5	12.2
CDCStratus	18	10.9	2.23	9.1	15.2
CEB 0149	12	9.8	1.44	7.9	11.2
Camas	27	11.1	1.78	8.8	13.7
Colter	33	9.1	1.14	6.9	10.9
Creel	33	9.4	1.85	7.1	13.2
Criton	18	10.2	1.51	8.6	12.5
Excel	12	8.6	0.69	7.9	9.6
Harrington	42	11.2	1.66	9.1	14.7
Idagold	12	11.5	1.55	9.9	13.4
Legacy	24	9.9	1.00	8.1	11.5
Merit	24	10.2	1.51	8.0	12.3
Millennium	15	10.3	0.64	9.4	11.3
Morex	18	10.4	1.00	9.6	12.4
Nebula	9	10.3	1.12	9.5	11.8
PB1952R522	21	10.7	1.59	8.8	13.4
Radiant	18	10.1	1.79	8.5	13.4
Steptoe	33	9.0	1.18	7.6	11.1
Tradition	24	10.1	0.98	8.6	11.4
Valier	18	10.6	1.83	8.5	14.1
WA1070149	12	9.6	1.43	7.8	11.6
WA850197	12	9.1	1.21	7.4	10.7
Xena	18	9.9	1.43	8.2	12.4

[1] Main effect of variety, P < 0.001.
[2] More than 6 observations/variety.

Within the reduced dataset (12 barley varieties grown at 6 locations), variety and region had no effect (P = 0.118 to 0.701) on barley protein degradation and ruminal protein escape (Table 3). Variety × region interaction, however, was significant for both rate of protein degradation and protein escape (P = 0.003 and 0.009, respectively). Thus, among barleys grown in Ashton, Harrington had a greater (P = 0.050 to 0.038) ruminal protein escape (61%) than ACMetcalfe (46%), CDCStratus (47%), Colter (49%), Morex (47%), and Radiant (42%). Harrington grown in Green creek and Moscow had only numerically greater (P > 0.05) protein escape (58 and 56%, respectively) compared with the

other barley varieties. Among barleys grown in Bonners Ferry, Steptoe had greater ($P < 0.001$ to 0.017) ruminal protein escape (61%) than most varieties, except Morex (58%; $P = 0.709$). Steptoe grown in Genesee, Green creek, and Soda Springs had also relatively high protein escape (57, 54, and 56%; statistically significant compared to some varieties). Another variety, which ranked relatively high in ruminal protein escape, was Baronesse. Grown in Ashton, Baronesse barley had greater ($P = 0.013$ to 0.054) ruminal protein escape than ACMetcalfe, CDCStratus, Morex, and Radiant (42%). Ruminal escape of Baronesse barley protein was only numerically greater ($P > 0.05$) than all other varieties grown in Green creek. Overall, no single barley variety had consistently greater ruminal protein escape throughout all locations. It appears, however, ruminal degradability of barley protein may depend on soil type, temperature, precipitations, elevation, and other unknown environmental factors.

Table 2. Variability in ruminal protein escape (% of CP) among selected barley varieties.

Variety	n	Average	SD	Min	Max
Overall[1]	732	50.2	11.95	17.7	82.7
Selected varieties[2]					
00ID1550	15	48.7	3.86	42.9	56.7
2B96-5057	12	46.5	13.3	26.0	65.5
85B-2323	12	51.2	15.1	28.5	74.4
ACMetcalfe	18	48.8	12.1	26.9	62.6
B5057	12	60.9	10.2	38.5	81.6
Baronesse	33	48.0	12.1	19.8	71.1
Bear	12	46.8	13.0	24.5	64.6
Bob	18	47.9	12.0	26.0	68.0
Burton	12	47.0	12.2	24.3	64.7
CDCStratus	18	46.1	9.9	28.1	59.8
CEB 0149	11	46.6	13.9	27.8	66.4
Camas	27	49.5	11.3	28.8	73.1
Colter	33	48.2	8.0	31.9	64.8
Creel	32	49.5	11.1	32.8	80.6
Criton	18	49.5	13.2	24.6	66.1
Excel	11	46.6	14.2	28.8	69.4
Harrington	42	52.3	14.1	17.7	79.1
Idagold	11	58.7	12.3	29.9	74.4
Legacy	23	56.7	12.5	30.6	79.1
Merit	23	52.3	11.8	26.6	71.6
Millennium	15	58.9	10.8	40.0	74.9
Morex	18	49.0	13.5	24.4	80.9
Nebula	8	58.5	6.0	50.4	66.5
PB1952R522	21	47.6	8.3	34.9	67.0
Radiant	18	47.3	12.1	26.8	65.2
Steptoe	33	51.1	12.4	26.0	82.7
Tradition	24	51.9	12.3	23.3	73.1
Valier	17	49.5	11.8	30.8	67.3
WA1070149	12	45.6	15.5	25.6	65.4
WA850197	11	45.3	12.5	28.8	62.6
Xena	18	49.1	11.5	29.3	64.6

[1] Main effect of variety, $P = 0.101$.
[2] More than 6 observations/variety.

Table 3. Effect of barley variety and region on rate of protein degradation and ruminal escape *in vitro* (n = 230).

Variable	DF	Pr > F
Rate of protein degradation		
Variety	11	0.574
Region	5	0.450
Variety × Region interaction	55	0.003
Ruminal protein escape		
Variety	11	0.118
Region	5	0.701
Variety × Region interaction	55	0.009

Implications

Barley varieties grown in Idaho vary significantly in crude protein content, but variability in ruminal degradation rate and protein escape was not significant among barleys tested in this study. Examination of a sub-set of barley varieties grown in six locations showed a significant interaction between variety and location on the rate of barley protein degradability and ruminal protein escape *in vitro*. Thus, results from this study suggest that, depending on environmental factors, some barley varieties may have decreased protein degradability in the rumen.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

IDENTIFICATION OF QUALITY CHARACTERISTICS OF FEED BARLEY AND RELATING THOSE CHARACTERISTICS TO DIGESTIBILITY IN FEEDLOT STEERS

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ABSTRACT: The objective of this study was to evaluate the variability of barley quality and to better characterize the relationship between variability in chemical composition and digestibility of barley. In 2004, 249 barley samples were obtained representing different varieties from 13 locations in Idaho. Whole barley was analyzed for bulk density (**BD**). Representative subsamples were ground through a 1-mm screen and analyzed for DM, NDF, ADF, ash, and starch. The ranges of NDF, ADF, starch, BD, and *in vitro* total digestibility (**IVTD**) were 11.8 to 25.6%, 2.2 to 8.8%, 48.2 to 72.5%, 569 to 784 g/L, and 66.7 to 85.1%, respectively. *In vitro* total digestibility was most closely correlated with NDF (r = -0.69, P < 0.001). This relationship improved when correlation was determined by variety (r = -0.81; P < 0.001) and location (r = -0.93, P < 0.001). Eight sources of barley were then selected to represent the spectrum of BD, IVTD, and chemical composition. Thirty-two steers were adapted to a finishing diet consisting of 80% dry-rolled barley (DM basis) from 1 of the 8 sources. Steers were randomly assigned to the 8 diets and digestibility measurements were obtained during 3 repeated experiments. Digestibility of DM, OM, and starch were not different between barley sources. *In vitro* total digestibility decreased (P < 0.001) quadratically with CP and NDF, but increased (P < 0.001) quadratically with starch. Despite the correlations noted between measures of chemical composition and IVTD, relationships between measures of chemical composition and *in vivo* digestibility were insignificant (r < 0.25). Results of this study support the notion that 1) there is a wide range in the chemical components of barley sources and 2) fiber appears to be the most reliable predictor of barley digestibility.

Key Words: *in vivo*, *in vitro*, digestible energy, variability

Introduction

Barley is an energy dense feed ingredient, but its nutritive value is considered to be less than that of corn or wheat (Taylor et al., 1985). One reason for this difference may be greater variability in the quality of the barley (Bowman *et al*., 2001). Bulk density is a popular method for estimating barley quality, but it may not accurately reflect chemical composition or quality of the barley. Christison and Bell (1975) noted little relationship between BD and DE of barley. Similarly, McDonnell et al. (2003) reported similar NE between barley having BD of 508 and 631 g/L. Accordingly, lack of relationship between BD and energy content should raise concern over the use of BD as an indicator of barley quality. Furthermore, this conundrum presents a challenge to livestock producers who purchase feed barley based on BD. The objective of this study was to evaluate the variability of barley quality, and to better characterize the relationship between the variability in chemical composition and the DE content of barley.

Materials and Methods

Laboratory Experiment

Two hundred and forty-nine barley grain samples representing multiple cultivars and growing locations throughout Idaho were collected from the 2004 cropping year. Varieties were not always represented at each growing location. Representative samples were analyzed for BD (Grain test weight scale, Seedburo Equipment Company, Chicago, IL), and subsequently ground through a 1-mm screen using a Thomas-Wiley laboratory mill (Model 4, Thomas Scientific, USA). Ground subsamples were analyzed for DM, ash (AOAC, 1990), IVTD (described later), and NDF and ADF (Goering and Van Soest, 1970). The IVTD method was according to Goering and Van Soest (1970) with some modifications: 1) the incubation period lasted 10-h, 2) samples were rinsed twice in boiling water containing 4 mL α-amylase, 3) incubated samples were rinsed once in boiling water, and 4) incubated samples were rinsed once in acetone. Additional subsamples were processed in a Retsch mixer mill (MM200; F. Kurt Retsch GmbH & Co. KG, Hann, Germany) for 2.5 min. at a frequency of 25 vibrations/s, and analyzed for total starch content (AOAC, 1995; total starch assay kit obtained from Megazyme Int. Ireland Ltd., Wicklow, Ireland).

In Vivo Digestibility Experiment

Thirty-two Angus crossbred steers (mean BW 305 kg) were adapted to a basal high concentrate diet containing 80% dry-rolled barley (DM basis; Table 1). The remainder of the diet DM was comprised of 6.25% chopped grass hay, 9.19% chopped alfalfa, and 4.56% supplement. Supplement DM was comprised of 46.26% dried distillers plus solubles, 19.66% ground limestone, 12.25% salt mix, 10.91% canola oil, and 10.91% urea. Water was included in order to attain a dietary moisture content of 34%. Steers were randomly assigned to experimental diets based on 1 of 8 different barley sources. Sources of barley were Nebula grown in Blackfoot, ID (**BN**), Xena grown in Blackfoot, ID (**BX**), Baronesse grown in Burley, ID (**BB**), Millennium grown in

Burley, ID (**BM**), Baronesse grown in Grace, ID (**GB**), Xena grown in Grace, ID (**GX**), Baronesse grown in Whitman County, WA (**PB**), and Baronesse grown in Soda Springs, ID (**SSB**). These barley sources were selected to represent the range in chemical composition and BD of the barley sources used in the laboratory study. Steers were fed in 2 equal portions at 0800 and 1600. Amount of diet fed was restricted to 2x NEm requirement (NRC, 1996). Water was provided *ad libitum* using automatic waterers. Animals were cared for and housed according to University of Idaho Animal Care and Use Committee guidelines.

Barley was dry-rolled in a Buhler Farm King Allied Y100STD roller mill (John Buhler Inc., Morden-Winnipeg, MB). In an attempt to prevent settling of fines as a result of a large supply of stored processed barley, barley was processes as needed. Roller setting was maintained to ensure each kernel was fractured into at least 4 pieces. Representative samples of barley, forages, and supplement were obtained 2 d prior to, and during the fecal collection period (described later). With the exception of supplement, samples were processed as described previously. Forages were analyzed for DM, ash, NDF, and ADF as previously described, and for CP by Kjeldahl N (AOAC, 1990). Whole barley samples were analyzed for BD, whereas ground samples were analyzed for DM, ash, NDF, ADF, and starch as described previously.

Steers were allowed to adapt to their respective diets over the course of 11 d at which point fecal samples were collected. Dry matter intake was recorded by steer daily. Fecal samples were collected over a 72 h period at predetermined times chosen to represent every 4 h within a 24 h cycle. Seven d prior to and during the fecal collection period, chromic oxide was included in the diet at a rate of 15g/steer/d. Fecal samples were frozen and later dried at 60°C in a forced-air oven. Dry samples composited by steer nd experimental diet were ground as described previously and analyzed for DM, ash, and starch as described previously and for Cr (Williams et al., 1962). Digestibility of DM, OM, and starch were calculated using the ratio of Cr in the diet and feces (Schneider and Flatt, 1975). Dietary DE content was computed using the TDN (NRC, 1996) and DM digestibility estimates of the individual feed ingredients.

Table 1. Composition of the basal feedlot finishing diet.

Item	Amount
Ingredient, % of dry matter	
Chopped grass hay	6.25
Chopped alfalfa hay	9.19
Dry-rolled barley	80.0
Supplement[a]	4.56
Chemical composition (calculated)[b]	
NEm, Mcal/kg	1.88
NEg, Mcal/kg	1.24
CP, %	12.90
DIP, % of CP[c]	66.98
Ca, %	0.48
P, %	0.35

[a]Supplement DM contained 46.3% dried distillers grains, 19.7% limestone, 12.3% trace mineralized salt, 10.9% canola oil, and 10.9% urea.
[b]Based on 1996 Beef NRC.
[c]DIP = Degradable intake protein

Statistical Analysis

Data from the laboratory experiment were analyzed as continuous data using the GLM procedure of SAS (Version 9.1, SAS Inst., Inc., Cary, NC). Growing location and cultivar were included as main effects in the model. The interaction between cultivar and growing location was not tested because many cultivars were not represented in more than one or two growing locations. Principle component analysis was conducted using the PRINCOMP procedure of SAS to evaluate what combination of variables might serve as unique independent variables during subsequent modeling. Ordinary linear regression models were invoked using the REG procedure of SAS to test models relating to IVTD.

The *in vivo* digestibility experiment was replicated 3 times, yielding at least 10 unique digestibility observations per dietary treatment. Using the MIXED procedure of SAS, the following statistical model was used to test the effects of dietary treatment on chemical composition and nutrient digestibility:

$$Yij(k) = \mu + \alpha i + \beta j + \gamma k + \varepsilon ij(k)$$

where

Y = observation,
μ = overall mean,
αi = random effect of steer,
βj = fixed effect of experiment,
γk = fixed effect of dietary treatment,
$\varepsilon ij(k)$ = residual error term that was assumed to be distributed normally.

Correlations (ρ) between measures of chemical composition and between measures of chemical composition and IVTD, *in vivo* digestibility, and DE were estimated by computing coefficients of determination (r) in the CORR procedure of SAS. Ordinary linear regression models were invoked using the REG procedure of SAS to compute models designed to test the relationships between measures of chemical composition, IVTD, and *in vivo* nutrient digestibility. Mean separation was conducted using the Fischer's least significant difference (LSD procedure of SAS) and differences were reported when $P < 0.05$.

Results and Discussion

Laboratory Experiment

A wide range in variability existed among measurements obtained from the laboratory samples. Neutral detergent fiber content ranged from 11.8 to 25.6%, mean was 19.0% and median was 18.9%. Mean ADF was 5.2%, median was 5.0% and ranged from 2.2 to 8.8%.

Starch content ranged from 48.2 to 72.5%, averaged 59.9%, and exhibited a median of 59.9%. The BD range for the sources of barley was from 569 to 784 g/L with a mean of 664.9 g/L and a median of 666.1 g/L. *In vitro* total digestibility ranged from 66.7 to 85.1%, averaged 76.6%, and had a median of 76.6%. Barley sources used in the laboratory trial were selected to represent a wide range in variability. Therefore, differences in chemical composition and BD noted among the barley sources may have been expected. Principle component analyses resulted in only the first principle component having an eigenvalue of greater than 1. Nevertheless, inclusion of the principle component did not improve model fitting and so only original measurements were included in the analysis. Neutral detergent fiber was negatively correlated to IVTD (r = -0.69 (P < 0.001; Figure 1), whereas the correlation between starch and IVTD was low (r = 0.36, P < 0.001; Figure 2). Bulk density was moderately correlated with IVTD (r = 0.51, P < 0.001; Figure 3). When computed by variety, correlation improved (r = -0.81; P < 0.001; data not shown). Likewise, correlation improved when the relationship between IVTD and NDF were computed by location (r = -0.93, P < 0.001; data not shown). Our observation that growing location influenced the relationship between NDF and IVTD may have been expected as growing condition typically has an impact on genotypic expression of barley cultivars. Nevertheless, Doornbos and Newman (1989) noted a larger proportion of variation in chemical properties of barley was attributed to variety rather than growing location. Addtionally, Reynolds *et al*. (1992) observed equal proportions of variability explained by variety and growing location.

In Vivo Digestibility Experiment

Crude protein was greater (P < 0.05) for SSB compared to BX, BB, BM, GX, and PB (Table 2). Baronesse grown in Whitman County, WA had lower (P < 0.05) CP compared to GX, BM, and BN. Neutral detergent fiber was lower (P < 0.05) for GX compared to BM and SSB and was greater (P < 0.05) for BM compared to BB and PB. Gace, ID Xena had lower (P < 0.05) ADF compared to PB, SSB, and BM, whereas BM had greater (P < 0.05) ADF compared to all barley sources but SSB. Starch content was lower (P < 0.05) for SSB compared to BX, GX, and PB and BD was lowest (P < 0.05) for SSB. Soda Springs, ID Baronesse had IVTD lower (P < 0.05) than BX, BB, GB, GX, and PB. As with the laboratory trial, barley sources used in the *in vivo* study were selected to represent the spectrum of BD and chemical composition which can be encountered in feed barley. Accordingly, it was not surprising to note differences in chemical composition among the different barley sources. *In vivo* digestibility of DM, OM, and starch are reported in Table 3. Interactions between barley sources and period were not detected for the digestibility values calculated. Although flow of DM (kg/d) tended to differ between barley sources (P = 0.10), DM digestibility was not different between barley sources. Similarly flow of OM (kg/d) tended to differ between barley sources (P = 0.08) without corresponding differences in starch or OM digestibility.

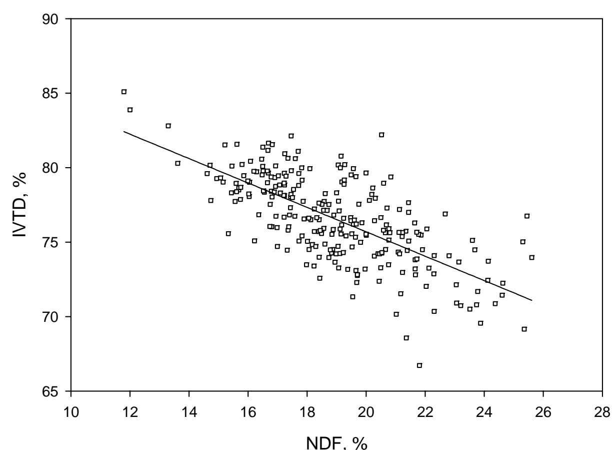

Figure 1. Relationship between NDF content and *in vitro* total digestibility (**IVTD**) of barley samples used in the laboratory trial (n = 249; IVTD, % = 92.075 – 0.8192*NDF; R^2 = 0.48; P < 0.001).

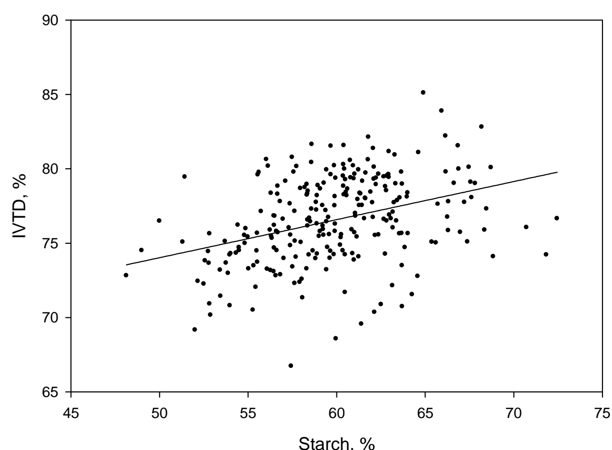

Figure 2. Relationship between starch content and *in vitro* total digestibility (**IVTD**) of preliminary barley samples (n = 249; IVTD, % = 61.2112 + 0.2565*starch; R^2 = 0.1320; P < 0.001).

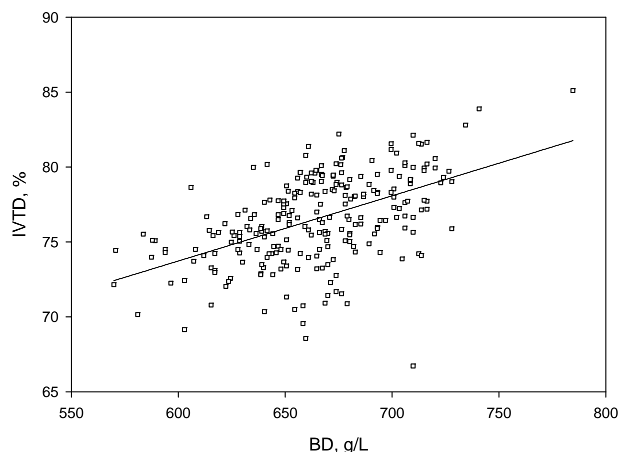

Figure 3. Relationship between bulk density (**BD**) and *in vitro* total digestibility (**IVTD**) of preliminary barley samples (n = 249; IVTD, % = 47.614 + 0.0435*BD; R^2 = 0.26; P < 0.001).

Table 2. Chemical composition, bulk density (**BD**), and *in vitro* total digestibility (**IVTD**) of barley sources used in the *in vivo* digestibility study.

	Barley Source§									
Item	BN	BX	BB	BM	GB	GX	PB	SSB	SE	*P*-value
Composition, % DM										
CP	12.8^{cd}	10.8^{abc}	11.4^{abc}	10.4^{ab}	11.8^{bcd}	10.2^{b}	9.5^{a}	13.7^{d}	0.38	0.001
NDF	22.9abc	21.5abc	21.3ab	24.7c	22.4abc	19.9^{a}	21.3^{ab}	24.5^{bc}	0.61	0.001
ADF	6.24abc	5.91ab	6.15ab	7.74d	5.88ab	5.32^{a}	6.57^{bc}	7.30^{cd}	0.20	0.001
Starch	51.5ab	56.0bc	53.7abc	53.2abc	53.3abc	57.6^{bc}	55.4^{bc}	50.4^{a}	0.87	0.001
BD, g/L	552.7b	651.9cd	686.7de	630.0c	623.6c	630.0^{c}	711.2^{e}	484.4^{a}	8.11	0.001
IVTD, %	72.7ab	75.6b	74.8b	72.0a	73.9b	74.9^{b}	74.5^{b}	69.5^{a}	0.75	0.001

§BN = Nebula grown in Blackfoot, ID; BX = Xena grown in Blackfoot, ID; BB = Baronesse grown in Burley, ID; BM = Millennium grown in Burley, ID; GB = Baronesse grown in Grace, ID; GX = Xena grown in Grace, ID; PB = Baronesse grown Whitman County, WA; SSB = Baronesse grown in Soda Springs, ID.

[a,b,c,d,e]Means within a row with unlike superscripts differ ($P < 0.05$).

Given the wide range in chemical composition represented by the barley sources used in this trial, we hypothesized that differences in digestibility of finishing diets based upon the different barley sources would be detected. On the contrary, *in vivo* digestibility of starch from the total digestive tract of steers did not differ between the different barley sources (range of 98.7 to 99.3%). Similarly, total tract DM and OM digestibility in steers was not influenced by barley source (range of 68.3 to 72.7% and 70.5 to 74.4% for DM and OM, respectively). It is not completely evident why *in vivo* digestibility differences were absent. One explanation may lie in the fact that steers did not consume diets at an *ad libitum* rate. Presumably, a relatively low feed intake could have provided greater retention time of barley in the digestive tract of steers. Greater retention time in turn may have afforded lower quality barley a greater opportunity to be digested. In cattle fed at *ad libitum* (i.e., potentially greater) intake, improved nutrient availability characteristic of a higher quality barley source, could have been of greater importance.

A total of 24 observations were collected for chemical composition and digestibility of the barley sources used in the *in vivo* trial (i.e., 8 sources; 3 replications). Using this data, we noted that starch increased ($P < 0.001$) quadratically with BD (Figure 4). Furthermore, IVTD was found to decrease ($P < 0.001$) quadratically with CP and NDF (Figures 5 and 6, respectively), but increased ($P < 0.001$) quadratically with starch (Figure 7). A weak correlation (r = 0.34) was noted between BD and IVTD. Results from other trials demonstrate BD is a poor indicator of animal performance (Mathison *et al*., 1991). Furthermore, Grimson (1987) proposed that differences in animal performance are influenced more by chemical composition of barley rather than bulk density. Despite the correlations noted between measures of chemical composition and IVTD, relationships between measures of chemical composition and *in vivo* digestibility were insignificant (r < 0.25). Our primary motive in conducting these studies was to identify those characteristics of barley

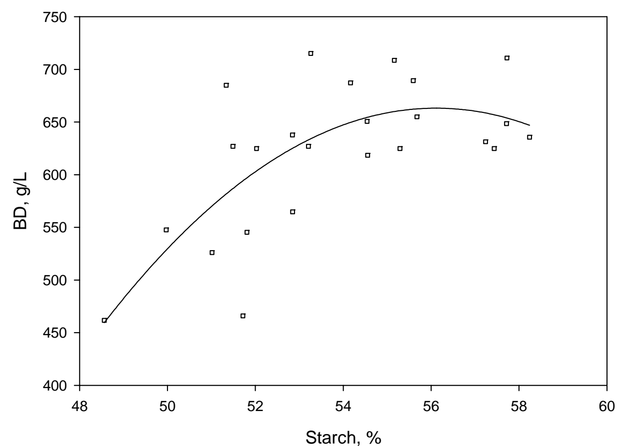

Figure 4. Relationship between starch and bulk density (**BD**) of barley grain sources for the *in vivo* digestibility trial (n = 24; BD, g/L = – 10,594.163 + 401.265*Starch – 3.576*Starch^2; $R^2 = 0.50$; $P < 0.001$).

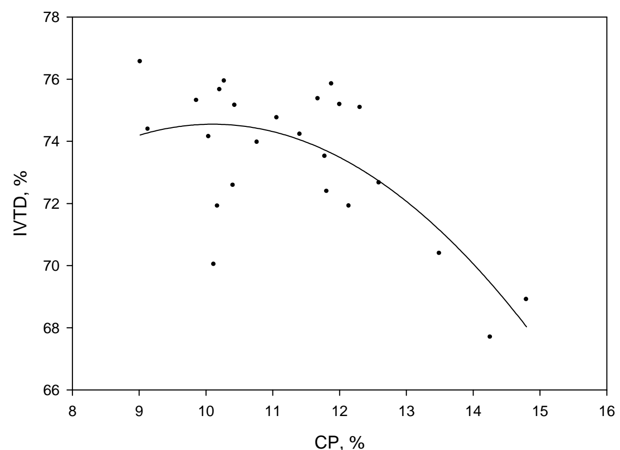

Figure 5. Relationship between CP and *in vitro* total digestibility (**IVTD**) of barley grain sources for the *in vivo* digestibility trial (n = 24; IVTD, % = 44.4002 + 5.9702*CP – 0.2955*CP^2; $R^2 = 0.5020$; $P < 0.001$).

that would be capable of predicting *in vivo* digestibility, and in essence, feeding value. Nevertheless, significant correlations were not apparent between chemical composition and measures of *in vivo* digestibility. Lack of relationship between these classes of variables might also have been explained by the same factor(s) responsible for precluding us from detecting differences in digestibility among the 8 barley sources. Even so, the relationship between IVTD and NDF was again present during our analysis of the *in vivo* data (Figure 6). Computing the first order derivate of this polynomial equation revealed that IVTD was maximized when NDF was equal to 20.1%. Whether this value for NDF is meaningful enough to apply to extraneous situations is not known; however, these findings do suggest that reliable prediction of feed barley quality may rest in characterizing fibrous components of barley rather than current methods such as BD. Further experimentation may be needed to solidify this concept.

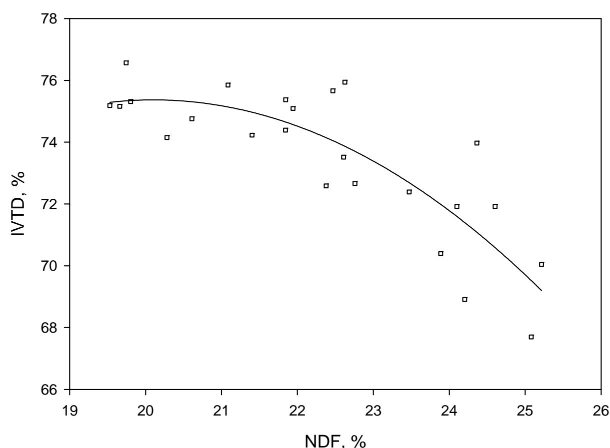

Figure 6. Relationship between NDF and *in vitro* total digestibility (**IVTD**) of barley grain sources for the *in vivo* digestibility trial (n = 24; IVTD, % = – 20.001 + 9.4879*NDF – 0.236*NDF^2; $R^2 = 0.70$; $P < 0.001$).

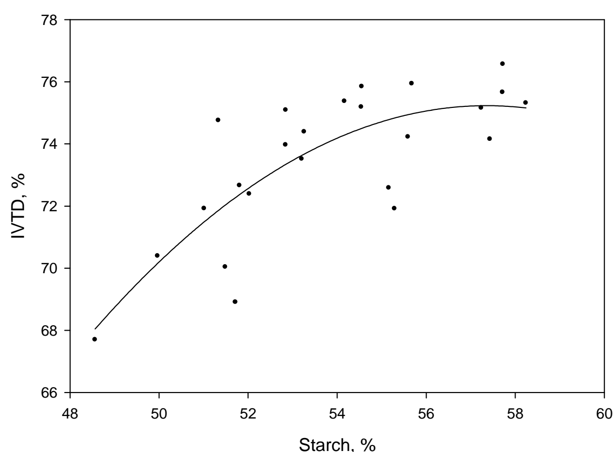

Figure 7. Relationship between starch and *in vitro* total digestibility (**IVTD**) of barley grain sources for the *in vivo* digestibility trial (n = 24; IVTD, % = -230.6126 + 10.6646*starch – 0.093*starch^2; $R^2 = 0.6124$; $P < 0.001$).

Implications

Fiber content, specifically the NDF fraction, was most highly correlated with IVTD. Additionally, BD and measures of chemical composition did not correlate well with measures *in vivo* digestibility. Results from these trials further emphasize the wide range in variation of barley and underscore the need to improve upon the current method for predicting feed value of barley in livestock.

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Table 3. *In vivo* DM, OM, and starch digestibility of feedlot diets based on different barley sources

	Barley Source§									Probability, *P*		
Item	BN	BX	BB	BM	GB	GX	PB	SSB	SE	Barley	Period	INT
DM												
Intake, kg/d	6.75	6.62	6.68	6.89	6.84	6.82	6.74	7.05	0.17	0.442	0.016	0.592
Flow, kg/d	1.87	1.91	2.00	2.17	1.97	1.91	2.12	2.09	0.09	0.097	0.423	0.384
Digestibility												
%	72.7	71.1	69.9	68.3	71.2	72.0	68.9	70.1	1.29	0.245	0.318	0.736
kg/d	4.84	4.71	4.68	4.74	4.85	4.92	4.65	4.97	0.16	0.597	0.025	0.854
OM												
Fecal, %	87.0	87.1	87.1	87.8	87.2	87.5	88.1	86.4	0.39	0.073	0.001	0.264
Flow, kg/d	1.62	1.66	1.75	1.90	1.72	1.67	1.87	1.81	0.08	0.079	0.670	0.356
Digestibility												
%	74.4	73.4	72.2	70.5	73.4	74.2	70.9	72.6	1.24	0.183	0.271	0.704
kg/d	5.00	4.86	4.83	4.89	5.00	5.06	4.78	5.14	0.16	0.544	0.016	0.839
Starch												
Fecal, %	1.69	2.57	2.14	1.51	1.80	1.43	1.80	1.68	0.28	0.109	0.055	0.393
Digestibility												
%	99.1	98.7	98.8	99.1	99.0	99.3	99.0	99.0	0.17	0.242	0.046	0.303
kg/d	6.67	6.53	6.60	6.82	6.77	6.78	6.68	6.98	0.17	0.412	0.012	0.692

§ BN = Nebula grown in Blackfoot, ID; BX = Xena grown in Blackfoot, ID; BB = Baronesse grown in Burley, ID; BM = Millennium grown in Burley, ID; GB = Baronesse grown in Grace, ID; GX = Xena grown in Grace, ID; PB = Baronesse grown Whitman County, WA; SSB = Baronesse grown in Soda Springs, ID.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

CHARACTERIZATION OF FORAGE TRACE MINERAL CONCENTRATION BY SEASON IN DIETS OF BEEF COWS GRAZING NATIVE RANGE IN EASTERN COLORADO[1]

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ABSTRACT: Concentrations of Se, Cu, Co, Fe, Mn, Mo, and Zn in diets of cows grazing native sandhills range in Eastern Colorado were characterized during a 21-month period in 2001 and 2002. After rumen evacuation and short-term grazing (approximately 30 min.), rumen grab-samples were collected from 2 fistulated beef cows. Samples were collected 27 times (once or twice monthly) and represented 4 seasons during each yr: winter (Nov. to Mar.), spring (April to May), summer (June to Aug.), and fall (Sept. to Oct.). Samples (n = 54) were analyzed for trace mineral concentration. In most (> 90%) samples, Co and Mo concentrations could not be quantified because they were below detection limits (0.5 and 1.0 mg/kg DM, respectively). Overall mean (± SD) concentrations (mg/kg DM) were: Se, 0.26 ± 0.097; Cu, 3.9 ± 1.84; Fe, 428.1 ± 530.06; Mn, 67.7 ± 25.05; and Zn, 18.3 ± 6.43. There was a tendency ($P = 0.10$) for a yr × season interaction for Mn concentration, but no yr × season interaction ($P > 0.48$) for Se concentration, so data were pooled across yr for Mn and Se. Concentration of Se tended to be greater in spring vs. summer ($P = 0.11$) and fall ($P = 0.06$), and Mn concentration tended ($P = 0.09$) to be greater in winter than spring. There were yr × season interactions for Cu ($P < 0.01$), Fe ($P < 0.05$), and Zn ($P < 0.001$) concentrations. In Yr 1, Cu concentration was greater ($P < 0.05$) in winter vs. summer and fall, and tended ($P < 0.09$) to be lower in fall vs. spring and summer. In Yr 2, Cu concentration was lower ($P < 0.05$) in winter vs. all other seasons. Concentration of Fe in Yr 1 was greater ($P < 0.05$) in winter than all other seasons. Winter Zn concentration in Yr 1 tended ($P = 0.08$) to be greater than spring, and was greater ($P < 0.05$) vs. summer and fall. Concentration of Zn was lower ($P < 0.05$) in winter vs. all other seasons in Yr 2, and greater ($P < 0.05$) in fall compared to all other seasons. Results suggest that beef cow diets in parts of eastern Colorado contain inadequate Cu and Zn concentrations, and concentrations of some trace minerals may differ by season.

Key words: Beef Cows, Native Range, Trace Minerals

Introduction

Trace minerals are necessary for normal growth, reproduction, and immune response in beef cattle (McDowell, 1992). During most of the year, native range forages provide the primary source of trace minerals for the majority of beef cows throughout the western U.S. As range forages mature during the summer and fall, nutrient content (fiber, protein, etc.) and digestibility undergo substantial changes. Thus, in many situations, nutrient supplementation to cattle is necessary in order to avoid deficiencies and maintain production. A major challenge faced by western beef cow/calf operators is determining when and how to supplement (Bohnert and DelCurto, 2004).

The ability of a beef cow to perform on western rangelands depends on 3 primary factors: 1) nutrient concentration and availability in forage, 2) forage intake, and 3) nutritional needs of the animal (Adams and Short, 1988). Nutrients required by beef cattle are well documented (NRC, 1996); however, information on the amount of minerals in beef cow diets is limited. Without these data, development of a low-cost trace mineral supplementation strategy to provide necessary trace minerals and reduce supplementation costs can be difficult.

Sprinkle and others (2000) documented that trace mineral concentrations in hand-clipped forages vary significantly at different stages of the growing season in Arizona. Similarly, year-to-year, month-to-month, and species-specific patterns of mineral concentration in 7 northern Great Basin rangeland grasses, also collected via clipping, are quite variable (Ganskopp and Bohnert, 2003). Earlier researchers using Texas native range evaluated the effect of plant selection by grazing livestock on the resulting concentration of macro minerals in the diet (Pinchak et al., 1989). The authors suggested that selection of live tissue vs. whole plants would result in a diet with maximum macro mineral concentrations.

Therefore, the objective of this study was to characterize seasonal effects on the forage concentrations of Se, Cu, Co, Fe, Mn, Mo, and Zn consumed by cows grazing native sandhills range in Eastern Colorado in order to determine if trace mineral supplementation strategies should be adjusted seasonally by beef cattle producers.

Materials and Methods

General procedures. Two mature, ruminally-fistulated beef cows were used during a 21-month period (January 2001 through September 2002) to collect masticated diet samples from native sandhills range at the Eastern Colorado Research Center (Akron, Colorado). The native range consisted primarily of blue grama (*Bouteloua gracilis*), prairie sandreed (*Calamovilfa longifolia*), and needle-and-thread grass (*Stipa comata*), which collectively comprised approximately 80% of the vegetation.

Sample collection was done as described by Olson (1991). Briefly, prior to sample collection, each rumen was completely evacuated by hand and the liquid portion was removed via a modified wet/dry vacuum cleaner. Each cow was then allowed to graze for approximately 30 min. in a designated 40 hectare pasture. After grazing, a diet sample (approximately 2 kg) was collected from each cow and frozen for later analysis. After grazed forages were sampled, each rumen was refilled with its original contents.

Samples were collected at 27 different times (once or twice monthly) during the trial, and represented 4 seasons during each yr: winter (Nov. to Mar.), spring (April to May), summer (June to Aug.), and fall (Sept. to Oct.). After freeze-drying, samples (n = 54) were ground to pass through a 2 mm screen and analyzed for trace mineral concentration via inductively coupled plasma atomic emission spectroscopy.

Data analyses. With animal as the experimental unit, data were analyzed using a restricted maximum likelihood-based, mixed effects model repeated measures analysis (PROC MIXED, SAS Inst. Inc., Cary, NC) to determine the effect of growing season on trace mineral concentration. Season and rumen-cannulated cows were the independent variables and concentration of trace mineral was the dependant variable.

Results and Discussion

In most (> 90%) of the samples, Co and Mo concentrations could not be quantified because they were below detection limits (0.5 and 1.0 mg/kg DM, respectively). Only 4 samples had Co concentrations above 0.5 mg/kg DM, and none were greater than 2.0 mg/kg DM. Similarly, of the 3 samples where Mo was quantified, the highest Mo concentration was 1.27 mg/kg DM. Based on these apparent low Mo concentrations, any antagonistic effect of Mo on Cu in a ruminant's diet would most likely be minimal (NRC, 1996).

For the other minerals, overall mean (± SD) concentrations (mg/kg DM) were: Se, 0.26 ± 0.097; Cu, 3.9 ± 1.84; Fe, 428.1 ± 530.06; Mn, 67.7 ± 25.05; and Zn, 18.3 ± 6.43. Relative to NRC (1996) recommendations for beef cows, mean trace mineral concentrations in masticate diet samples collected at the 27 sampling times were adequate for Se and Fe every time, adequate in Mn 26 times, and adequate in Zn only 2 times. Mean copper concentration was not adequate at any of the collection times.

In terms of the effect of season on trace mineral concentration, there was a tendency ($P = 0.10$) for a yr × season interaction for Mn concentration, but no yr × season interaction ($P > 0.48$) for Se concentration, so data were pooled across yr for both Mn and Se (Table 1). Concentration of Se tended to be greater in spring vs. summer ($P = 0.11$) and fall ($P = 0.06$). In contrast, Mn concentration was not affected by season, but tended ($P = 0.09$) to be greater in winter than spring.

Mean Cu, Fe, and Zn concentrations are reported in Table 2. There were yr × season interactions for Cu ($P < 0.01$), Fe ($P < 0.05$), and Zn ($P < 0.001$) concentrations, and therefore data are reported by yr. In Yr 1, Cu concentration was greater ($P < 0.05$) in winter vs. summer and fall, and tended ($P < 0.09$) to be lower in fall vs. spring and summer. In Yr 2, Cu concentration was lower ($P < 0.05$) in winter vs. all other seasons. Concentration of Fe in Yr 1 was greater ($P < 0.05$) in winter than all other seasons. In Yr 2, Fe concentrations were not different among the 4 seasons. Winter Zn concentration in Yr 1 tended ($P = 0.08$) to be greater than spring, and was greater ($P < 0.05$) vs. summer and fall. Concentration of Zn was lower ($P < 0.05$) in winter vs. all other seasons in Yr 2, and greater ($P < 0.05$) in fall compared to all other seasons. Interestingly, mean fall Zn concentration in Yr 2 was the only season during the entire experiment when dietary Zn concentration was adequate relative to NRC (1996) recommendations for beef cows.

Results for Zn, Cu, Fe and Mn in the current experiment are similar to those reported in a large survey by Corah and others (1996) where 352 forage samples collected from 18 states (including Colorado) were evaluated for trace mineral concentration. In general, relative to beef cow dietary needs, the authors reported widespread deficiencies of Se and Zn, marginal Cu deficiency and elevated concentrations of Cu antagonists (Fe, Mo, and S), and adequate concentrations of Mn and Fe.

In a regional experiment, a smaller-scale survey conducted to quantify forage trace mineral concentrations in New Mexico (Mathis and Sawyer, 2004) via hand-plucked samples reported similar results. The authors observed that overall trace mineral concentrations varied greatly, based on more than a ninefold range in concentrations for all minerals evaluated. When compared to NRC (1996) recommendations for beef cows, forage samples were inadequate in Cu (40% of samples), Mn (16%), Se (92%), and Zn (77%) concentration. However, unlike the current experiment, trace mineral concentrations were higher in fall than late winter in most cases.

Also in agreement with our results, Sprinkle et al. (2000) reported that hand-clipped range forage samples in Arizona were marginally deficient in Cu (7.0 mg/kg DM) and Zn (20.5 mg/kg DM). Although, in contrast to our results, the authors observed that Se was deficient (0.05 mg/kg DM) throughout the 2-yr study. In that experiment, which evaluated how Cu, Se, and Zn varied by season, only Cu and Zn concentrations varied during the yr, apparently due to winter and summer precipitation levels.

Consistently low Cu (1.75 mg/kg DM) and Zn concentrations (12.1 mg/kg DM) have also been reported in northern Great Basin grasses sampled monthly over a 2-yr period (Ganskopp and Bohnert, 2003). Also, Fe concentration was adequate and Mn concentration was generally adequate (38.6 mg/kg DM). However, Mn was the only trace mineral that varied by season by increasing as grass matured.

Grings et al. (1996) also observed widespread inadequate concentrations of Zn and Cu and adequate concentrations of Mn for beef cattle. In that experiment, northern Great Plains mineral concentrations were characterized in clipped range samples by plant species, date, and tissue class (live vs. dead). Similar variability in mineral concentration to the current experiment was observed, as well as low and mostly-undetectable Mo concentrations (< 1.0

mg/kg DM) that did not exceed 2.0 mg/kg DM. In general, the authors reported no obvious patterns of mineral concentration change during the growing season.

In conclusion, results of the current experiment indicate that beef cow diets in areas of eastern Colorado contain very low concentrations of Mo and Co, inadequate Cu and Zn concentrations, and adequate concentrations of Fe, Mn, and Se. In addition, trace mineral concentrations in beef cow diets can differ by season, but not consistently across years or minerals. It should be noted that trace mineral values reported are from masticate samples, and the digestibility of the trace minerals within the masticate samples was not determined. Therefore, caution should be taken when formulating trace mineral supplements based on masticate trace mineral analysis or, for that matter, any forage sample.

Implications

Limited information is available on the concentrations of trace minerals in diets of beef cows grazing western range. However, data suggest that trace mineral concentrations vary substantially due to several variables. In general, among beef cows in the western U.S., researchers have consistently shown that copper and zinc concentrations are widely inadequate, manganese and iron concentrations are mostly adequate, and selenium concentrations are extremely variable. In addition, seasonal variation in trace mineral concentration does occur, but does not follow a consistent pattern. Therefore, site-specific forage collection and analysis at more than one time during the year is warranted prior to development of a trace mineral supplementation program.

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Table 1. Least squares means for Se and Mn concentrations (± SE) in beef cow diets on native range in Eastern Colorado[a]

Season of year	Se concentration (mg/kg DM)	SE	Mn concentration (mg/kg DM)	SE
Winter	0.26	0.024	75.1	6.129
Spring	0.32	0.030	58.1	7.661
Summer	0.26	0.020	68.2	5.082
Fall	0.22	0.040	63.3	10.279

[a] There was no yr × season interaction for Se concentration ($P = 0.48$), and a tendency ($P = 0.10$) for a yr × season interaction for Mn concentration; therefore, data were pooled across yr for Se and Mn.

Table 2. Least squares means for Cu, Fe, and Zn concentrations (± SE) in beef cow diets on native range in Eastern Colorado[a]

Season of year	Cu concentration (mg/kg DM)	SE	Fe concentration (mg/kg DM)	SE	Zn concentration (mg/kg DM)	SE
Year 1						
Winter	5.38[b]	0.506	937.8[b]	153.99	21.93[b]	1.680
Spring	4.10[bc]	0.653	232.1[c]	198.80	17.08[bc]	2.168
Summer	3.86[c]	0.506	140.7[c]	153.99	15.75[c]	1.680
Fall	2.19[c]	0.800	199.5[c]	243.48	15.35[c]	2.656
Year 2						
Winter	1.87[b]	0.653	318.3	198.80	12.42[b]	2.168
Spring	4.20[c]	0.800	536.3	243.48	20.93[c]	2.656
Summer	3.71[c]	0.462	434.0	140.57	19.84[c]	1.533
Fall	4.84[c]	1.131	440.0	344.33	30.25[d]	3.756

[a] There was a yr × season interaction for Cu ($P < 0.01$), Fe ($P < 0.05$), and Zn ($P < 0.001$) concentration; therefore data are reported by yr for Cu, Fe, and Zn.

[b,c] Within a year for each mineral, values in the same column without common superscripts are different ($P < 0.05$).

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

INFLUENCE OF SUPPLEMENTAL WHOLE FLAXSEED LEVEL ON FORAGE INTAKE AND SITE AND EXTENT OF DIGESTION IN BEEF HEIFERS CONSUMING NATIVE GRASS HAY

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ABSTRACT[1]: The objectives of this study were to evaluate the influence of supplemental whole flaxseed level on intake and site and extent of digestion in beef cattle consuming native grass hay. Nine Angus heifers (avg. BW 303 ± 6.7 kg) fitted with ruminal and duodenal cannulas were used in a triplicated 3 × 3 Latin square. Cattle were fed ad libitum chopped native grass hay (8.7% CP and 70.0% NDF, DM basis). All animals were randomly allotted to one of three experimental treatments being either no supplement (Control); 0.91 kg whole flaxseed; or 1.82 kg whole flaxseed on a DM basis. Supplemental flaxseed tended to decrease (linear, $P = 0.06$) forage OM intake. However, total OM intake did not differ ($P = 0.29$) due to flaxseed inclusion. Total duodenal OM flow increased (linear, $P = 0.05$) with additional flaxseed in the diet and no differences were observed for microbial ($P = 0.29$) OM flow. True ruminal OM disappearance was not affected ($P = 0.14$) by supplemental flaxseed. Apparent lower tract OM digestibility was greater for supplemented versus Control cattle ($P = 0.03$) and increased (linear, $P = 0.01$) with level of whole flaxseed. Apparent total tract OM digestibility was not different ($P = 0.41$) among treatments. Nitrogen intake increased ($P < 0.001$) with supplemental flaxseed. Total duodenal N flow tended ($P = 0.08$) to increase with additional dietary flaxseed. Therefore, true ruminal N digested (g/d) tended ($P = 0.07$) to be greater for flax fed cattle, however, true ruminal N digestibility did not differ ($P = 0.11$) across treatment. Supplemental whole flaxseed did not influence ruminal ($P = 0.13$) or total tract ($P = 0.14$) NDF digestibility. An increase in the duodenal supply of 18:3*n-3* ($P < 0.001$), total unsaturated fatty acids ($P < 0.001$) and total fatty acids ($P < 0.001$) was observed with additional dietary whole flaxseed. Overall, the inclusion of 1.82 kg of flaxseed does not appear to negatively influence nutrient digestibility of a forage-based diet and therefore can be used as an effective supplement to increase intestinal supply of key fatty acids important to human health.

Key Words: Beef cattle, Digestion, Fatty acid, Forage, Flaxseed

Introduction

Feeding beef cattle diets high in *n-3* fatty acids is warranted for livestock producers interested in enhancing the human healthfulness of meat products (Weill et al., 2002) or reducing reproductive losses (Ambrose et al., 2006). Feeding flaxseed is a viable option for bolstering *n-3* fatty acid concentration in livestock diets. Unfortunately, there are limitations as to the level which fat can be added to ruminant diets due to reductions in intake and digestibility (Jenkins, 1993) however, flaxseed seems to be unique in this regard. Because, Zhang et al. (2005) observed an increase in DM digestibility for lactating ewes fed a silage-based diet that contained 8% flaxseed compared to diets that contained no oilseed or Canola. In feedlot diets, flaxseed has been reported to not affect (Maddock et al., 2006) or increase (Drouillard et al., 2004) dietary intake with an overall improvement in ADG when included at 5 – 8 % of the diet. However, little information exists regarding the use of flaxseed in hay-based diets. Our objectives were to evaluate site and extent of digestion in beef heifers fed increasing amounts of whole flaxseed and consuming native grass hay.

Materials and Methods

Animals and diets

Nine Angus heifers (avg. BW 303 ± 6.7 kg) fitted with ruminal and duodenal cannulas were used in a triplicated 3 × 3 Latin square. Cattle were fed ad libitum chopped native grass hay (8.7% CP and 70.0% NDF, DM basis). Previous to the initiation of the experiment heifers were given ad libitum access to both forage and whole flaxseed for 21 d in an effort to determine the level at which the animal would chose to consume flaxseed. It was determined that the average maximal amount of whole flaxseed consumed was approximately 1.82 kg/d. Therefore, all animals were randomly allotted to one of three experimental treatments being either no supplement (Control); 0.91 kg whole flaxseed; or 1.82 kg whole flaxseed on a DM basis. All experimental procedures were reviewed and approved by the Northern Great Plains Research Laboratory, Animal Care and Use committee. Animals were housed in a temperature controlled barn within a 3.3 m × 2.7 m pen equipped with cup waterers. Heifers were fed twice daily at 0600 and 1800. Hay and flax was fed in a separate feeders and hay was fed at a level to achieve 5% orts each day. As a marker of digesta flow, boluses containing 5 g of TiO_2 were dosed intraruminally at each feeding. Each experimental period was 21 d with 17 d adaptation to ensure adequate adjustment of the gastrointestinal tract to the new dietary treatment and 4 d of intensive sampling.

Sampling and laboratory analysis

Beginning at 0400 on d-18 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 so that samples were collected 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

[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.

within heifer for each period. Duodenal digesta samples were composited (equal vol.) within heifer for each period and immediately frozen. Duodenal digesta samples were then lyophilized (Freezemobile 25SL Freeze Dryer, The VirTis Co., Gardiner, NY) and ground (Wiley mill; 1-mm screen).

Immediately before the 0600 feeding and again every 3 h on d 20 until d 21 approximately 200 mL of whole ruminal contents were collected. Immediately after each collection whole ruminal contents were processed for subsequent isolation of ruminal bacteria and analysis of purines as described by Scholljegerdes et al. (2004).

All feed, microbes, 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. (2005).

Feed was prepared for fatty acids analysis via direct transesterification (Whitney et al., 1999) with methanolic-HCl (Kucuk et al., 2001) and duodenal digesta fatty acids were prepped for fatty acid analysis as outlined by Lake et al. (2006). Separation of fatty acid methyl esters was achieved by GLC (Model CP-3800, Varian Inc., Palo Alto, CA) with a 100 m capillary column (SP-2560, Supelco, Bellefonte, PA) and H_2 as a carrier gas at 1.0 mL/min for feedstuffs and 1.5 mL/min for duodenal digesta. Oven temperature was maintained at 120° C for 2 min and then ramped to 210° C at 6° C/min. Oven temperature was then ramped to 250° C at 5° C/min. Injector temperature was 260° C and flame ionization detector temperature was 300° C. Identification of peaks was accomplished using purified standards (Sigma-Aldrich, St. Louis, MO; Nu-Chek Prep, Elysian, MN; Matreya, Pleasant Gap, PA). Furthermore, based on the chromatogram published by Loor et al. (2004) the identification of 18:1*trans 13+14* peak was based on peak location relative to a peak identified and confirmed as 18:1*cis-9* using a commercially available standard.

Statistical analysis

All data were analyzed using the MIXED model of SAS (SAS Inst. Inc., Cary, NC) as a triplicated 3 × 3 Latin square experiment. The model included animal as the random variable. Single degree of freedom orthogonal contrasts were used to compare effects of Control vs. supplemented and orthogonal polynomial contrasts were used to compare linear and quadratic responses to level of flax intake (Steel and Torrie, 1980).

Results and Discussion

The addition of whole flaxseed tended to reduce OM intake (P = 0.10) compared to Control and tended to be linearly reduced (P = 0.06) with increasing levels of flaxseed (Table 1). However, total OM intake did not differ (P = 0.29) across treatments due to the addition of whole flaxseed. Likewise, increasing the level of supplemental flaxseed linearly increased (P = 0.05) total duodenal OM flow. A depression in forage intake with fat supplementation has been well documented by others (Palmquist and Jenkins, 1980; Jenkins and Palmquist, 1984) and is generally attributed to a reduction in ruminal digestibility. However, in the current trial, no differences (P = 0.14) were observed in true ruminal OM digestibility. Therefore, the reduction in forage intake may have been due to the increase in cholecystokinin **(CCK)** observed with an increase in fat intake (Chelikani et al., 2004). Specifically, these authors observed an increase in circulating levels of CCK with a decrease in intake when canola oil was fed or infused into the abomasums of dairy cows. Apparent lower tract OM digestibility was increased (P = 0.03) with flaxseed supplementation and increased linearly (P = 0.01) with level of flaxseed. Apparent total tract OM digestibility did not differ (P = 0.38) across treatment due to the lack of differences in ruminal digestibility combined with differences observe for lower tract OM digestibility. Contrary to this result, Ueda et al (2003) reported an increase in total tract OM digestibility when dairy cattle were fed a 65% forage diet and linseed oil. In the current experiment, whole flaxseed made up 13.0 and 24.8% of the total OM intake for 0.91 and 1.82 treatments, respectively.

Overall, N intake increased linearly (P < 0.001) with additional flaxseed. However, additional flaxseed only tended (quadratic, P = 0.08) to increase the amount of N reaching the duodenum. Duodenal supply of microbial N did not differ (P = 0.22) across treatments. Others have also indicated that duodenal microbial N supply (g/d) was unaffected by fat feeding (Brokaw et al., 2001; Scholljegerdes et al., 2004) with forage-based diets. Whereas, nonmicrobial N flow did not differ (P = 0.25) between Control and supplemented treatments but did increase linearly (P = 0.02) with inclusion of whole flaxseed. Overall, true ruminal N digestibility did not differ (P = 0.14) across treatments. However, apparent lower tract N digestibility did increase (P = 0.02) for flax-fed heifers and increased linearly (P = 0.003) with level of flaxseed. Thereby indicating that N supplied by whole flaxseed is readily available in the small intestine. Apparent total tract N digestibility was greater (P < 0.001) for heifers supplemented with whole flaxseed.

No differences were observed (P = 0.20 to 0.92) for NDF intake or duodenal and fecal NDF flow. In turn, the inclusion of whole flaxseed had no affect on ruminal, lower tract or total tract NDF digestibility (P = 0.13 to 0.90). This agrees with Ueda et al. (2003) who fed dairy cows a 65:35 forage:concentrate diet containing 3.0% linseed (4.7% total fatty acids on an OM basis). The fat levels fed in the current experiment were 0.91, 4.1 and 6.7% total fatty acids (DM basis) for Control, 0.91 or 1.82 kg treatments, respectively.

Due to experimental design, fatty acid intake increased (P < 0.001) for all dietary fatty acids (Table 2). Duodenal supply of 16:0 increased (P < 0.001) across treatment (Table 3). Due to ruminal biohydrogenation of unsaturated fatty acids duodenal supply of 18:0 increased (P < 0.001) with inclusion of flaxseed and increased quadratically (P < 0.001) as flaxseed intake went from 0 to 1.82 kg per day. No differences (P = 0.50) were observed for total 18C biohydrogenation (data not shown). The approximate 20

fold increase in intestinal supply of 18:0 compared to intake was due to biohydrogenation of 18C fatty acids averaging 82.1% across treatments. Previous research has reported as much as 91% of the total 18C fatty acids from linseed oil are biohydrogenated (Scollan et al., 2001). It was thought that feeding whole oilseeds would protect dietary fatty acids from biohydrogenation (Jenkins, 1993). However, Keele et al. (1989) reported little to no protection from biohydrogenation of whole cotton seed, because of mastication of the seed. Duodenal supply of all 18:1 isomers increased ($P < 0.001$) in flax-fed cattle compared to unsupplemented control and there was a quadratic increase ($P < 0.01$) for 18:1*trans-9*, 18:1*trans-11*, and 18:1*trans13+14* whereas 18:1*n-9* increased linearly ($P < 0.001$) with increasing levels of whole flaxseed. This agrees with Loor et al. (2004) who reported an increase in duodenal supply of 18:1 isomers when linseed oil was added to either a low concentrate or high concentrate dairy-type diet. Flax feeding increased ($P < 0.001$) intestinal supply of 18:3*n-3*. The addition of flaxseed to the diet increased ($P < 0.001$) duodenal supply of MUFA and exhibited a quadratic ($P = 0.02$) response to increasing levels of flaxseed. Intestinal PUFA and total unsaturated fatty acid supply increased ($P < 0.001$) with flaxseed supplementation.

Implication

Although the addition whole flaxseed up to 1.82 kg per day did reduce forage intake, the impact on diet digestibility was minimal. Therefore, adding whole flaxseed at the levels described herein is a viable option for increasing the supply of key fatty acids to ruminant tissues in cattle consuming a forage-based diet.

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Table 1. Influence of supplemental whole flaxseed level on OM, N, and NDF intake, flow, and digestibility in beef heifers consuming native grass hay

	Treatments[a]				Contrasts		
Item	Control	0.91	1.82	SE[b]	Control vs Supplemented	Linear	Quadratic
OM Intake, g/d							
Forage	6358	5771	5236	435.0	0.10	0.06	0.96
Total	6358	6636	6966	435.8	0.37	0.29	0.96
Duodenal OM Flow, g/d	3963	4275	4721	253.5	0.11	0.05	0.83
Microbial OM Flow, g/d	1127	1346	1208	138.0	0.37	0.67	0.29
Fecal OM Flow, g/d	2461	2548	2633	161.3	0.51	0.45	1.0
OM Digestibility							
True ruminal, % intake	54.4	54.5	49.0	3.2	0.40	0.14	0.37
Lower tract, % of duodenal flow	37.6	40.9	44.1	1.6	0.03	0.01	0.97
Total tract, % of intake	60.1	61.7	62.1	1.7	0.38	0.41	0.75
N Intake, g/d	83.6	104.9	120.1	5.8	0.001	<0.001	0.88
Duodenal N Flow, g/d	135.5	146.3	161.8	10.1	0.15	0.08	0.85
Microbial N Flow, g/d	92.3	108.1	84.7	13.6	0.75	0.72	0.22
Nonmicrobial N Flow, g/d	44.2	38.2	77.1	11.2	0.25	0.02	0.06
Fecal N Flow, g/d	48.4	48.6	47.2	3.9	0.88	0.75	0.79
N Digestibility							
True ruminal, % intake	47.0	60.3	39.3	11.4	0.80	0.55	0.14
Lower tract, % of duodenal flow	64.2	66.0	70.7	2.1	0.02	0.003	0.38
Total tract, % of intake	40.5	54.3	63.2	3.0	<0.001	<0.001	0.47
NDF Intake, g/d	4466	4331	4262	307.8	0.62	0.60	0.92
Duodenal NDF flow, g/d	2379	2120	2349	147.2	0.44	0.89	0.20
Fecal NDF flow, g/d	1863	1724	1672	119.1	0.27	0.26	0.76
NDF digestibility							
Ruminal, % intake	44.5	50.4	43.9	3.3	0.50	0.90	0.13
Lower tract, % of duodenal flow	21.0	19.4	27.3	3.4	0.59	0.22	0.28
Total tract, % of intake	56.4	60.6	60.4	2.1	0.14	0.21	0.40

[a]Treatments = Control = Chopped native grass hay only; 0.91 = Chopped native grass hay plus 0.91kg (DM basis) whole flaxseed; 1.82 = Chopped native grass hay plus 1.82 kg (DM basis) of whole flaxseed

[b]n = 9

Table 2. Influence of supplemental whole flaxseed level on fatty acid intake (g/d) in beef heifers consuming native grass hay

	Treatments[a]				Contrasts		
Fatty acid	Control	0.91	1.82	SE[b]	Control vs. Supplemented	Linear	Quadratic
16:0	21.1	32.2	43.1	1.5	<0.001	<0.001	0.96
18:0	2.62	10.1	17.6	0.40	<0.001	<0.001	0.98
18:1*n-9*	4.98	43.3	81.6	0.78	<0.001	<0.001	0.99
18:2*n-6*	11.9	50.0	88.4	1.4	<0.001	<0.001	0.96
18:3*n-3*	16.4	144.2	272.1	1.9	<0.001	<0.001	0.99
Total saturated fatty acids[c]	23.8	42.3	60.7	1.8	<0.001	<0.001	0.97
MUFA[d]	4.98	43.3	81.6	0.78	<0.001	<0.001	0.99
PUFA[e]	28.3	194.3	360.5	3.3	<0.001	<0.001	0.98
TUFA[f]	33.4	237.6	442.1	4.1	<0.001	<0.001	0.98
Total	60.5	284.6	508.7	5.8	<0.001	<0.001	1.00

[a]Treatments = Control = Chopped native grass hay only; 0.91 = Chopped native grass hay plus 0.91kg (DM basis) whole flaxseed; 1.82 = Chopped native grass hay plus 1.82 kg (DM basis) of whole flaxseed

[b]n = 9

[c]Total saturated fatty acids = 16:0 + 18:0

[d]MUFA = 18:1*n-9*

[e]PUFA = 18:2*n-6* + 18:3*n-3*

[f]Total unsaturated fatty acids = MUFA + PUFA

Table 3. Influence of supplemental whole flaxseed level on duodenal fatty acid flow (g/d) in beef heifers consuming native grass hay

	Treatments[a]				Contrasts		
Fatty acid	Control	0.91	1.82	SE[b]	Control vs Supplemented	Linear	Quadratic
12:0	0.74	0.80	0.89	0.05	0.11	0.05	0.83
14:0	2.77	4.33	3.94	1.07	0.31	0.45	0.47
14:1	2.64	2.62	2.39	0.08	0.18	0.04	0.26
15:0	2.53	2.68	2.69	0.37	0.71	0.75	0.84
15:1	1.83	0.07	0.16	0.94	0.16	0.23	0.43
16:0	21.3	36.1	84.5	8.40	0.02	<0.001	0.12
16:1	1.03	0.89	0.78	0.08	0.07	0.05	0.87
17:0	1.38	1.78	1.64	0.08	0.01	0.04	0.02
18:0	42.6	251.1	365.9	19.3	<0.001	<0.001	<0.001
18:1*trans-9*	0.0	2.46	2.36	0.16	<0.001	<0.001	<0.001
18:1*trans-11*	2.65	13.9	15.5	1.31	<0.001	<0.001	0.01
18:1*trans13+14*	0.03	13.7	17.5	1.28	<0.001	<0.001	0.004
18:1*n-9*	5.36	27.3	46.2	2.86	<0.001	<0.001	0.66
18:2*trans-9 trans-12*	0.0	1.14	1.67	0.20	<0.001	<0.001	0.20
18:2*n-6*	3.5	12.7	19.9	2.13	<0.001	<0.001	0.67
20:0	2.16	2.27	2.74	0.19	0.16	0.05	0.46
18:3*n-3*	3.15	27.2	50.4	6.31	<0.001	<0.001	0.95
21:0	0.39	0.67	0.27	0.13	0.58	0.52	0.04
22:0	1.91	2.22	1.80	0.29	0.74	0.76	0.25
20:3*n-6*	0.0	0.0	0.11	0.06	0.49	0.24	0.49
23:0	0.27	0.35	0.32	0.06	0.43	0.59	0.51
22:2	0.59	0.31	0.61	0.22	0.60	0.92	0.23
24:0	1.89	2.42	1.58	0.31	0.72	0.40	0.04
20:5*n-3*	0.58	0.65	0.13	0.32	0.20	0.05	0.34
24:1	0.26	0.02	0.30	0.11	0.47	0.77	0.07
Other	17.9	39.6	43.3	2.86	<0.001	<0.001	0.02
Total saturated FA[c]	78.0	304.7	466.2	21.2	<0.001	<0.001	0.23
MUFA[d]	13.9	61.0	85.3	3.71	<0.001	<0.001	0.02
PUFA[e]	8.17	42.2	74.7	8.25	<0.001	<0.001	0.93
TUFA[f]	22.1	103.2	160.0	10.7	<0.001	<0.001	0.29
Total	117.1	446.7	668.6	28.2	<0.001	<0.001	0.14

[a]Treatments = Control = Chopped native grass hay only; 0.91 = Chopped native grass hay plus 0.91kg (DM basis) whole flaxseed; 1.82 = Chopped native grass hay plus 1.82 kg (DM basis) of whole flaxseed

[b]n = 9

[c]Total saturated fatty acids = 12:0 + 14:0 + 15: 0 + 16:0 + 17:0 + 18:0 + 20:0 + 22:0 + 23:0 + 24:0

[d]MUFA = 14:1 + 15:1 + 16:1 + 18:1*trans-9* + 18:1*trans-11* + 18:1*trans 13+14* + 18:1*n-9* + 24:1

[e]PUFA = 18:2*trans-9 trans-12* + 18:2*n-6* + 18:3*n-3* + 20:3*n-6* + 22:2 + 20:5*n-3*

[f]Total unsaturated fatty acids = MUFA + PUFA

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

BACKGROUNDING CALVES WITH ANNUAL FORAGE CROPS

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ABSTRACT: Cereal forages harvested as hay have become popular winter forage for livestock producers in Montana. This may be explained in part by use of cereal forages as an emergency crop in times of drought. Study objectives were to evaluate animal performance of newly released winter cereal crops in comparison to barley hays. In 2005, 'Willow Creek' winter wheat (WW) hay and 'Willow Creek' WW silage were compared to 'Hays' and 'Haybet' barley hays. Eighty Angus cross steers were allotted to 16 pens in a randomized complete block design. Steers were given ad libitum access to their cereal forage source, 3.96 kg· head^{-1}·d^{-1} of cracked feed barley, and 0.45 kg· head^{-1}·d^{-1} of a commercial 32% CP supplement. Pen (rep = 4) was the experimental unit in a 66 d trial. Steers were weighed and diet, ort, and fecal samples were obtained on d 34 and upon completion (d 66) of the trial. Diet and fecal samples were composited by pen and analyzed for DM, OM, N, NDF, ADF, and IADF. Insoluble acid detergent fiber was used to estimate fecal output. Steers fed Haybet and Hays diets had higher ADG than steers fed WW silage and WW hay (1.29, 1.28, 1.08, and 1.15 kg· head^{-1}·d^{-1}, respectively, $P < 0.01$). Dry matter intake was greatest ($P < 0.01$) for steers fed WW silage, intermediate for Hays and WW hay and lowest for steers fed Haybet (avg. 15.5 vs. avg. 8.9 kg· head^{-1}·d^{-1}, respectively). Gain: feed ratio was highest ($P < 0.01$) for Haybet, intermediate for Hays and WW hay and lowest for WW silage (15.7 vs. avg. 11.22 kg gain·100 kg feed, respectively). No difference was seen in DM, N, or ADF digestibility ($P > 0.10$). While the barley hay based diets resulted in higher ADG and intake, WW hay and silage would still have acceptable animal performance for backgrounding rations.

Key Words: Backgrounding, Cereal forages, Steers, Winter Wheat

Introduction

Backgrounding is often used to add value to calves by using an inexpensive feed source to increase gain prior to entering a feedlot. Cereal forages have proven to be an alternative addition to backgrounding diets. Previous research at MSU has found steers fed diets composed primarily of hay barley to gain 1.2 to 1.5 kg· head^{-1}·d^{-1} (Robison et al., 2001 and Todd et al., 2003).

Cereal forages harvested as hay are a significant source of winter forage for livestock producers in Montana. Cereal forages are not consistent across species with regards to forage quality. Cherney and Martin (1982), found barley forage to have higher forage quality in comparison with wheat, oat, and triticale.

Winter cereals are ideal alternative forages for livestock producers. They are consistently higher yielding and have better water use efficiency than spring cereals. They provide flexible management options for end point uses (i.e. grazing, harvested as hay or grain etc.). Winter cereals provide advantages in redistributing annual workloads, taking pressure off of spring workloads. Winter cereals can also provide ideal pasture. Preliminary grazing trials at MSU measured the regrowth of 'Willow Creek' winter wheat after being grazed at three different stages of its growth cycle. In mid June, prior to grazing, WW plots had 1921 kg·ha of DM forage available. Sheep were allowed to graze plots until the forage was at one inch stubble height. In mid July, when plots were hayed, those which were grazed in June had a regrowth of 1260 kg·ha (Cash, 2007).

Limited data is available using winter wheat harvested as hay as a roughage source for backgrounding steers. The objectives of this study were to evaluate animal performance of newly released winter cereal crops in comparison to barley hays. In 2005, Willow Creek winter wheat (WW) hay and Willow Creek WW silage were compared to 'Hays' and 'Haybet' barley hays.

Materials and Methods

Eighty Angus cross steers (average initial weight 343.2 kg) were allotted to 16 pens in a randomized complete block design. Four backgrounding diets based on 1) Haybet hay barley; 2) Hays hay barley; 3) Willow Creek WW hay; and 4) Willow Creek WW silage were fed. Cultivars were seeded at the recommended rates for soil type and environment and were grown under similar irrigated conditions near Bozeman, MT. Forages were harvested as hay at soft dough stage of maturity.

A new variety of WW called Willow Creek was released by MSU in 2005. It is a hard red wheat variety that is tall, late-maturing, awnletted, and fine stemmed. Haybet, a two-row, hooded forage barley, was released jointly by the Montana Agricultural Experiment Station (MAES) and Agricultural Research Service in 1990 (Hockett et al., 1990). Hays is a two-rowed hooded forage barley derived from a cross between Stark and Baronesse barley varieties, released by MAES in 2002.

Steers were given ad libitum access to their roughage source, 3.96 kg· $head^{-1}·d^{-1}$ of cracked feed barley, and 0.45 kg·$head^{-1}·d^{-1}$ of a commercial 32% CP supplement. All roughage was chopped to 5.1 cm prior to feeding. Steers were implanted at the beginning of the study with Synovex® (Fort Dodge Animal Health, Overland, KS). Steers were weighed and diet, ort, and fecal samples were obtained midway (d 34) and upon completion (d 66) of the trial. Initial and final weights were the average of weights obtained on two consecutive days. Diet and fecal samples were composited by period and pen and analyzed for DM, OM, N (AOAC, 2000); NDF, ADF (Van Soest et al, 1991); and by a modified insoluble acid detergent fiber (IADF) procedure outlined by Bohnert et al. (2002). Insoluble acid detergent fiber was used as an internal marker to estimate fecal output.

Data were analyzed by the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) to test the main effects of forage variety. Block (side of feedlot) was included in the statistical model. Least square means were separated by the LSD method if the F-test was significant ($P < 0.10$).

Results and Discussion

Composition of the cereal forages is displayed in Table 1. Cereal forages can be susceptible to nitrate accumulation and make feeding problematic. Nitrate concentrations of 0.035 to 0.11% NO_3-N are considered safe for non-pregnant livestock and should be diluted to 50% of ration for pregnant livestock. Levels of 0.226% NO_3-N and above are considered toxic (Cash et al., 2002). Hays and WW hay utilized in our study had nitrate levels that would warrant dilution if fed to pregnant livestock (avg. 0.05% NO_3-N). The WW silage would be considered safe for all classes of livestock. Haybet had very high levels of NO_3-N as well as high ISDMD, 90.7%. Steers under the Haybet treatment may have gradually adapted to the high levels of NO_3-N. Surber (2006) suggests that there may be a positive relationship between ISDMD and NO_3-N. Animals consuming high levels of NO_3-N may have higher levels of microbial growth. Ruminal micro organisms treated in a culture environment with NO_3-N produced less methane and increased microbial growth (Russell, 2002). The high levels of NO_3-N in Haybet may have influenced its ISDMD. If high nitrate forages are to be utilized their portion of the ration must be increased very gradually in order to allow the rumen environment time to adapt. There was a significant amount of readily digestible carbohydrates in the diet (i.e. 3.6 kg of cracked barley). This factor would also aid in the steers ability to adapt to high nitrate in the diet. Routine testing of all cereal forages for NO_3-N is crucial to avoid the dangers of feeding a high NO_3-N diet.

Performance and intake by steers is presented in Table 2. Steers fed WW diets had fairly consistent results with those fed hay barley diets. No difference ($P > 0.10$) was seen in ending weight between Haybet, Hays, WW hay, and WW silage diets (avg. 393 kg). Steers fed Haybet and Hays had the highest ($P < 0.01$) 66-d ADG, WW hay was intermediate, and WW silage the lowest (1.29 vs. 1.15 and 1.08 kg· $head^{-1}·d^{-1}$). Gain:feed ratio was highest ($P < 0.01$) for Haybet, followed by Hays, WW hay, and WW silage was lowest (15.7 vs. avg. 11.22 kg gain·100 kg feed, respectively). Dry matter intake for the 66-d study was greatest ($P < 0.01$) for steers fed WW silage when compared to the other treatments (15.53 vs. avg. 8.9 kg· $head^{-1}·d^{-1}$, respectively). Winter wheat silage had the highest ($P < 0.01$) intake as %BW, followed by Hays, WW hay, and Haybet (3.35 vs. avg. 1.86 %). Similar results were found in a 2005 backgrounding trial conducted by NDSU, Hettinger Research Extension Center (Stamm et al., 2006). In their study, steers were fed barley hay and silage alongside wheat and oat hay and no difference was seen in ADG between barley hay and wheat hay

In vivo nutrient digestibility and intake for backgrounding steers fed cereal forages are presented in Table 3. Dry matter intake was greatest ($P = 0.04$) for WW silage compared to WW hay, Hays, and Haybet diets (13.92 vs. avg. 8.72 kg· $head^{-1}·d^{-1}$, respectively). No differences ($P > 0.10$) were seen in ADF or NDF intake. Nitrogen intake was greatest ($P = 0.04$) for WW silage followed by WW hay, Hays, and lowest for Haybet (0.30 vs. avg. 0.19 kg· $head^{-1}·d^{-1}$). No differences ($P > 0.10$) were seen in DM, N, or ADF digestibility. Winter wheat silage had the lowest ($P = 0.07$) NDF digestibility while Haybet, Hays, and WW hay were all similar (45.63 vs. avg. 52.16 % respectively). Despite WW silage having the highest levels of DMD and N intake, steers on the WW silage had the lowest performance.

Implications

The winter wheat hay resulted in acceptable animal performance for backgrounding rations. Results from this trial as well as other MSU and NDSU trials indicate few differences between WW hay and hay barley as a backgrounding diet. The combined advantages of winter cereals make it a very appealing forage source. Winter wheat hay could easily be substituted for barley or oat hay, providing livestock producers increased management options. High water use efficiency, increased competition for weeds, and high forage yields make it ideal for MT growing conditions. Willow Creek winter wheat can be a suitable alternative to more traditional cereal forage crops in backgrounding rations.

Acknowledgments

We would like to thank the South Dakota Agricultural Experiment Station and the Four-State Ruminant Consortium for funding this project.

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Table 1. Composition of cereal forages harvested as hay (DM basis)

Item	Haybet	Hays	WW Silage	WW Hay
DM, %	87.07	92.74	90.91	89.84
CP, %	15.30	13.00	13.70	15.10
NDF, %	41.26	49.61	52.67	41.14
ADF, %	32.00	31.20	42.00	36.90
NO_3-N, %	0.26	0.05	0.02	0.05
ISDMD at 48 h, %	90.71	64.66	75.19	87.67

Table 2. Performance and intake by steers fed cereal forages harvested as hay.

Item	Haybet	Hays	WW Silage	WW Hay	SEM	*P*-value
No. of pens	4	4	4	4		
Weight, kg						
Initial	315.1	315.2	315.4	312.5	3.89	0.95
34-d	360.9	359.9	353.7	353.4	4.66	0.53
66-d	400.2	399.4	386.5	388.6	4.85	0.10
ADG, kg						
Per. 1	1.53[a]	1.49[a]	1.28[a]	1.36[a]	0.65	0.03
Per. 2	1.31	1.32	1.09	1.18	0.74	0.13
Overall	1.29[a]	1.28[a]	1.08[c]	1.15[b]	0.04	0.002
DMI, kg	8.37[b]	9.39[b]	15.53[a]	8.84[b]	1.11	0.009
FE, kg of gain/100 kg of feed	15.70[a]	13.60[b]	6.95[c]	13.05[b]	1.29	0.001
Intake, % BW	1.74[b]	1.96[b]	3.35[a]	1.89[b]	0.28	0.006

[a,b,c]Within a row, means lacking a common superscript letter differ ($P < 0.10$).

Table 3. *In vivo* nutrient digestibility by backgrounding steers fed cereal forages harvested as hay

Item	Haybet	Hays	WW Silage	WW Hay	SEM	*P*-value
Intake						
DM, kg	7.97[b]	9.29[b]	13.92[a]	8.90[b]	1.79	0.048
N, kg	0.17[b]	0.19[b]	0.30[a]	0.20[b]	0.04	0.046
NDF, kg	4.16	4.98	7.14	4.85	0.99	0.99
ADF, kg	2.30	2.73	4.28	2.78	0.59	0.40
Apparent total tract *in vivo* digestibility						
DM, %	55.82	53.34	53.60	57.64	1.60	0.78
N, %	45.20	39.63	44.59	52.29	3.16	0.10
NDF, %	51.13[a]	52.32[a]	45.63[b]	53.03[a]	1.91	0.075
ADF, %	46.41	51.24	44.70	51.17	4.19	0.88

[a,b,c]Within a row, means lacking a common superscript letter differ ($P < 0.10$).

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECTS OF BACTERIAL ENDOTOXIN AND DIETARY PROTEIN ON SERUM HORMONES AND PLASMA AMINO ACIDS IN GROWING STEERS

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ABSTRACT: Bacterial lipopolysaccharide (LPS) mimics clinical and metabolic responses to gram(-) bacterial infection in cattle. Effects of LPS and dietary protein on serum prolactin (PRL), triiodithyronine (T_3), thyroxine (T_4), insulin, insulin-like growth factor-1 (IGF-1) and plasma AA were evaluated in 24 steers (250 ± 2.8 kg BW). Treatments were a 2 × 3 factorial arrangement of LPS (0 vs 1.5 µg/kg BW; **-LPS** vs **+LPS**) and diets containing (DM basis): 1) 14.5% CP, 11.6% ruminally degradable protein (RDP) and 2.9% ruminally undegradable protein (RUP; **CP14.5CON**); 2) 16.3% CP, 13.4% RDP and 2.9% RUP (**CP16RDP**); and 3) 16.1% CP, 11.2% RDP and 4.9% RUP (**CP16RUP**). Casein was used to alter dietary RDP, and fish meal and corn gluten meal were used to alter dietary RUP. Steers were adapted to diets (1.2 Mcal/kg NEg fed DM at 1.8% BW) for 14 d, and steers were infused (i.v. 1 mL/min) with LPS (in 100 mL saline) on d 15. Blood samples were collected before LPS infusion and every 2 h for 12 h thereafter. No LPS × diet × hour interactions ($P > 0.23$) were observed. Serum PRL in +LPS steers was elevated from 2 to 4 h after LPS infusion, and insulin increased 4 h after LPS infusion. Both PRL and insulin of +LPS steers returned to pre-LPS concentrations and were similar to -LPS steers by 6 h after infusion (LPS × hour, $P \leq 0.05$). Serum IGF-1, T_3, and T_4 decreased in +LPS steers 2 h after infusion and remained lower than -LPS steers for 12 h (LPS × hour, $P \leq 0.05$). Plasma Met, Ile, and Thr of +LPS steers decreased at 2 h, and plasma Leu and Trp decreased at 4 h after LPS infusion. These AA remained lower in +LPS than -LPS steers for 12 h (LPS × hour, $P \leq 0.05$). Plasma Phe was lower ($P \leq 0.05$) for +LPS than -LPS steers at 4 through 6 h after infusion. Concentrations of Leu were greater ($P \leq 0.05$) for steers fed CP16RUP than CP14.5CON and CP16RDP. Results imply that altering concentration or source of dietary protein does not affect serum hormones, but serum hormones and essential AA are altered in steers exposed to bacterial endotoxin.

Key Words: Cattle, Protein, Stress

Introduction

Clinical and metabolic alterations associated with gram(-) bacterial infection occur due to recognition of the lipopolysaccharide (**LPS**) component of bacterial cell walls (Cullor, 1992) and can be induced experimentally by administration of purified LPS (Steiger et al., 1999). During immunological stress, absorbed AA are directed away from lean tissue growth because of increased metabolic demands for the synthesis of acute phase response proteins and(or) glucose in monogastric species (Reeds and Jahoor, 2001; Le Floc'h et al., 2003). Therefore, increasing the amount of dietary protein during immunological stress should be beneficial to the animal and facilitate enhanced immune function and performance. However, this practice has been inexplicably associated with increased morbidity in feedlot cattle, despite the increases in performance reported with increasing CP concentration in the diets of newly received feedlot calves (Galyean et al., 1999).

The objective of this study was to evaluate the effects of dietary protein on serum hormone and plasma AA concentrations in growing beef steers following an endotoxin challenge.

Materials and Methods

Animals and Facilities. Procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Twenty-four Angus-cross steers (250 ± 2.8 kg initial BW) were individually housed in tie stalls of a metabolism building with evaporative cooling and continuous lighting.

Design and Treatments. The experiment was a randomized block design and lasted 15 d, which allowed 14 d for adaptation to dietary treatments, and 1 d for blood collections. Treatments were a 2 × 3 factorial arrangement of endotoxin challenge (LPS infusions) and dietary protein concentration and source. Infusions of LPS included no LPS (**-LPS**) vs a prolonged low-dose of LPS (**+LPS**) via indwelling catheters (J-457A; Jorgensen Laboratories, Loveland, CO) that were placed in the jugular vein on d 14. At 3 h after feeding on d 15, LPS (*E. Coli* O55:B55; Sigma Chem. Co., St. Louis, MO) dissolved in 100 mL of sterile saline was infused (1 mL/min via i.v. catheter) at 1.5 µg/kg BW for +LPS steers. An equal volume of sterile saline was administered at a similar rate to -LPS steers.

Dietary treatments consisted of a basal diet (Table 1) to which casein (source of ruminally degradable protein; **RDP**) or a combination of fish meal and corn gluten meal (source of ruminally undegradable protein; **RUP**) were mixed before feeding. The basal diet was limit-fed (1.8% of BW, DM basis) to steers at 0700 and 1900 each day, and the addition of protein sources before feeding allowed dietary treatments (Table 2) to contain (DM basis): 1) 14.5% CP, 11.6% RDP and 2.9% RUP (**CP14.5CON**) 2) 16.3% CP, 13.4% RDP and 2.9% RUP (**CP16RDP**); and 3) 16.1% CP, 11.2% RDP and 4.9% RUP (**CP16RUP**). Compared to CP14.5CON, the CP16RDP diet provided greater RDP and similar RUP amounts, whereas the

CP16RUP diet provided similar RDP and greater RUP amounts.

Table 1. Composition of the basal diet

Item	% of DM
Ingredient	
Wheat Grain, Ground	28.1
Soybean Hulls	20.0
Sorghum Silage	16.0
Corn Grain, Cracked	15.0
Alfalfa Hay	12.0
Molasses	4.0
Tallow	2.5
Urea	0.50
Minerals[1]	1.83
Vitamins[2]	0.03
Rumensin-80[3]	0.02
Nutrient	
NE_g, Mcal/kg	1.20

[1]Supplied (% of DM): Limestone (0.50), dicalcium phosphate (0.50), sodium bicarbonate (0.50), salt (0.30), Na selenite premix-0.06% Se (0.015), zinc sulfate (0.009), copper sulfate (0.004).
[2]Supplied 1,500 IU vitamin A and 100 IU vitamin E.
[3]Supplied 33 mg monensin per kg diet DM.

Table 2. Ingredients mixed with the basal diet (Table 1) before feeding to supply three dietary protein treatments

Item	CP14.5 CON	CP16 RDP	CP16 RUP
Ingredient, g/d			
Casein	60	160	-
Fish Meal	-	-	150
Corn Gluten Meal	-	-	100
Nutrient, % of diet DM			
CP, analyzed	14.5	16.3	16.1
RDP, calculated	11.6	13.4	11.2
RUP, calculated	2.9	2.9	4.9

Collections and Analysis. On d 15 of the experiment, blood samples were collected via catheters into vacuum tubes (Corvac serum separator and Monoject 15% EDTA, Kendall, Ontario, CA) before LPS infusion and every 2 h for 12 h thereafter. Blood samples for serum were allowed to coagulate at room temperature for 30 min, whereas samples for plasma were immediately placed on ice. All samples were centrifuged (Sorvall RT600B, Thermo Electron Corp., Asheville, NC) at 1,500 × *g* for 20 min at 10°C and frozen for later analysis.

Serum concentrations of prolactin (**PRL**; Spoon and Hallford, 1989), insulin like growth factor-1 (**IGF-1**; Berrie et al., 1995), insulin (Reimers et al., 1982), triiodothyronine (**T_3**; Wells et al., 2003), and thyroxine (**T_4**; Richards et al., 1999) were determined via RIA. Inter and intra-assay CV were less than 12% for all serum hormones. Plasma AA concentrations were determined via gas chromatography (Varian CP-3800, Varian, Walnut Creek, CA) using a commercially available kit (EZ:FAAST; Phenomenex, Torrance, CA).

Statistics. Data were analyzed using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Serum PRL, T_3, T_4, insulin, IGF-1 and plasma AA were analyzed as repeated measures (covariance structure = autoregressive order one). The model included all possible combinations of LPS, diet, and hour. Data are presented as least squares means and differences were considered significant at $P \leq 0.05$.

Results

No LPS × diet × hour interactions ($P > 0.23$) were observed for serum hormones or plasma AA concentrations. Serum PRL (Figure 1) increased in +LPS steers remaining elevated above -LPS steers from 2 to 4 h after LPS infusion and then returned to pre-LPS infusion levels at 6 h (LPS × hour, $P \leq 0.05$). Insulin (Figure 1) increased 4 h after LPS infusion in +LPS steers and returned to pre-LPS concentrations by 6 h after infusion (LPS × hour, $P \leq 0.05$). Serum IGF-1, T_3, and T_4 concentrations (Figure 2) declined in +LPS steers 2 h after infusion and remained lower than -LPS steers for 12 h (LPS × hour, $P \leq 0.05$).

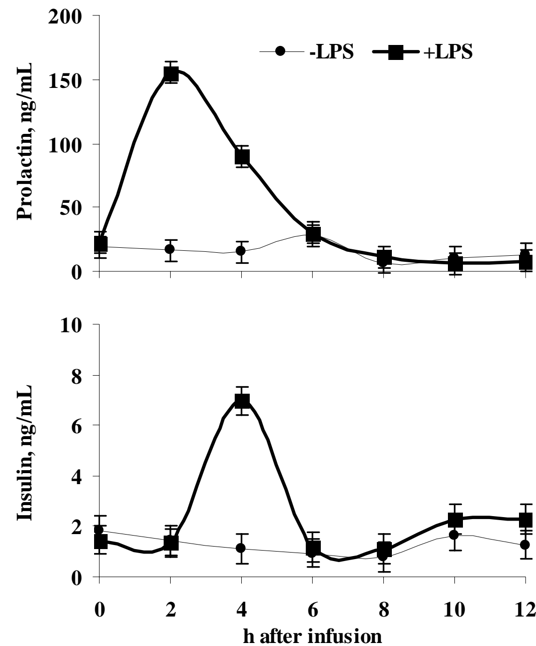

Figure 1. Serum insulin and prolactin concentrations in response to bacterial endotoxin challenge (LPS) in steers (LPS × hour, $P \leq 0.05$).

Plasma concentrations of Met, Thr, Ile, Leu, Val, Trp, and Lys declined in +LPS steers compared to -LPS (Figure 3). Plasma concentrations of Met, Ile, and Thr in +LPS steers decreased 2 h after infusion, whereas plasma Lys, Leu, and Trp concentrations decreased 4 h after LPS infusion. These AA remained lower in +LPS than -LPS steers for 12 h (LPS × hour, $P \leq 0.05$). Plasma Phe concentrations were lower for +LPS than -LPS steers from 4 to 6 h after LPS infusion (LPS × hour, $P \leq 0.05$). Concentrations of Leu were greater ($P \leq 0.05$) for steers fed CP16RUP than CP14.5CON and CP16RDP (data not shown), and His concentrations were not affected by LPS or diet.

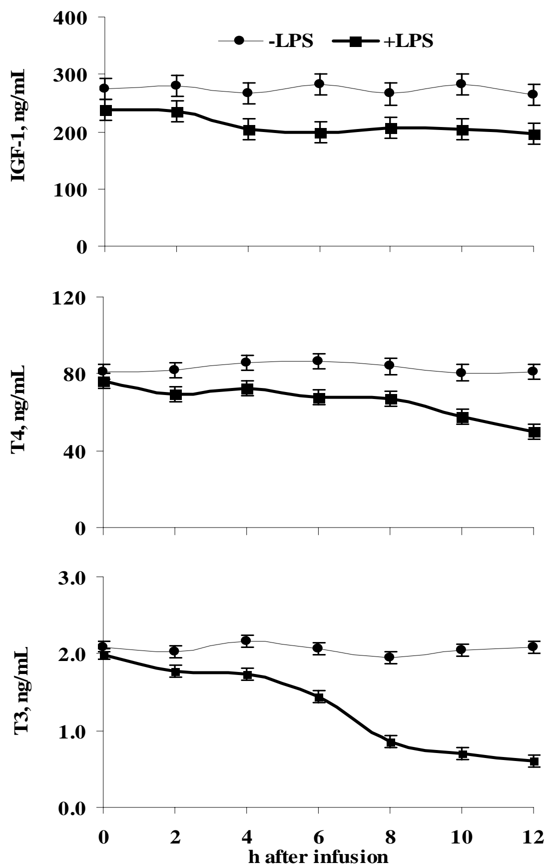

Figure 2. Serum triiodothyronine (T_3), thyroxine (T_4) and insulin like growth factor 1 (IGF-1) concentrations in response to bacterial endotoxin challenge (LPS) in steers (LPS × hour, $P \leq 0.05$).

Discussion

Increases in serum concentrations of PRL and insulin as well as the declines in IGF-1, T_3 and T_4 concentrations in +LPS vs -LPS steers are indicative of the ability of LPS to stimulate the hormonal changes associated with stress and infection. Prolactin is viewed as a lactogenic hormone for development of mammary tissue and lactation. However, immune cells (e.g. lymphocytes) also posses receptors for PRL, which has been reported to stimulate lymphocyte proliferation and function (Arkins et al., 1993). The observed increases in serum PRL likely occurred as a result of stimulation of the hypothalamus by cytokines released in response to LPS.

An increase in serum insulin concentrations for steers receiving LPS is similar to reports for dairy heifers (Steiger et al., 1999). During stress and infection, insulin resistance develops in peripheral tissues due to interactions among cytokines and insulin receptors. These interactions block glucose uptake by peripheral tissues as a means of redirecting glucose to the immune system (Spurlock, 1999).

A reduction in serum concentrations of IGF-1 and the thyroid hormones in response to immunological stress has been reported previously (Elsasser et al., 1987; Kahl et al., 1999). The decline in IGF-1 following exposure to LPS is attributed to reduced production of IGF-1 by the liver and skeletal muscle (Spurlock, 1999). Kahl et al. (1999) reported that LPS reduced hepatic concentrations of the enzyme responsible for converting T_4 to T_3, which may in part explain the observed decline in serum T_3 in steers challenged with LPS. Collectively, these hormones regulate protein metabolism, growth and nutrient utilization. The lack of an effect of diet implies that altering dietary protein does not influence the changes in serum hormone concentrations associated with immunological stress in cattle.

A decrease in plasma concentrations of Met, Thr, Lys, Leu, Ile, Val, Trp, and Phe for +LPS compared to -LPS steers is similar to the decline in plasma AA concentrations that we reported previously for growing beef steers exposed to LPS (Waggoner et al., 2006). These consistent decreases in plasma AA concentrations could be due to hepatic catabolism in response to an increased demand for glucogenic precursors. However, Reeds and Jahoor (2001) reported that human plasma proteins (e.g. acute phase response proteins) contain high quantities of Lys, Phe, Trp, Cys, and Ser. Therefore, the observed decreases in plasma Lys, Phe, and Trp concentrations for LPS-challenged steers could also be because of an increased demand for the synthesis of acute phase response proteins. The decrease in plasma Met concentrations could be due to an increase in Met transsulfuration to supply Cys for incorporation into acute phase response proteins. Also, Cys is required for glutathione synthesis, and S-adenosylmethionine may stimulate the production of cytokines involved in the immune response (Grimble and Grimble, 1998). Decreases in the branched-chain AA, Leu, Ile, and Val, may be explained in part by their essentiality for protein synthesis by B-lymphocytes to produce antibodies, an integral component of the immune response (Calder, 2006).

Bergen (1979) proposed that blood concentrations of an AA would remain low if supply was below the animal's requirement, and that the AA concentration in blood would increase as the supply of that AA exceeded requirements. In our study, increasing dietary protein concentration (14.5 vs 16% CP) or altering dietary protein source (RDP vs RUP) did not affect plasma AA concentrations (with the exception of Leu) in either -LPS and +LPS steers. Therefore, the metabolizable supply of AA from all dietary treatments was likely below the animals' AA requirements given availability of other nutrients (e.g. dietary energy).

Implications

The results of this study imply that increasing dietary protein concentration from 14.5 to 16%, or increasing ruminally undegradable protein does not affect the changes observed in serum hormone and plasma AA concentrations associated with exposure to bacterial endotoxin in cattle.

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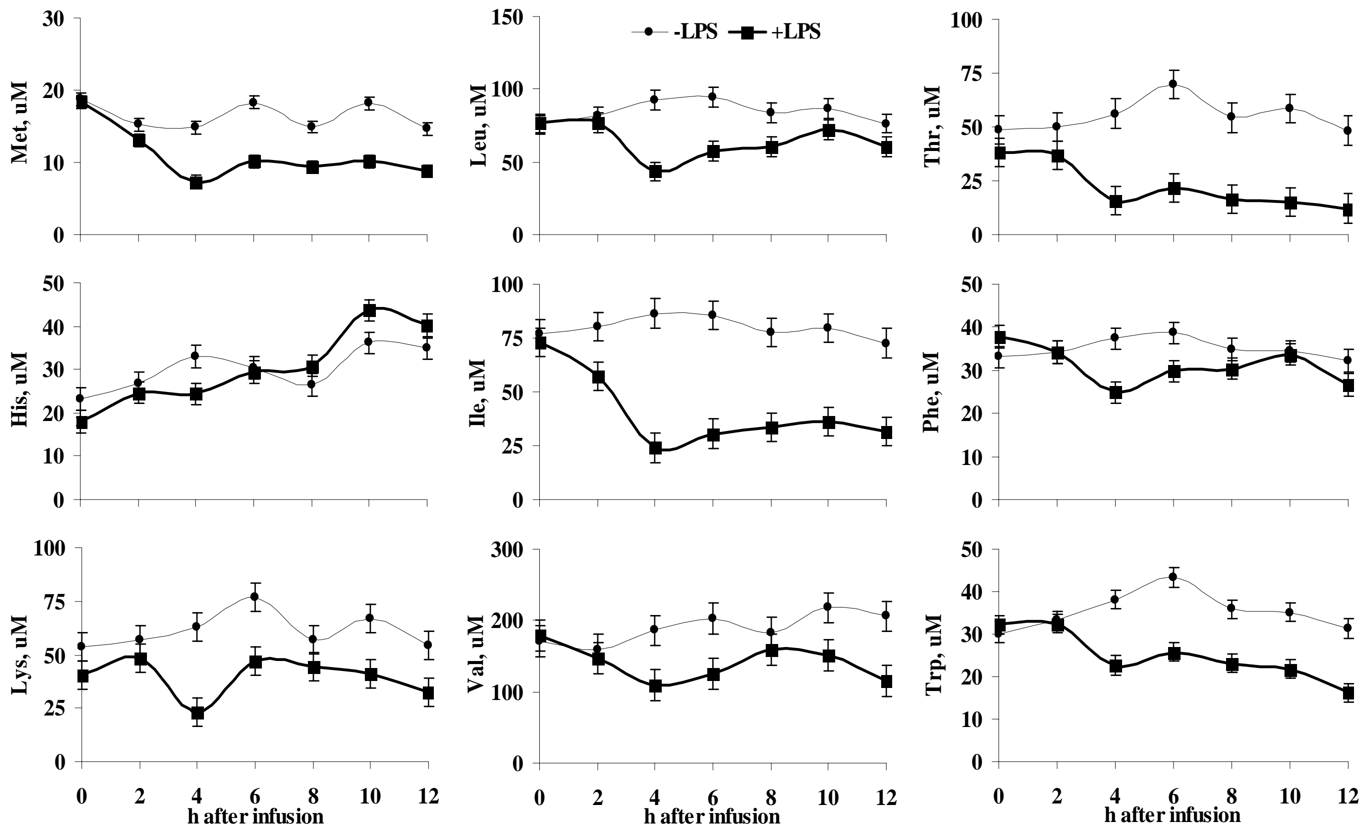

Figure 3. Plasma AA concentrations in response to bacterial endotoxin challenge (LPS) in steers. Effects of LPS × hour ($P \leq 0.05$) for plasma Met, Leu, Ile, Thr, Phe, Trp, and effects of LPS ($P \leq 0.05$) for Met, Val, Leu, Ile, Lys, Thr, and Trp.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

IMPACTS OF SUPPLEMENTAL GLUCOGENIC PRECURSORS AND COW AGE ON POSTPARTUM RANGE COW PERFORMANCE

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ABSTRACT: Altering nutrient partitioning after calving from milk production to positive energy balance may improve reproductive performance. A 2004 study conducted at the Corona Range and Livestock Research Center evaluated responses of 2- (n = 17), 3- (n = 23), and 4-yr-old (n = 31) postpartum cows grazing native range (11.3% CP and 80% NDF, OM basis) to 3 protein supplements with increasing glucogenic potential (**GP**). Supplements were fed at 1,135 $g \cdot cow^{-1} \cdot d^{-1}$ twice weekly for 65 d postpartum and provided: 1) 341 g CP, 142 g ruminally undegradable protein (**RUP**), 57 g GP (**RUP0**), 2) 341 g CP, 151 g RUP + 80 g propionate salt (NutroCAL™, Kemin Industries, Inc.), 121 g GP (**RUP80**), or 3) 341 g CP, 159 g RUP + 160 g propionate salt, 185 g GP (**RUP160**). A supplement × age interaction occurred for days to first estrus ($P = 0.10$). Days to first estrus was longest for 2-yr-old cows fed RUP0 and then decreased with cow age ($P \leq 0.04$), while for RUP80 and RUP160, return to estrus was similar for 2- and 3-yr-old cows ($P \geq 0.16$) and shorter for 4-yr-old cows ($P \leq 0.10$). Milk production exhibited a quadratic ($P = 0.03$) response to increasing supplemental GP, with cows fed RUP80 producing the least amount of milk at 55 d postpartum (9,982, 8,439, and 9,620 ± 473 g/d for RUP0, RUP80, and RUP160, respectively). Milk production differences did not impact 205-d calf weight ($P = 0.96$; 251 ± 5 kg). Days from BW nadir to estrus decreased linearly with cow age ($P < 0.01$; 33, 22, and 1 ± 4 d for 2-, 3-, and 4-yr-old cows, respectively). Milk production increased linearly with cow age ($P < 0.01$; 7,856, 9,407, and 10,777 ± 509 g/d for 2-, 3-, and 4-yr-old cows respectively. Calf 205-d weight reflected cow age differences in milk production (linear $P < 0.01$; 229, 249, and 274 ± 6 kg for 2-, 3-, and 4-yr-old cows, respectively). Moderate amounts of supplemental GP shifted nutrients away from milk production. Older cows returned to estrus at the same time they reached BW nadir, while younger cows needed to regain weight to return to estrus. Glucogenic precursor addition to protein supplements decreased days to first estrus in postpartum 2-year-old range cows.
Key Words: Beef Cattle, Glucogenic, Reproduction

Introduction

Protein supplementation is often necessary to meet maintenance nutrient requirements of cows grazing dormant range forage and greater nutrient demands during gestation and lactation amplify the need for supplementation. Young, supplemented range cows often experience a period of negative energy balance and weight loss before and after parturition (Waterman et al., 2006), and poor reproductive performance of first- and second-calf cows is a challenge faced by cow-calf producers in the southwestern US and other regions. Supplementing ruminally undegradable protein (**RUP**) once ruminally degradable protein (**RDP**) requirements are met (NRC, 2000) can encourage nutrient repartitioning from lactation to synthesis of maternal tissues for maintenance, growth and reproduction by way of improved nutrient use (Hunter and Magner, 1988; Triplett et al., 1995; Waterman et al., 2006). It has been suggested that increased supply of protein as RUP may result in alterations in glucose supply and metabolism (Bell and Bauman, 1997; Waterman et al., 2006). Waterman et al. (2006) found that 2-yr-old cows fed protein supplement containing glucogenic precursors provided as glucogenic amino acids from RUP plus 100 g/d propionate salt (NutroCal™, Kemin Industries, Inc.) while grazing dormant range were more sensitive to insulin and returned to estrus 9 d earlier than cows fed traditional cottonseed meal-based protein supplements with no additional glucogenic precursors. The objectives of the current research was to investigate if 2-, 3-, and 4-yr-old postpartum cows would benefit if the amount of glucogenic precursors in the supplements was increased and to determine if cow age influenced response to increased supplemental glucogenic precursors. To accomplish these objectives, we evaluated return to estrus, milk production, weight change responses, and insulin sensitivity of postpartum 2-, 3-, and 4-yr-old range beef cows to supplements with increasing glucogenic potential (**GP**) provided as 0, 80, or 160 g/d propionate salt.

Materials and Methods

The experiment was conducted during spring and summer 2004 at the Corona Range and Livestock Research Center, Corona, NM (average elevation = 1900 m; average annual precipitation = 400 mm) located 300 km northeast of Las Cruces, NM. Predominant forages in experimental pastures included blue grama (*Bouteloua gracilis*) and wolftail (*Lycurus phleoides*), as well as other less dominant grasses and forbs (Forbes and Allred, 2001). Three ruminally cannulated cows were used to collect diet samples for analysis of CP (AOAC, 2000) and NDF (Van Soest et al., 1991), which averaged 11.3% and 80%, respectively (OM basis).

All animal handling and experimental procedures were conducted in accordance with guidelines of the Institutional Animal Care and Use Committee of New Mexico State University. Cows (n = 71) were 2 (n = 17), 3

(n = 23), and 4 (n = 31) years of age and predominantly Angus with some Hereford influence. First-calf heifers were wintered separately from older cows and all cows were wintered on pasture with protein supplementation. Within age, cows were assigned to treatment by calving date so that similar age distribution and days postpartum were reflected in each treatment group. Supplements were cubed and milled at Hi-Pro Feeds, Friona, TX and were individually fed at a rate of 1135 $g \cdot cow^{-1} \cdot d^{-1}$ for 65 d postpartum. Supplementation ceased when breeding season began (15 May). Supplements provided 1) 341 g CP, 142 g RUP, 57 g GP (**RUP0**), 2) 341 g CP, 151 g RUP + 80 g propionate salt (NutroCAL™, Kemin Industries, Inc.), 121 g GP (**RUP80**), or 3) 341 g CP, 159 g RUP + 160 g propionate salt, 185 g GP (**RUP160**). Glucogenic potential was calculated by the equation of Preston and Leng (1987), where 40% of the RUP is considered to be glucogenic (Overton et al., 1999; Vanhatalo et al., 2003). NutroCal™ contains 80% propionate, which was assumed to be 95% glucogenic (Steinhour and Bauman, 1988).

Blood samples were collected twice weekly on supplementation days (Monday and Friday) via coccygeal venipuncture beginning approximately 40 d postpartum for analysis of progesterone to determine days to first estrus (2 or more consecutive progesterone concentrations > 1 ng/mL). Samples were analyzed for progesterone by solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) as described by Schneider and Hallford (1996). Inter- and intra-assay coefficients of variation were less than 10%. Cows were diagnosed for pregnancy via rectal palpation at weaning (24 September).

A subsample of cows (n = 20) were milked with a portable milking machine approximately 56 d postpartum on a day following supplementation using a modified weigh-suckle-weigh technique (Appeddu et al., 1997). Milk weight was recorded to estimate milk yield. Milk subsamples were collected in preservative-coated vials for analysis of protein, lactose, butterfat, and solids non-fat by an independent laboratory (Pioneer Dairy Labs, DHIA, Artesia, NM).

Cows were weighed weekly until termination of breeding season and at weaning. Days to BW nadir were determined from lowest BW obtained postpartum. Pre-planned intervals of weight change were calculated and included beginning of supplementation to BW nadir, BW nadir to end of supplementation, BW nadir to end of breeding, end of supplementation to end of breeding, and end of breeding to weaning. Body condition scores (**BCS**; 1 = emaciated, 9 = obese) were assigned to each cow by visual observation and palpation at beginning and end of supplementation, beginning and end of breeding season, and at weaning. Calf birth weights were obtained in the field within 3 d after birth with a portable platform scale, and body weight was measured at branding and weaning. Calf weights at branding were adjusted to average calf age at branding (45 d) and adjusted 205-d weaning weights were calculated with no adjustment for sex of calf or age of dam.

A glucose tolerance test (**GTT**) was conducted approximately 65 d postpartum on a subsample of cows (n = 20; same cows used for milk production) on a day after supplementation. A 50% dextrose solution was infused at 0.5 mL/kg BW via indwelling jugular catheter inserted the morning of the GTT. Blood samples were collected at –1, 0, 3, 6, 9, 12, 15, 20, 40, 60, 80, 100, 120, 140, 160, and 180 min relative to infusion. Glucose was analyzed with a commercial kit (enzymatic endpoint method, Thermo DMA, Louisville, CO). Insulin was analyzed by solid-phase radioimmunoassay (DCP kit, Diagnostic Products Corp., Los Angeles, CA) as validated by Reimers et al. (1982). Intra- and inter-assay coefficients of variation were less than 10%. Serum glucose and insulin areas under the curve (**AUC**) were calculated using trapezoidal summation. Glucose half-life was estimated by determining time required for 50% decrease in peak serum glucose concentration.

Data were analyzed as a completely randomized design by analysis of variance using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) with cow as the experimental unit, using the Kenward-Roger degrees of freedom method. The model included fixed effects of supplement, cow age, and their interaction. Covariates were used when appropriate and included calving date, days on supplement, and calf gender. When appropriate, orthogonal polynomial contrasts were used to test for linear and quadratic effects of increasing supplemental glucogenic precursors and cow age. Pregnancy data were analyzed using the GENMOD procedure of SAS. Significance was determined at $P \leq 0.10$.

Results and Discussion

A supplement × age interaction occurred for days to first estrus ($P = 0.10$; Table 1). Two-yr-old cows fed RUP0 took longer to return to estrus than any other group ($P \leq 0.04$), and RUP0-fed cows exhibited decreasing days to first estrus with increasing cow age. Cows fed RUP80 and RUP160 had similar patterns of return to estrous cyclicity where days to first estrus were similar for 2- and 3-yr-old cows ($P \geq 0.16$) and shorter for 4-yr-old cows ($P \leq 0.10$). Increasing glucogenic precursor delivery to postpartum cows had the most beneficial effect on return to estrus in 2-yr-old cows. Pregnancy rates were above 95% for all supplement groups, and all cows fed RUP80 were pregnant in the fall. Calving intervals were slightly over a year in length and were similar ($P = 0.99$) regardless of supplement group.

Milk production exhibited a quadratic ($P = 0.03$; Table 2) response to increasing supplemental GP, with cows fed RUP80 producing the least amount of milk at 55 d postpartum. Milk lactose and solids non-fat production exhibited the same quadratic trend ($P \leq 0.03$) as 24-h milk production, while milk butterfat and protein production were similar regardless of supplement group ($P \geq 0.23$). Decreased milk production when 80 g of propionate salt was added to the supplement suggests that this combination of glucogenic precursors (metabolizable protein from RUP plus propionate salt) shifted nutrient partitioning away from milk production. Similarly, Waterman et al. (2006) found a 9% decrease in milk production when cows were fed

bypass protein + 100 g/d propionate salt compared with bypass protein without additional propionate. In contrast however, Waterman et al. (2006) found a 25% reduction in butterfat secretion for cows supplemented with additional propionate while butterfat was similar for all treatment groups in the current experiment. Similar results to Waterman et al. (2006) were observed for dairy cows fed calcium propionate in a total mixed ration; decreased milk fat was attributed to decreased adipose tissue mobilization (Mandebvu et al., 2003). Milk production differences did not impact calf weights at branding ($P = 0.69$) or weaning (205-d weight; $P = 0.96$).

Cow BW were similar for all supplement groups at all measurement times during the experiment ($P \geq 0.58$; Table 2). Days to BW nadir increased linearly ($P < 0.01$) with increasing supplemental glucogenic precursors, but magnitude of BW nadir was similar across supplement groups ($P = 0.87$), and all other BW change intervals were similar as well ($P \geq 0.52$). Days from BW nadir to first estrus were similar for all supplement groups ($P = 0.21$). Cow BCS were similar for all treatment groups at beginning and end of supplementation ($P \geq 0.68$), and tended ($P = 0.15$) to exhibit a quadratic relationship ($P = 0.06$) at termination of breeding, when RUP80-fed cows had the highest BCS. A supplement × cow age interaction occurred at weaning ($P = 0.10$; Table 3). Two-yr-old cows fed RUP80 had higher BCS at weaning than their counterparts. For 3-yr-old cows, the RUP 160 group had higher BCS than RUP0-fed cows, with RUP80-fed cows intermediate. Four-yr-old cows had similar BCS at weaning regardless of supplement.

Cows had similar response to GTT regardless of supplement ($P \geq 0.32$; Table 2). Glucose half-lives for all cows were similar to those in Waterman et al. (2006) and these authors determined that cows fed supplements with increased GP in the form of additional metabolizable protein (i.e., RUP) and/or propionate salt had shorter glucose half-lives and were more sensitive to insulin than cows fed a traditional cottonseed meal-based supplement with no additional glucogenic precursors (avg 63 vs 100 min, respectively). All cows in the present study were fed supplements with additional GP from metabolizable protein and/or propionate salt and exhibited similar glucose half-lives to those of the previous study. Cows in both studies were considered insulin-resistant, as glucose half-lives were approximately 2-fold higher than the normal value of 35 min described by Kaneko (1997).

All 2-yr-old cows were pregnant in the fall, with all pregnancy rates above 90% (Table 4). The lowest pregnancy rates were for 3-yr-old cows. Calving intervals were slightly over a year in length for all cow ages ($P = 0.49$), which might be unexpected based on shorter days to first estrus observed for 4-yr-old cows.

Milk production and milk constituents increased linearly with cow age ($P < 0.01$). Three-yr-old cows produced ~20% more milk than 2-yr-old cows, and 4-yr-old cows produced ~15% more milk than 3-yr-olds. These milk production differences resulted in a linear increase in calf branding and 205-d weights with cow age ($P < 0.01$). For each year increase in cow age, a corresponding 20-25 kg increase in calf 205-d weight was observed.

Cow BW at supplementation start increased linearly ($P < 0.01$) with cow age as expected. At subsequent measurement times, BW increased in a quadratic fashion with increasing cow age. Throughout the experiment, 3-yr-old cows were approximately 73 kg heavier than 2-yr-old cows, compared to a 24 kg BW difference between 3- and 4-yr-old cows. Cows lost increasing amounts of weight from start of supplementation to BW nadir with increasing cow age ($P < 0.01$). Similar gains were observed from BW nadir to end of supplementation regardless of age ($P = 0.70$), but a quadratic pattern of BW gain was observed for the period from BW nadir to end of breeding ($P \leq 0.01$). During this time period, 3-yr-old cows gained more weight than 2- or 4-yr-old cows. A similar trend ($P = 0.15$) was noted from the end of supplementation to end of breeding, but all cows gained similar amounts of weight from the end of breeding to weaning ($P = 0.69$). Days to BW nadir were similar ($P = 0.46$) regardless of cow age. Days from BW nadir to estrus decreased linearly with cow age ($P < 0.01$). Two-yr-old cows returned to estrus approximately 1 mo after reaching BW nadir, compared to 3-yr-old cows who returned to estrus approximately 3 wk after BW nadir. Interestingly, 4-yr-old cows returned to estrus at essentially the same time as they reached BW nadir, implying that BW loss has less of an impact on reproductive performance in mature cows. Cow BCS at start of supplementation exhibited a quadratic ($P = 0.10$) pattern to increasing cow age, where 4-yr-olds were in higher body condition than 2- and 3-yr-old cows. At the end of supplementation and end of breeding, cow BCS increased linearly with cow age ($P \leq 0.02$).

Glucose AUC, insulin AUC, and insulin:glucose ratio were similar ($P \geq 0.53$) for all cows, regardless of age. A weak linear trend (overall $P = 0.24$; linear $P = 0.10$) was detected for decreasing glucose half-life with increasing cow age, implying increased metabolic efficiency.

Implications

Glucogenic precursor addition to protein supplements decreased days to first estrus in postpartum 2-year-old range cows, but did not have the same effect in 3- and 4-yr-old cows. Moderate amounts of supplemental GP shifted nutrients away from milk production regardless of cow age. Older cows returned to estrus at the same time they reached BW nadir, while younger cows needed to regain weight to return to estrus. Strategic supplementation with a combination of glucogenic precursors may be best suited to shift nutrient partitioning in young postpartum range cows grazing dormant forage, and may serve as a tool to enhance cow longevity and sustainability of extensively managed ranches in the western United States.

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Table 1. Supplement × cow age interaction ($P = 0.10$) for days to first estrus for 2-, 3-, and 4-yr-old postpartum cows grazing native range and fed supplements containing increasing amounts of glucogenic potential (0, 80, 160 g/d propionate salt).

	Supplement					
Cow Age	RUP0	SEM	RUP80	SEM	RUP160	SEM
2	90^{ax}	6	68^{bx}	7	70^{bxy}	8
3	70^{ay}	6	63^{ax}	6	74^{ax}	5
4	46^{az}	5	50^{ay}	5	55^{ay}	5

[a,b]Within row, values with different superscripts differ ($P \leq 0.10$).
[x,y,]Within column, values with different superscripts differ ($P \leq 0.10$).

Table 2. Effect of supplements containing increasing amounts of glucogenic potential (0, 80, or 160 g/d propionate salt) on reproduction, milk production, calf weight, cow weight and body condition score, and glucose tolerance test responses for 2-, 3-, and 4-yr-old postpartum cows grazing native range.

	Supplement							Contrast	
Response	RUP0	SEM	RUP80	SEM	RUP160	SEM	*P*-value	Linear	Quadratic
Pregnancy Rate, %	96	--	100	--	96	--	0.24	--	--
Ratio	22/23	--	24/24	--	24/25	--	--	--	--
Calving interval, d	370	3	371	3	371	3	0.98	0.86	0.99
Milk, g/d									
24-h production	9,982	443	8,439	473	9,620	437	0.08	0.56	0.03
Butterfat	342	27	297	28	361	25	0.26	0.62	0.13
Protein	271	16	232	16	262	15	0.23	0.67	0.10
Lactose	513	28	412	29	485	26	0.06	0.47	0.02
Solids non-fat	879	49	718	50	835	44	0.09	0.51	0.03
Calf BW, kg									
Branding	69	2	69	2	71	2	0.69	0.51	0.57
Weaning (205-d)	252	5	250	5	250	5	0.96	0.86	0.84
Cow BW, kg									
Begin supplementation	415	9	412	9	402	9	0.60	0.32	0.80
BW Nadir	361	7	355	8	350	8	0.58	0.30	0.97
End supplementation	395	9	393	9	389	9	0.89	0.64	0.90
End breeding	446	9	450	9	441	9	0.80	0.69	0.59
Weaning	500	10	504	11	500	11	0.96	0.96	0.77
Cow BW change, kg									
Begin supp – Nadir	-54	4	-56	4	-53	4	0.87	0.87	0.61
Nadir – End supp	34	5	37	5	39	5	0.70	0.40	0.95
Nadir – End breed	85	5	93	5	88	5	0.52	0.70	0.28
End supp – End breed	52	6	56	6	49	6	0.76	0.75	0.50
End breed – Wean	54	4	54	4	58	4	0.75	0.53	0.67
Days to BW nadir	45	1	47	1	50	1	0.01	< 0.01	0.42
Days from nadir to estrus	24	4	14	4	18	4	0.21	0.26	0.18
Cow BCS									
Begin supplementation	4.2	0.10	4.2	0.10	4.1	0.10	0.82	0.58	0.75
End supplementation	4.4	0.11	4.5	0.11	4.4	0.11	0.68	0.68	0.43
End breeding	4.6	0.12	4.9	0.12	4.5	0.12	0.15	0.54	0.06
GTT Response									
Glucose half-life, min	62	13	60	14	66	13	0.95	0.82	0.82
Glucose AUC	6,434	1,002	6,635	1,047	7,317	1,002	0.81	0.54	0.85
Insulin AUC	171	16	174	17	157	16	0.75	0.55	0.65
Insulin:glucose ratio	0.034	0.004	0.028	0.005	0.024	0.005	0.32	0.15	0.82

Table 3. Supplement × cow age interaction (P = 0.10) for body condition score at weaning for 2-, 3-, and 4-yr-old postpartum cows grazing native range and fed supplements containing increasing amounts of glucogenic potential (0, 80, 160 g/d propionate salt).

	Supplement					
Cow Age	RUP0	SEM	RUP80	SEM	RUP160	SEM
2	4.4^{ax}	0.14	4.9^{bxy}	0.16	4.5^{ax}	0.16
3	4.3^{ax}	0.15	4.6^{bx}	0.13	4.7^{bx}	0.12
4	4.9^{aby}	0.11	5.0^{ay}	0.10	4.7^{bx}	0.11

^{a,b}Within row, values with different superscripts differ ($P \leq 0.10$).

^{x,y,}Within column, values with different superscripts differ ($P \leq 0.10$).

Table 4. Effect of cow age on reproduction, milk production, calf weight, cow weight and body condition score, and glucose tolerance test responses for 2-, 3-, and 4-yr-old postpartum cows grazing native range and fed supplements containing increasing amounts of glucogenic potential (0, 80, or 160 g/d propionate salt).

	Cow Age							Contrast	
Response	2	SEM	3	SEM	4	SEM	P-value	Linear	Quadratic
Pregnancy Rate, %	100	--	91	--	97	--	0.30	--	--
Ratio	17/17	--	21/23	--	30/31	--	--	--	--
Calving interval, d	369	4	368	3	373	2	0.49	0.50	0.45
Milk, g/d									
24-h production	7,856	509	9,407	476	10,777	477	< 0.01	<0.01	0.88
Butterfat	244	29	347	30	408	28	< 0.01	< 0.01	0.60
Protein	208	15	250	16	308	16	< 0.01	< 0.01	0.71
Lactose	383	30	475	31	553	29	< 0.01	< 0.01	0.86
Solids non-fat	653	51	815	53	964	50	< 0.01	< 0.01	0.92
Calf BW, kg									
Branding	61	2	68	2	79	2	< 0.01	< 0.01	0.38
Weaning (205-d)	229	6	249	5	274	4	< 0.01	< 0.01	0.62
Cow BW, kg									
Begin supplementation	340	12	418	9	472	8	< 0.01	< 0.01	0.27
BW Nadir	297	9	368	8	399	6	< 0.01	< 0.01	0.04
End supplementation	335	12	406	9	436	8	< 0.01	< 0.01	0.07
End breeding	390	10	466	9	482	7	< 0.01	< 0.01	0.01
Weaning	445	12	519	10	539	9	< 0.01	< 0.01	0.04
Cow BW change, kg									
Begin supp – Nadir	-42	5	-50	4	-72	3	< 0.01	< 0.01	0.12
Nadir – End supp	33	5	37	5	39	4	0.70	0.38	0.86
Nadir – End breed	79	5	100	5	87	4	0.01	0.26	< 0.01
End supp – End breed	46	7	62	6	48	5	0.14	0.76	0.05
End breed – Wean	53	6	54	4	58	4	0.69	0.51	0.72
Days to BW nadir	47	1.0	47	1.0	48	0.9	0.46	0.42	0.46
Days from nadir to estrus	33	4	22	4	1	3	< 0.01	< 0.01	0.24
Cow BCS									
Begin supplementation	4.0	0.13	4.0	0.09	4.5	0.08	< 0.01	< 0.01	0.10
End supplementation	4.1	0.14	4.4	0.11	4.8	0.10	< 0.01	< 0.01	0.70
End breeding	4.5	0.15	4.6	0.12	4.9	0.10	0.03	0.02	0.45
GTT Response									
Glucose half-life, min	81	13	59	13	49	14	0.24	0.10	0.72
Glucose AUC	7, 655	1,002	6,555	1,002	6,175	1,047	0.57	0.32	0.77
Insulin AUC	174	16	164	16	164	17	0.87	0.67	0.79
Insulin:glucose ratio	0.026	0.005	0.033	0.005	0.027	0.005	0.53	0.88	0.27

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

INVESTIGATION OF THE BIOAVAILABILITY OF MANGANESE FROM ORGANIC VS. INORGANIC SUPPLEMENTS

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ABSTRACT: This study investigated the bioavailability of Mn-Glycine chelate vs. inorganic manganese (Mn) in lactating dairy cattle. Groups of 15 cows each were fed TMR containing mineral supplements with Mn as 1) 100% Mn oxide (0% group), 2) 50% Mn oxide and 50% Mn chelate (50% group), or 3) 100% Mn chelate (100% group) for a formulated total Mn concentration of 70 ppm, in a complete randomized block design. The feeding study lasted 8 wk. Clinical condition, serum Mn, whole blood Mn, liver Mn, and milk Mn were monitored prior to the study, at 4 wk, and at 8 wk. Also, milk production, somatic cell counts, milk protein, and milk fat were tested weekly. No differences were observed in clinical health, somatic cell counts, milk fat, or milk production among the treatments. No differences were observed for serum Mn at 0 and 4 wk, but cows receiving both chelate treatments had significantly greater serum Mn than those in the 0% group at wk 8 ($P < 0.05$). The whole blood Mn of the 100% group was significantly greater than the 0% group at 4 wk, but did not differ from the 50% group ($P < 0.10$). Similarly, the whole blood Mn of the 50% group was significantly greater than the 0% group, but did not differ from the 100% group at 8 wk ($P < 0.10$). No changes in milk Mn contents were observed at wk 4. By wk 8, cows receiving both Mn chelate diets had significantly greater milk Mn than those in the 0% group ($P < 0.05$), but the two chelate groups did not differ. Liver Mn of both chelate diet groups was significantly greater than the 0% group at wk 4 ($P < 0.05$). The liver Mn of the 50% group was significantly greater than the 0% group, but did not differ from the 100% group at wk 8. Percent milk protein was quite variable across the 8 weekly tests, with the 100% group being lower than the other 2 groups on wk 2, lower than the 50% group on wk 4, and lower than the 0% group on wk 5 ($P < 0.05$). The 50% group had significantly more milk protein content than the other two groups at wk 3 ($P < 0.05$). In summary, the chelated Mn resulted in increased bioavailability as indicated by increased systemic Mn concentrations in various matrices.
Keywords: Mn, chelate, dairy cattle

Introduction

Manganese is an essential component of bovine nutrition and has a variety of functions. Common processes that require manganese include carbohydrate metabolism, bone growth, normal brain function, growth, reproduction, and a variety of enzymatic systems (Ho, *et al*., 1984). Some of the enzymatic functions dependent on manganese include some pathways involved in amino acid metabolism, energy metabolism, and enzyme activation. Manganese deficiency in cattle has been associated with retarded/poor bone growth, skeletal abnormalities, enlarged/swollen joints, ataxia of the newborn, reduced birth weight, knuckling of the joints, physical weakness in calves, paralysis, and defects in lipid and carbohydrate metabolism (Hurley and Keen, 1987). In addition, manganese deficiency in bovine females also causes disturbed or depressed reproductive efficiency, silent heats, delayed estrus, poor/reduced conception, and abortions. The actual expression of deficiency varies with the degree of the deficiency and with the timing in which the deficiency occurs.

Manganese is a trace element included at low concentrations for diets of dairy cows (Hood, 1996). Supplemental manganese is usually found in the oxidized form in supplements for prepared rations, which likely decreases absorptive potential. The systemic absorption of manganese is usually less than one percent, but about 20 ppm manganese in the diet is reportedly adequate for growth and satisfactory reproductive performance. The U.S. National Research Council lists the following requirements for manganese 1-10 ppm for growing and finishing beef cattle, 20 ppm for dry pregnant cows, and 40 ppm for dairy cattle (Hurley and Keen, 1987).

When manganese is absorbed it is stored primarily in the liver. The liver has been shown to be important in the homeostatic control of certain trace elements, including manganese, and their excretion in bile (Symonds, *et al.*, 1982). Manganese is absorbed in the upper small intestine and, according to Howes and Dyer, is better absorbed under conditions of low manganese concentrations (1971). Much of the manganese taken up by the intestinal mucosa of cattle is not transported to the blood but in some way reenters the intestinal contents soon after uptake (Hood, 1996). Almost all the absorbed manganese is removed from the blood during its first passage through the liver and, in this way, systemic manganese concentrations are maintained at between 0.09 and 0.36 µmol per liter (Hall, *et al*., 1982). Bile flow is the main route of excretion for manganese to prevent excessive accumulation in the liver (Symonds and Hall, 1983). The peak rates of excretion of manganese occur principally around the period of feeding, as indicated by Symonds, *et al*. in 1982. Normally, hepatic

*This research was supported by the Utah Agricultural Experiment Station, Utah State University, Logan, Utah Approved as journal paper no. 7883

concentrations of manganese are low and excess absorbed manganese is rapidly excreted into the bile (Symonds and Hall, 1983).

Manganese deficiency is a very common finding in dairy cattle, but not in beef cattle (Hall, 2006). The normal serum manganese concentration for cattle is 0.006 to 0.070 ppm (Puls, 1994). Additionally, the normal liver concentration is 2.5 to 6 ppm. Diagnostic cases at the Utah Veterinary Diagnostic Laboratory have demonstrated that >90% of submitted dairy cattle serum samples are sub-normal, while this occurs in < 5% of beef cattle sera. In comparison, >50% of dairy cattle liver samples are manganese sub-normal, while <1% of beef cattle livers are sub-normal. It has been suggested that high dietary calcium and phosphorous, two highly supplemented minerals in dairy cattle rations, directly interferes with manganese absorption (NRC, 1988, Puls, 1994). This may partially explain the occurrence of manganese deficiency in dairy, but not beef, cattle.

With this research, we investigated the comparative bioavailability of manganese oxide, manganese-glycine chelate, and combined manganese oxide + manganese-glycine chelate in lactating dairy cattle. The relative bioavailability was evaluated with repetitive sampling and manganese quantification of liver, serum, whole blood, and milk over an 8 week feeding period. In addition, we compared milk production parameters of somatic cell counts, average daily milk weight, milk protein content, and milk fat content.

Materials and Methods

Cows. Lactating Holstein dairy cows between 30 and 110 day in lactation were utilized. The cows were pen fed as study groups to more closely mimic normal dairy production practices. Cows were first stratified as to lactation number, then ranked within lactation number as to days in milk. The cows were grouped as three animals on the same lactation number with similar days in milk, and then randomly assigned to one of the three treatments. The three treatments had a total of 15 animals per treatment or 45 total animals.

Milk Production and Animal Health. General animal health was evaluated twice daily for the duration of the study period. Milk weights were automatically collected for each animal at every milking.

Water. Cows were provided water *ad lib* for the duration of the study.

Feed. Cows were provided daily total mixed rations (TMR) in their pens *ad lib*. The three TMRs contained supplemented mineral packages with manganese as 1) 100% manganese oxide, 2) 50% manganese oxide and 50% manganese-glycine chelate, or 3) 100% organic manganese-glycine chelate. This feeding protocol lasted for 8 weeks. Six sub-samples of each TMR were collected (three each at 4 weeks and 8 weeks), dried, analyzed for mineral content by Inductive Coupled Plasma- Mass Spectroscopy (ICP-MS), and averaged (Table 1). The target manganese concentration in the TMRs was 70 ppm, which equaled the concentration which was being fed prior to the onset of the study.

Serum, Whole Blood, and Milk Manganese Analyses. Serum, milk, and whole blood was collected prior to the feeding study, at 4 weeks, and at 8 weeks of the study. Serum and whole blood samples were collected by venipuncture of the tail vein. Milk and whole blood samples were held refrigerated until analyzed. Serum was separated from the clot within 3 hours and held frozen until analyzed. The samples were analyzed for manganese content by ICP-MS at the Utah Veterinary Diagnostic Laboratory. Briefly, the samples were digested (1:1) in trace mineral grade nitric acid at 90 deg. C for 1 hour. The digests were diluted with ultra-pure water to a 5% nitric acid content, which provided a matrix match for the analytical standards and quality control samples.

Liver Manganese Analyses. Liver biopsy samples were collected by the paracostal, percutaneous needle biopsy method prior to the feeding study, at 4 weeks, and at 8 weeks of the study. Samples were weighed within 2 hours of collection and digested the day of collection. The samples were analyzed for manganese content by ICP-MS at the Utah Veterinary Diagnostic Laboratory. Briefly, the samples were digested in 0.5 ml of trace mineral grade nitric acid at 90 deg. C for 1 hour. The digests were diluted with ultra-pure water to a 5% nitric acid content, which provided a matrix match for the analytical standards and quality control samples.

Milk Composition and Somatic Cell Counts. Milk samples were collected prior to the study and weekly throughout the study from each animal. The milk samples were delivered to the DHIA testing laboratory within 2 hours of collection. Milk protein, milk fat, and somatic cell counts were evaluated by DHIA on the weekly individual animal samples.

Statistical Evaluation. Raw data for the manganese content of serum, whole blood, milk, and liver were compared across treatments by PROC MEAN and PROC GLM analysis using SAS Statistical Software 9.1.3 (SAS Institute, Inc., Cary, NC). Statistical outliers were removed from milk protein, milk fat, and somatic cell count samples prior to analysis and plotting. Significance was set at $p < 0.05$ for all analyses except whole blood manganese content. Significance for whole blood manganese content was set at $p < 0.10$ due to the physiologic effects of blood cell turnover.

Results and Discussion

Milk Production and Animal Health. Although the study was controlled for lactation number and days in milk, the randomization of animals was not controlled for total milk production. The 100% chelate treatment group had lower daily milk production at the outset of the study, which continued throughout the 8 week feeding period (Figure 1). No significant changes or trends in total daily milk production were identified among the three treatment groups. The 100% treatment group had a minor decrease in total milk production at a single sampling period, week 4, but was not significantly different than the proceeding weeks. In comparison, the 100% chelate group also had a minor trend towards increased milk production at weeks 6, 7, and 8, which followed the same occurrence in the other

two treatment groups.

The overall animal health did not change during the 8 week study period. Two cows in each of the treatment groups were treated for sub-clinical mastitis during the 8 week study period. This degree of sub-clinical mastitis was consistent with the overall herd incidence. Thus, no adverse health effects were identified across the three treatment groups.

Feed. The mean mineral concentrations were consistent across the three TMRs (Table 1). The mineral packages for the TMRs were mixed to achieve a 70 ppm manganese content. The average manganese content of the collected TMR sub-samples was 68.7, 66.5, and 65.7 ppm, for the 100% manganese oxide, 50% manganese oxide and 50% manganese chelate, and the 100% manganese chelate, respectively. No observed change in dietary intake was observed with the initiation of the differing TMRs.

Serum Mn Analyses. Although the study was controlled for lactation number and days in milk, the randomization of animals was not controlled for serum manganese content. The 0% chelate treatment group had a slightly lower serum manganese concentration than the other two treatment groups at the outset of the study, but there was not a statistically significant difference among the groups at that time (Figure 2). All three treatment groups had mean serum manganese concentrations that were deficient (normal – 0.006 to 0.070 ppm; deficient - < 0.005 ppm (Puls, 1994)) at the beginning of the study.

Slight numerical increases in serum manganese content were observed at week 4 in all three treatment groups, but they were still below the normal range. By week 8, cows receiving both chelate containing treatments had serum manganese concentrations that were significantly greater than those in the 0% chelate group ($p < 0.05$). However, the two chelate treatment groups did not differ from each other. All three treatment groups had serum manganese concentrations in the normal range by week 8.

Whole Blood Mn Analyses. Although the study was controlled for lactation number and days in milk, the randomization of animals was not controlled for whole blood manganese content. The 0% chelate treatment group had a slightly lower numerical whole blood manganese concentration than the other two treatment groups at the outset of the study, but there was not a statistically significant difference among the groups at that time (Figure 3). All three treatment groups had mean whole blood manganese concentrations that were deficient (normal – 0.02 to 0.09 ppm; deficient - < 0.02 ppm (Puls, 1994)) at the beginning of the study.

Numerical increases in whole blood manganese content were observed at week 4 and 8 in all three treatment groups. Both the 50% manganese chelate and the 100% manganese chelate groups were within the normal range at both 4 and 8 weeks, but the 0% chelate group was still in the deficient range at both 4 and 8 weeks. Significant differences were not observed at 0, 4, or 8 weeks ($p < 0.05$). However, with RBC turnover being a more long term change in whole blood, evaluation was also made at $p < 0.10$. At 4 weeks, whole blood of the 100% manganese chelate diet group was significantly greater than the 0% chelate group, but did not differ from that of the 50% chelate group. Similarly, at 8 weeks, whole blood of the 50% manganese chelate diet group was significantly greater than the 0% chelate group, but did not differ from that of the 100% chelate group.

Milk Mn Analyses. Although the study was controlled for lactation number and days in milk, the randomization of animals was not controlled for milk manganese content. There was no significant difference among the groups at that time (Figure 4). All three treatment groups had mean milk manganese concentrations that were at the very low end of normal (normal – 0.02 to 0.07 ppm (Puls, 1994)) at the beginning of the study.

No changes in milk manganese contents were observed at week 4 for any of the treatment groups. By week 8, cows receiving both manganese chelate containing treatments had milk manganese concentrations that were significantly greater than those in the 0% chelate group ($p < 0.05$). However, the two chelate treatment groups did not differ from each other.

Liver Mn Analyses. Although the study was controlled for lactation number and days in milk, the randomization of animals was not controlled for liver manganese content. The 0% chelate treatment group had a slightly lower numerical liver manganese concentration than the other two treatment groups at the outset of the study, but there was not a statistically significant difference among the groups at that time (Figure 5). All three treatment groups had mean liver manganese concentrations that were at the low end of normal (normal – 2.5 to 6.0 ppm (Puls, 1994)) at the beginning of the study.

Numerical increases in liver manganese content were observed in all three treatment groups at 4 weeks and again at 8 weeks in all three treatment groups. Significant differences were not observed at 0 weeks ($p < 0.05$). However, at 4 weeks, liver of both manganese chelate diet group was significantly greater than the 0% chelate group ($p < 0.05$). In contrast, at 8 weeks, liver manganese content of the 50% chelate group was significantly greater than the 0% chelate group, but did not differ from the 100% group. As manganese concentrations rise into the normal range, much less effect is anticipated, due to the efficient biliary elimination of manganese (Symonds and Hall, 1983).

Somatic Cell Counts. Milk somatic cell counts were quite variable across the 8 week study period (Figure 6). Weekly somatic cell count results were so variable that each treatment group had individual weeks in which the cows of the treatment group had significantly higher or significantly lower somatic cell counts than each of the other treatments. Overall, for the 8 week study, there was no significant difference or discernable trends among the treatments for weekly somatic cell counts.

Milk Composition. Milk percent fats were somewhat variable across the 8 week study period (Figure 7). Overall, for the 8 week study, there was no significant difference or trends among the treatments for weekly percent milk fat.

Percent milk protein contents were quite variable across the 8 week study period (Figure 8). Weekly percent milk protein results were so variable that no real discernable trends among the treatments were observed, although individual weeks had some significant differences.

At week 2, the 100% groups had significantly lower protein than the other two groups ($p < 0.05$). At week 3, the 50% group had significantly higher protein content than the other two groups ($p < 0.05$). And, on weeks 4 and 5, the 100% group had significantly lower protein content than the 50% and 0% groups ($p < 0.05$), respectively.

Implications

We did not identify any significant improvement/suppression or overall trends, in milk production, general animal health, or milk component parameters in this study. Minor overall milk production increases were observed across time for all three treatments, suggestive of a trend associated with days in milk and not a treatment associated effect. However, we did not evaluate reproductive performance as part of this study. Increased incidence of cystic ovaries and reproductive failure have been associated with manganese deficiency in cattle (Puls, 1994, NRC 2006), but an 8 week feeding window starting 30 to 110 days post-partum was not considered appropriate for evaluating reproductive performance. Thus, beneficial health effects in terms of reproduction should be evaluated with the manganese-glycine chelate.

Different chemical forms of manganese have been shown to result in significantly different bioavailability. Manganese oxides and carbonates had much lower relative bioavailabilities than manganese sulfate or chlorides. And these lowered bioavailabilities were more pronounced in sheep than in monogastrics. In contrast, manganese amino acid complexes and proteinates had greater relative bioavailability than manganese sulfate in poultry. Thus, the chemical form can be very important it terms of systemic bioavailability.

In our study, the manganese-glycine chelate increased serum, whole blood, milk, and liver manganese concentrations in comparison to the manganese oxide.

An interesting observation involved the cross comparison of the different matrices evaluated for total manganese content. The mean serum and whole blood manganese concentrations were sub-normal at the onset of the study, while the mean liver and milk manganese concentrations were within, although at the lower end, of the published normal ranges for cattle (Puls, 1994). This may indicate a need to reevaluate the "normal" ranges for these matrices. Or, it may indicate a poorer reliability of certain of these matrices in evaluating true, physiologic deficiencies.

In our study, manganese-glycine chelate in the diet of lactating dairy cattle significantly increased systemic manganese concentrations, as compared to manganese oxide, but did not significantly alter production parameters of milk production, milk fat, milk protein, somatic cell counts, or general animal health. Manganese deficiency is associated with poor reproductive function, and decreased metabolic efficiency. With significant numbers of dairy cattle having deficient manganese concentrations in systemic biologic matrices, these results strongly support the use of manganese-glycine chelate as a means of increasing systemic manganese concentrations.

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Table 1. Average mineral content of the three treatment TMRs that had manganese oxide in the added mineral packages replaced with 0%, 50%, and 100% Mn-Glycine chelate as the manganese source for an 8 week feeding period.

	TMR Control Concentration (ppm)	TMR 50% Chelate Concentration (ppm)	TMR 100% Chelate Concentration (ppm)
Ag	<0.01	<0.01	<0.01
Al	60.05	36.99	46.01
As	0.10	0.07	0.06
B	32.06	18.56	20.48
Ba	17.22	9.97	11.42
Be	0.02	0.01	<0.01
Ca	10518.32	9691.79	11242.62
Cd	0.17	0.10	0.10
Co	0.29	0.28	0.26
Cr	1.45	1.44	1.76
Cu	19.01	26.97	21.12
Fe	143.67	137.99	136.06
K	20998.72	20033.87	21219.41
Li	0.66	0.37	0.44
Mg	3271.12	3386.13	3662.00
Mn	68.67	66.51	65.67
Mo	1.17	0.71	0.77
Na	2900.17	3152.73	3430.95
Ni	1.11	1.18	1.17
P	3202.42	3121.32	3124.40
Pb	0.20	0.12	0.15
Sb	0.09	0.03	0.03
Se	0.54	0.59	0.56
Si	196.67	132.59	127.44
Sn	0.02	0.02	0.02
Sr	41.53	26.13	28.16
Tl	0.02	0.02	0.03
V	0.30	0.32	0.31
Zn	61.38	73.65	62.61

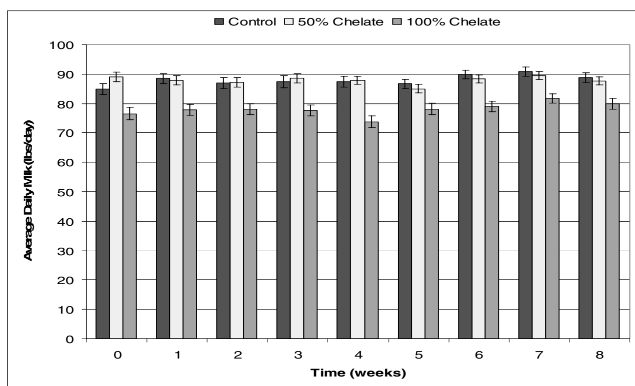

Figure 1. Average daily milk production of lactating dairy cattle when the mineral package for their TMR included 0%, 50%, and 100% Mn-Glycine chelate as the manganese source for 8 weeks. Error bars represent standard error of the mean.

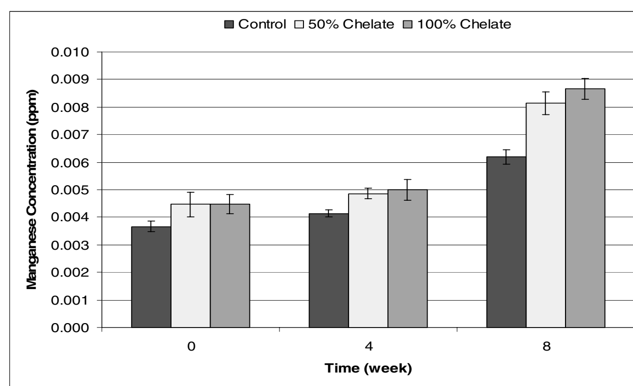

Figure 2. Serum manganese content of lactating dairy cattle when the mineral package for their TMR included 0%, 50%, and 100% Mn-Glycine chelate as the manganese source for 8 weeks. Error bars represent standard error of the mean.

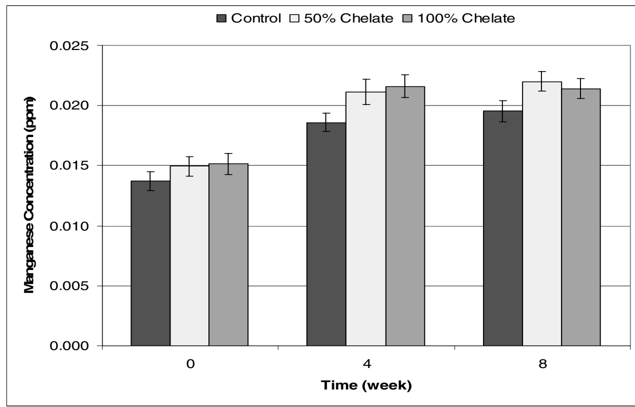

Figure 3. Whole blood manganese content of lactating dairy cattle when the mineral package for their TMR included 0%, 50%, and 100% Mn-Glycine chelate as the manganese source for 8 weeks. Error bars represent standard error of the mean.

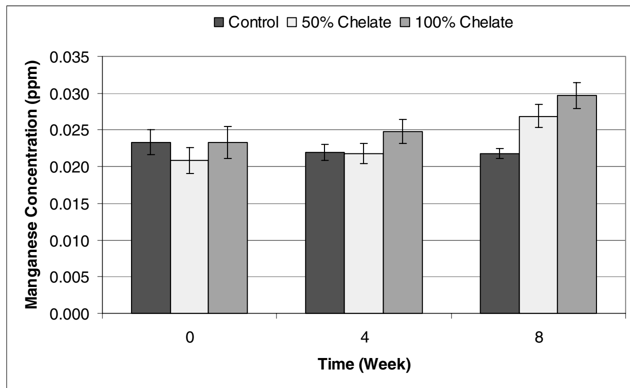

Figure 4. Milk manganese content of lactating dairy cattle when the mineral package for their TMR included 0%, 50%, and 100% Mn-Glycine chelate as the manganese source for 8 weeks. Error bars represent standard error of the mean.

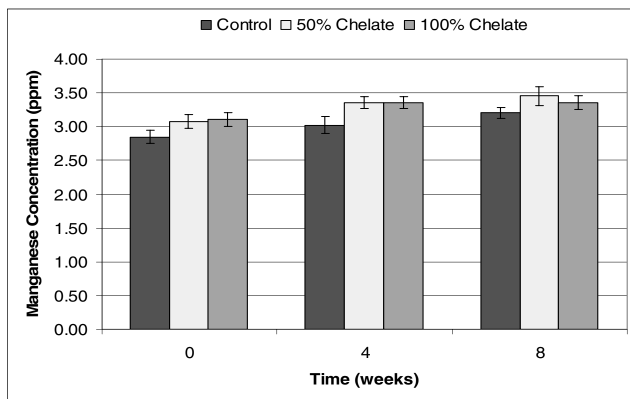

Figure 5. Whole blood manganese content of lactating dairy cattle when the mineral package for their TMR included 0%, 50%, and 100% Mn-Glycine chelate as the manganese source for 8 weeks. Error bars represent standard error of the mean.

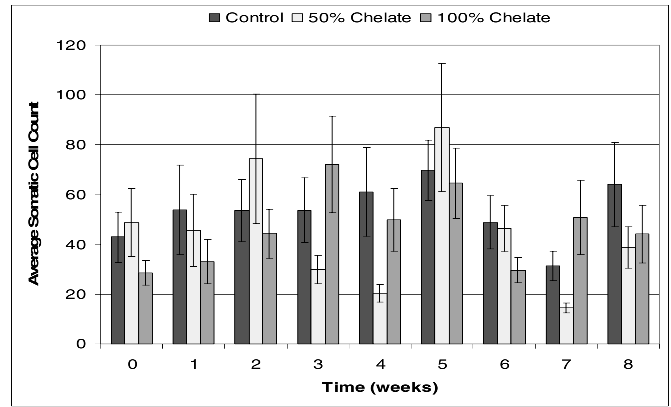

Figure 6. Weekly average daily somatic cell counts of lactating dairy cattle when the mineral package for their TMR included 0%, 50%, and 100% Mn-Glycine chelate as the manganese source for 8 weeks. Error bars represent standard error of the mean.

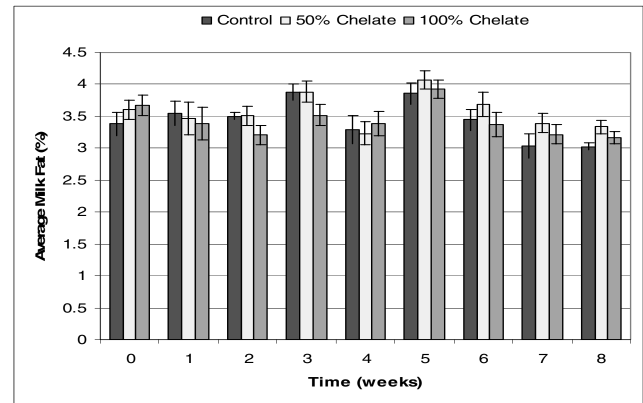

Figure 7. Weekly average milk fat percentage of dairy cattle when the mineral package for their TMR included 0%, 50%, and 100% Mn-Glycine chelate as the manganese source for 8 weeks. Error bars represent standard error of the mean.

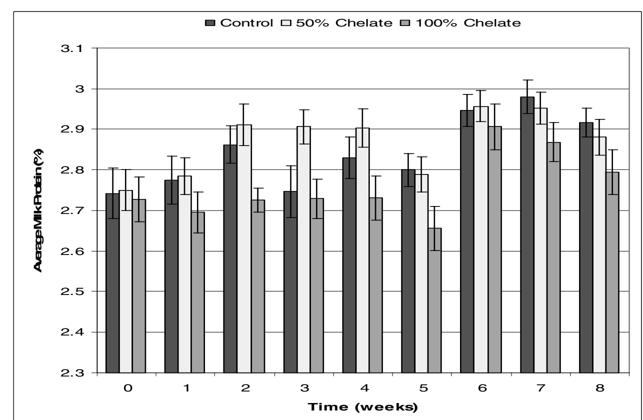

Figure 8. Weekly average milk protein percentage of dairy cattle when the mineral package for their TMR included 0%, 50%, and 100% Mn-Glycine chelate as the manganese source for 8 weeks. Error bars represent standard error of the mean.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

INTAKE AND DIGESTIBILITY OF BETA-GLUCAN FROM 'VALIER' BARLEY IN YOUNG CALVES[1]

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ABSTRACT: The mammalian immune system has been stimulated with oral beta-glucan (BG) doses of 0.10 to 0.22 g BG/kg BW; however, BG are highly digestible in the rumen of adult cattle. We conducted 2 research trials to estimate BG intake and digestibility of a 'Valier' barley-based starter pellet by young dairy calves. In Trial 1, diet and fecal samples from five 2- to 9-wk old calves (43 kg) fed starter pellets containing 60% ground Valier barley or corn were analyzed for DM, BG, N, ADF, and starch. Data were analyzed using PROC MIXED with treatment, week, and their interaction in the model. Means were separated using LSMEANS when $P < 0.10$. In Trial 2, three 12-wk old calves (108 kg) were individually dosed with sustained release chromic oxide sheep boluses. Digesta samples collected from the beginning and end of small intestine and feces were analyzed for DM, BG, and Cr. In Trial 1, pre-weaning, calf ADG and pellet DMI did not differ ($P > 0.78$) between treatments, averaging 0.48 kg/d and 337 g/d, respectively. Post-weaning calf ADG did not differ ($P = 0.61$) between treatments averaging 1.0 kg/d. Post-weaning intake of DM, N, ADF, and starch by calves did not differ ($P > 0.12$) between treatments averaging 1,643, 52, 138, 634 g/d, respectively. Beta-glucan intake by barley-fed calves ranged from 0.10 to 0.73 g BG /kg BW throughout the study. Post-weaning total tract DM and starch digestibility did not differ ($P > 0.10$) between treatments averaging 75.6 and 99.0%, respectively. Digestibility of N and ADF was greater ($P < 0.10$) in calves fed barley than corn (68.5 vs 62.2% for N, and 54.1 vs 37.0 for ADF). Beta-glucan digestibility was lower ($P = 0.09$) in corn-fed than barley-fed calves on d 58 and 65. In Trial 2, calves consumed 0.94 g BG/kg BW and amounts of BG present at the beginning and end of small intestine and in the feces averaged 0.07, 0.005, and 0.009 g BG/kg BW, respectively. Digestibility to the beginning of the small intestine, from the beginning to end of the small intestine, and for the total tract was 1.9, 81.8, and 69.5%, respectively for DM, and 93.0, 91.9, and 99.0%, respectively for BG. A calf starter pellet containing 60% ground Valier barley delivered an amount of BG to the small intestine of young calves at the low end of oral beta-glucan doses used to stimulate the immune system of other mammals.

Key Words: Barley, Beta-glucan, Calves

Introduction

The mammalian immune system has been stimulated with oral beta-glucan (BG) doses of 0.10 to 0.22 g BG/kg BW (Yun et al., 2003; Davis et al., 2004). It has been hypothesized that mucosal cells in the intestine may be able to absorb beta-glucan and transport it to the blood and immune system while preserving its original active conformation (Delaney et al., 2003 based on work by Owen, 1999). Ninety-eight percent of orally administered SSG (soluble BG from *Sclerotinia sclerotiorum*) was found in the gastrointestinal tract suggesting that beta-glucan can act directly from the gut to stimulate the immune system (Miura, 2005). While the exact mechanism is unknown, we are assuming that it is necessary to deliver beta-glucan to the small intestine in order to stimulate the immune system of mammals. Little research has been conducted using beta-glucans as an immunostimulant in ruminants, because beta-glucans are highly digestible in the rumen of adult cattle (Grove et al., 2006). We hypothesized that beta-glucan from 'Valier' (a low DMD barley) would escape ruminal digestion in young dairy calves. The objectives of this study were to estimate beta-glucan intake and digestibility of a 'Valier' barley-based starter pellet by young dairy calves.

Materials and Methods

In Trial 1, seven < 1 wk old dairy calves (43 kg) were obtained and assigned to one of 2 treatments: corn-based starter pellet (0.12% beta-glucan, n = 4) or 'Valier' barley-based starter pellet (2.8% beta-glucan, n = 3). Chemical composition of the grains is presented in Table 1. Grains were ground and pellets were formulated based on requirements for young dairy calves (NRC, 2001) using 60% grain along with soybean meal, beet pulp, molasses, vitamins, and minerals. Animals were cared for under protocols approved by the Montana State University Animal Care and Use Committee.

Calves were fed only milk replacer the first week of life and offered their assigned treatment pellets beginning on d 7. Calves were weighed once a week throughout the study. Calves were weaned off milk replacer beginning on d 36 and were consuming only pellets by d 42. Diet, ort, and fecal samples were collected from each calf on d 16, 23, 30, 37 (pre-weaning from milk), 44, 51, 58, and 65 (post-weaning from milk). Diet and fecal samples were dried in a 60° C forced-air oven, ground through a Wiley mill (1-mm screen), and analyzed for DM (AOAC, 1999), N (Leco Corporation, St. Joseph, MI), ADF (Van Soest et al., 1991), starch (Megazyme, Sidney, Australia), beta-glucan (McCleary kits; Megazyme, Sidney, Australia), and AIA (4N HCl method; Van Keulen and Young, 1977). Acid insoluble ash was used as an internal marker to estimate fecal output and calculate apparent nutrient

[1] The authors wish to thank Kerri Rask and Jim Thompson for their valuable animal care expertise on this project.

digestibility. Pre-weaning and post-weaning data were analyzed separately using PROC MIXED with treatment, day, and their interaction in the model (Littell et al., 1998). Means were separated using LSMEANS when $P < 0.10$.

In Trial 2, three 9-wk-old calves (108 kg) were offered the Valier barley-based starter pellet described in Trial 1 in order to quantify the amount of beta-glucan available along the digestive tract. Calves were group-fed pellets and a small amount of hay and were individually dosed with sustained release chromic oxide sheep boluses (Captec, New Zealand, Australia) on d 16. Diet and orts were monitored daily and samples were collected on d 16, 23, and 28. On d 28, calves were sacrificed and digesta samples were collected from the beginning of the small intestine, end of the small intestine, and feces. Digesta samples were frozen after collection. Diet and digesta samples were dried in a 60° C forced-air oven, ground through a Wiley mill (1-mm screen), and analyzed for DM and BG (as previously described), and Cr using inductively coupled plasma emission spectroscopy (Ellis et al., 1982).

Results

Trial 1. Prior to weaning, calf ADG did not differ ($P = 0.78$) between treatments, averaging 0.48 kg/d (Table 2). Pellet intake did not differ ($P = 0.85$) between treatments averaging 337 g/d (Table 3). By design, BG intake was greater ($P = 0.001$) by calves fed barley than corn ranging from 0.10 to 0.34 g BG/kg BW in pre-weaned barley-fed calves and 0.03 to 0.08 g BG/kg BW in pre-weaned corn-fed calves. Total tract BG digestibility prior to weaning did not differ ($P = 0.20$) between treatments and was greater than 97% on all days.

Post-weaning calf ADG did not differ ($P = 0.61$) between treatments averaging 1.0 kg/d (Table 2). Post-weaning intake of DM, N, ADF, and starch by calves did not differ ($P > 0.12$) between treatments averaging 1,643, 52, 138, 634 g/d, respectively (Table 4). By design, beta-glucan intake was greater ($P = 0.001$) by barley-fed than corn-fed calves ranging from 0.47 to 0.73 g BG/kg BW for barley-fed calves and being 0.03 g BG/kg BW on all days for corn-fed calves. Total tract DM and starch digestibility did not differ ($P > 0.10$) between treatments averaging 75.6 and 99.0%, respectively. Digestibility of N and ADF was greater ($P < 0.10$) in barley than corn-fed calves (68.5 vs 62.2% for N, and 54.1 vs 37.0 for ADF). Beta-glucan digestibility was lower ($P = 0.09$) in corn-fed than barley-fed calves on d 58 and 65. Negligible amounts of BG were recovered in the feces of calves pre- and post-weaning (0 to 0.005 g BG/kg BW).

In Trial 2, calves consumed 0.94 g BG/kg BW and amounts of BG present at the beginning and end of small intestine and in the feces averaged 0.07, 0.005, and 0.009 g BG/kg BW, respectively. Digestibility to the beginning of the small intestine, from the beginning to end of the small intestine, and for the total tract was 1.9, 81.8, and 69.5%, respectively for DM, and 93.0, 91.9, and 99.0%, respectively for BG.

Discussion

Grove et al. (2006) reported 91.6% and 98.1% total tract BG digestibility in steers fed 80% Valier barley-based finishing diets at 42- and 107-d on feed, respectively. We hypothesized that BG would escape digestion in pre-ruminant dairy calves; however, we recovered almost no beta-glucan in the feces of young dairy calves with ruminal and total tract digestibility estimates similar to those observed in mature cattle. High digestibility of BG in the young calves was unexpected; however, the calf starter pellet contained 60% ground barley whereas the finishing diets consumed by mature steers contained 80% cracked barley. Grinding the feed could have increased nutrient digestibility even in young calves. Similar to our research in cattle, no beta-glucan was detected in the feces of pigs (Graham et al., 1986) or rats (Dongowski et al., 2002) consuming barley-based diets. Graham et al. (1986) also reported that 27% of BG was digested at the duodenum and 70% was digested in the small intestine of pigs consuming barley-based diets. In Graham et al. (1986), pigs averaging 40 kg BW were fed diets containing 3.12% BG at 4% of their BW (1.6 kg feed). Therefore, BG intake was 50 g/d (or 1.25 g BG /kg BW). If 27% of this was digested in the stomach, then 0.91 g BG/kg BW reached the small intestine of these pigs. Other researchers have stimulated the immune system of mice with oral beta-glucan doses as low as 0.10 to 0.22 g BG/kg body weight (Yun et al., 2003; Davis et al., 2004). In Trial 2, 2 out of 3 calves did have beta-glucan concentrations at the beginning of the small intestine equal to the low end of doses reported in the literature for immune system stimulation. Extrapolating the 93% beta-glucan digestibility to the beginning of the small intestine from Trial 2 to the finishing steers in Grove et al. (2006) and pre- and post-weaned calves in Trial 1 of the current study, the quantity of BG reaching the small intestine was estimated to be 0.24 to 0.27, < 0.01 to 0.02, and 0.02 and 0.05 g BG/kg BW, for steers, pre-weaned calves, and post-weaned calves respectively. Only finishing steers and calves > 9 wk had had amounts of beta-glucan present in small intestine comparable to doses used to stimulate the immune system of other mammals.

Low DM digestibility in Trial 2 could be due to inconsistent digesta flow in young calves or external marker methodology. We also recognize that the BG concentration in digesta is at the low end of detection by our assay.

Implications

Pellet intake and ADG were similar by calves consuming corn- or Valier barley-based starter pellets. Beta-glucan delivery to the small intestine of young calves was lower than oral beta-glucan doses used to stimulate the immune system of other mammals. Beta-glucan delivery to the small intestine could be increased by including more barley in the calf starter, slowing digestibility by formulating a texturized calf starter containing cracked rather than ground barley, or using a barley variety with higher beta-glucan content.

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Table 1. Nutrient and ingredient composition of milk replacer and calf starter pellets based on corn or 'Valier' barley

Item	Corn	Valier	Milk replacer
DM, %	86.47	87.85	95.91
N, %	3.29	3.09	3.79
ADF, %	7.10	9.95	1.15
Starch, %	43.04	34.07	4.52
BG, %	0.12	2.80	0.04
Ingredient, % as-fed			
Corn, ground	60	-	-
Valier barley, ground	-	60	-
Soybean meal	26.75	18.5	-
Beet pulp	6.25	14.75	-
Molasses	5	5	-
Calcium carbonate	1.1	1	-
Dicalcium Phosphate	0.5	0.5	-
Salt	0.225	0.125	-
Vitamin E	0.06	0.06	-
Mineral mix	0.05	0.05	-
Vitamin mix	0.008	0.008	-

Table 2. Body weight and average daily gain by dairy calves consuming starter pellets based on corn or "Valier" barley

	Treatment			*P*-value
Item	Valier	Corn	SE	Trt
Start wt, kg	43.1	42.6	1.91	0.88
End wt, kg	81.4	82.9	1.03	0.40
ADG, kg/d				
Pre-weaning	0.46	0.49	0.06	0.78
Post-weaning	1.00	1.05	0.06	0.61
Overall	0.66	0.69	0.02	0.31

Table 3. Pre-weaning nutrient intake (DM basis) and digestibility by dairy calves consuming starter pellets based on corn or 'Valier' barley

Item		Treatment		Day					*P*-value		
		Barley	Corn	16	23	30	37	SE	Trt	Day	Trt*Day
Intake, g/d											
Milk replacer		476	476	544	544	544	272		-	-	-
Pellet		328.4	345.0	92.4^{a}	253.3^{b}	302.2^{b}	703.6^{c}	61.02	0.85	0.001	0.81
Beta-glucan, g/kg BW											
Intake	Barley			0.10^{aB}	0.15^{bB}	0.15^{bB}	0.34^{cB}	0.024	0.005	0.001	0.001
	Corn			0.03^{aA}	0.06^{abA}	0.04^{aA}	0.08^{bA}				
Fecal output		0.001	0.002	0^{a}	0.002^{ab}	0^{a}	0.003^{b}	0.0009	0.45	0.09	0.72
Digestibility, %											
DM	Barley			95.1^{c}	90.1^{b}	90.9^{bA}	75.2^{aB}	1.26	0.98	0.001	0.003
	Corn			96.5^{b}	-	94.1^{bB}	70.7^{aA}				
Beta-glucan		99.4	97.4	98.9^{b}	99.2^{b}	98.6^{b}	97.5^{a}	0.83	0.20	0.09	0.13

[abc] Within a row and effect, means lacking a common superscript letter differ ($P < 0.10$).
[AB] Within a column and item, means lacking a common superscript letter differ ($P < 0.10$).

Table 4. Post-weaning nutrient intake (DM basis) and digestibility by dairy calves consuming starter pellets based on corn or 'Valier' barley

Item		Treatment		Day					*P*-value		
		Barley	Corn	44	51	58	65	SE	Trt	Day	Trt*Day
Intake, g/d											
DM		1629.4	1655.7	1021.8^{a}	1493.0^{b}	1858.4^{c}	2207.4^{d}	156.34	0.92	0.001	0.71
N		50.3	54.6	32.3^{a}	49.0^{b}	59.2^{c}	70.9^{d}	4.99	0.57	0.003	0.55
ADF		157.8	117.2	86.7^{a}	126.9^{b}	155.9^{c}	164.4^{c}	12.15	0.13	0.001	0.94
Starch		551.8	715.6	405.0^{a}	581.5^{b}	735.9^{c}	878.0^{d}	60.68	0.14	0.003	0.67
Beta-glucan, g/kg BW											
Intake	Barley			0.47^{aB}	0.62^{bB}	0.70^{cB}	0.73^{dB}	0.021	0.002	0.001	0.001
	Corn			0.03^{A}	0.03^{A}	0.03^{A}	0.03^{A}				
Fecal output		0.002	0.002	0^{a}	0^{a}	0.005^{b}	0.002^{ab}	0.0009	0.37	0.03	0.52
Digestibility, %											
DM		76.6	74.5	71.0	80.5	73.5	76.4	2.81	0.44	0.26	0.35
Beta-glucan	Barley			100.1	99.9	99.2^{B}	99.8^{B}	3.56	0.01	0.06	0.09
	Corn			96.5^{bc}	97.7^{c}	86.3^{aA}	87.9^{abA}				
N		68.5^{b}	62.2^{a}	53.7^{a}	73.5^{b}	65.8^{b}	65.7^{b}	3.94	0.08	0.04	0.79
ADF		54.1^{b}	37.0^{a}	54.6^{b}	59.6^{b}	44.6^{b}	16.6^{a}	8.43	0.09	0.07	0.18
Starch		99.4	98.6	99.0	99.0	98.5	99.1	0.36	0.11	0.70	0.80

[abcd] Within a row and effect, means lacking a common superscript letter differ ($P < 0.10$).
[AB] Within a column and item, means lacking a common superscript letter differ ($P < 0.10$).

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECTS OF RUMINAL PROTEIN DEGRADABILITY AND SUPPLEMENTATION FREQUENCY ON EXPRESSION AND DISTRIBUTION OF UREA TRANSPORTER-B IN LAMBS FED LOW-QUALITY FORAGE

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ABSTRACT: Thirteen Dorset wether lambs (initial BW = 34 ± 4 kg) were used in a completely randomized designed experiment to examine the effects of ruminal protein degradability and supplementation frequency on the expression and distribution of urea transporter-B (UT-B) in tissues important to N recycling. Lambs were fed crested wheat grass hay (4.2% CP, 59% NDF) for ad libitum consumption plus one of four isonitrogenous supplements: 1) ruminally degradable protein (RDP) fed daily (n=3), 2) RDP fed on alternate days (n=3), 3) ruminally undegradable protein (RUP) fed on alternate days (n=3) or 4) a 50:50 mixture of RDP and RUP fed on alternate days (n=4). After 18 d, lambs were euthanized and samples (5 g) taken from the gastrointestinal tract, liver, kidney, and parotid salivary gland were snap frozen and later processed for Western blot analyses for UT-B. Immunoblotting using a rabbit polyclonal antibody to UT-B confirmed the presence of distinct 32 kDa (consistent with a non-glycosylated UT-B protein) and 47 kDa (probable N-glycosylated form of UT-B) protein bands in all nine tissues. The liver, dorsal rumen, reticulum, and ventral rumen displayed strong bands at 32 kDa and lighter bands at 47 kDa. The spiral colon, cecum, parotid salivary gland, large colon, and kidney had slight bands at 32 kDa and visible bands at 47 kDa. Although the abundance of the 47 kDa UT-B band in the ventral rumen was greater (P = 0.03) in lambs fed RDP daily, no other treatment differences ($P \geq 0.16$ to 0.99) in the abundance of the 32 or 47 kDa UT-B proteins or the 32 kDa/47 kDa ratio within tissue were observed. However, the 32 kDa/47 kDa ratio was greatest ($P \leq 0.001$) for the liver with no difference (P = 0.63 to 0.99) among the remaining tissues. Although protein supplementation strategy had little effect on UT-B expression in tissues other than the ventral rumen, differences in N-glycosylation among tissues may provide insight into the regulation f UT-B function.

Key Words: lambs, nitrogen recycling, urea transporters

Introduction

Decreasing the frequency of protein supplementation to ruminants consuming low quality forages has generally resulted in minimal impact on nutrient intake or digestion (Bohnert et al., 2002a), or subsequent animal performance (Bohnert et al., 2002a; Ludden et al., 2002). Hunt et al. (1989) suggested that infrequent protein supplementation may stimulate recycling of endogenous N into the rumen. Because of the positive relationship that exists between ruminally degradable protein (RDP) supplementation and forage utilization, most supplements fed infrequently include high levels of RDP. However, recent work in our laboratory (Atkinson, 2005, 2006a, 2006b) suggests that replacing a portion of the supplemental RDP with ruminally undegradable protein (RUP) may further enhance the N recycling process by altering the timing of N return to the rumen relative to the time of supplementation. The recent identification of urea transporter-B (**UT-B**) in the gastrointestinal tract tissues of sheep (Marini et al., 2004) and cattle (Marini and Van Amburgh, 2003; Stewart et al., 2005) provide a potential mechanism by which the N recycling process could be regulated. Despite early research, little is known about the expression, tissue distribution, and physiological role of UT-B in the N recycling process. Moreover, the effects of dietary protein on UT-B expression requires further investigation, given the importance of N recycling in ruminant livestock consuming low quality (low protein) forages. We hypothesize that UT-B is widely distributed in the gastrointestinal tract and associated tissues of ruminants, and that UT-B expression will be up-regulated in infrequently-supplemented animals, particularly when those supplements contain higher levels of RUP. Therefore, our objectives are to 1) evaluate the expression and distribution of UT-B within the gastrointestinal tract, liver, kidney, and parotid salivary gland of lambs consuming low quality forage, and 2) to assess the role of ruminal protein degradability and supplementation frequency on UT-B abundance in these tissues.

Materials and Methods

Animals and diets

Thirteen Dorset wether lambs (34 ± 4 kg initial BW) were randomly assigned to one of four treatments within a completely randomized design. All procedures were approved by the University of Wyoming Animal Care and Use Committee. Wethers were housed in individual metabolism crates (1.4 × 0.6 m) in a temperature controlled room (20ºC) under constant lighting. Details on the basal diet and the formulation of supplements fed in this experiment are described in Atkinson et al. (2005). Briefly, wethers were fed a basal diet of low-quality mature crested wheatgrass hay (4.2% CP, 59% NDF, and 42% ADF) for ad libitum consumption in two portions daily at 0630 and 1600. Wethers were supplemented at 0600 daily with one of four supplemental protein treatments: 1) a high RDP supplement (Table 1) based upon isolated soy protein fed daily (**RDP-D**), 2) the high RDP supplement provided on alternate days (**RDP-D**), 3) a high RUP supplement based upon corn gluten meal fed on an isonitrogenous basis to the RDP supplement, provided on alternate days (**RUP-A**), or

[1]This project was supported in part by the University of Wyoming Faculty Grant-In-Aid Program.

4) a 50:50 mixture of the RDP and RUP supplements provided on alternate days (**MIX-A**). The RDP and RUP supplements were fed at the daily equivalent rate of 0.236 and 0.285% of BW, respectively throughout the experiment, with alternate-day treatments fed at twice that of daily supplementation.

Table 1. Composition of supplements

	RDP[1]	RUP
Ingredient, % of DM		
Isolated soy-protein[2]	73.1	
Corn gluten meal		75.8
Calcium carbonate	11.7	11.1
Vitamin premix[3]	10.2	8.1
Dried molasses	5.0	5.0
Chemical		
DM, %	95.8	93.9
CP, % of DM	73.9	54.3

[1]RDP = ruminally degradable protein, RUP = ruminally undegradable protein
[2]ARDEX® AF, Archer Daniels Midland Company, Decator, IL.
[3]Contained 3,628,739 vitamin A 3,628,739 vitamin D3 and 18,144 vitamin E IU/kg.

Tissue Collection and Sample Analyses

After receiving their supplemented treatments for 18 days, wethers were randomly euthanized by injection of Beuthanasia-D Special (Schering-Plough Animal Health, Union, NJ) according to label directions. The lambs were immediately eviscerated, and tissue samples (5 g) collected from the dorsal and ventral rumen, reticulum, cecum, large colon, small (spiral) colon, liver, kidney, and parotid salivary gland. Samples were rinsed with PBS, and immediately snap frozen in liquid N. Samples were stored at -80ºC until protein isolation and Western blot analyses were performed.

Western blot procedures used in this experiment are described in detail by Stohrer et al. (2007). Briefly, samples (500 mg tissue) were homogenized in RIPA buffer and 25 μg of protein was loaded per lane on 12% polyacrylaminde SDS gels. The electrophoresed protein was transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA). Membranes were then probed with purified polyclonal anti-UT-B antibody (Stohrer et al. (2007) and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG. Immunoreactive protein bands were visualized by chemiluminescent substrate (Pierce, Rockford, IL). All Western blots within a tissue were processed together, with exposure time dependent upon intensity of signal within tissue type. Autoradiographs were digitized (UN-SCAN-IT_{TM}, Orem, UT) to obtain total pixel counts for the 32 kDa and 47 kDa UT-B bands as well as a 42 kDa β-actin band. Blots were normalized by dividing the total pixels for each band (32 kDa or 47 kDa) by the total pixels obtained for the corresponding β-actin of the same lane/sample. Results were expressed as arbitrary densitometry units per 32kDa and 47kDa UT-B band.

Statistical Analyses

All densitometry data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) using the model for a completely randomized design. Because the same tissue sample from all lambs was included in a single set of Western blots assayed simultaneously, the model used to detect treatment differences in the 32 and 47 kDa protein bands consisted of treatment only, and was conducted within a given tissue type. The model used to detect differences in the 32 kDa/47 kDa ratio also included the effects of tissue type and the treatment × tissue type interaction. No treatment × tissue type interactions ($P \geq 0.93$) were detected, and thus only main effects of treatment and tissue type will be discussed. When necessary, separation of treatment means was accomplished using least squares means and Fisher's protected LSD ($P \leq 0.05$).

Results and Discussion

Immunoblotting confirmed the presence of two distinct UT-B protein bands (32 kDa and 47 dKa) in all nine tissues analyzed. Despite their presence in all tissues analyzed, the visual intensity of these UT-B bands differed among tissues. A strong 32 kDa band and a light 47 kDa band were detected in the dorsal, ventral rumen, reticulum (Figure 1), and liver. Conversely, the small (spiral) colon, cecum, large colon, and kidney displayed slight bands at 32 kDa and more visible/intense bands at 47 kDa. The parotid salivary gland displayed similar bands to the lower GI tract and the kidney (Figure 2). This is one of the first known studies (Stohrer et al., 2007) identifying UT-B within the parotid salivary gland of any species.

Figure 1. Western blot of samples from the reticulum.

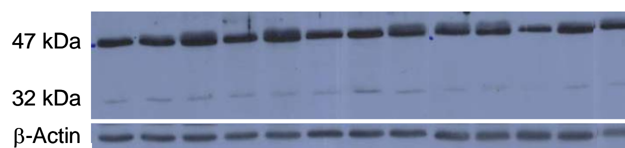

Figure 2. Western blot of samples from the parotid salivary gland.

Other researchers have reported UT-B proteins similar in size to our 47 kDa UT-B protein in rumen papillae of dairy heifers (Marini and Van Amburgh, 2003), the duodenum, ileum, and cecum of lambs (Marini et al., 2004), and in the rumen of slaughter cattle (Stewart et al., 2005). However, we believe that the two distinct protein bands detected by our anti-UT-B antibody represent *N*-glycosylated (47 kDa) and non-glycosylated (32 kDa) forms of the UT-B protein. Lucien et al. (2002) predicted that the structure of human UT-B1 contained a single unique functional *N*-glycosylation site, which is consistent with the presence of two distinct UT-B bands as observed in the current study and that of others. Similarly, Timmer et al. (2001) and Olives et al. (1995) reported that

deglycosylation of UT-B reduced the size of a larger 45-60 kDa UT-B band in human erythrocytes to 32 or 36 kDa, which is consistent with the two protein bands detected in the current experiment.

Contrary to our initial hypothesis that UT-B abundance would be greater in animals supplemented on alternate days, particularly with RUP, the only difference observed in UT-B abundance occurred in the ventral rumen. Although the abundance of the 47 kDa UT-B band in the ventral rumen was greater ($P = 0.03$) in lambs fed RDP daily, no other treatment differences ($P \geq 0.16$) in the abundance of the 32 or 47 kDa UT-B proteins (Table 2) was observed. Similarly, Marini et al. (2004) reported no differences in UT-B abundance in the rumen in response to increasing dietary N. In contrast, Marini and Van Amburgh (2003) observed greater expression of UT-B (based upon visual evaluation) in ruminal papillae collected from the ventral sac of the rumen in dairy heifers fed a high N diet (2.97 - 3.4% N) as compared to a low N diet (1.45 – 1.89% N). The increased abundance of the 47 kDa UT-B band in the ventral rumen when lambs were fed RDP daily could be the result of high rumen ammonia concentrations associated with feeding excess RDP on the day of supplementation (Atkinson et al., 2005). Marini and Van Amburgh (2003) suggested that when a high N diet is fed and ruminal ammonia is high, urea diffuses into the gastrointestinal tract via the paracellular space and may return to the blood via UT-B. Therefore, it is possible that the level of RDP fed to RDP-D lambs was sufficient to increase UT-B abundance in the ventral rumen in the manner observed by Marini and Van Amburgh (2003). Furthermore, because it has been demonstrated that ruminal epithelial and duodenal mucosal cells are capable of undergoing ureagenesis in vitro (Oba et al., 2004), UT-B expression in these tissues may function as an excretory route for urea, rather than recycling of urea into the gastrointestinal tract.

Dietary treatment had no influence ($P \geq 0.14$) on the abundance of the 32 kDa/47 kDa ratio within tissues. However, the 32 kDa/47 kDa ratio differed ($P = 0.06$) across tissues, being greatest ($P \leq 0.001$) for the liver with no difference ($P \geq 0.63$) among the remaining tissues. While differences in the 32 kDa/47 kDa ratio between the liver and the remaining tissues reflect the relative differences in the degree of *N*-glycosylation of UT-B the physiological importance of *N*-glycosylation to the function of UT-B remains largely unexplained. In other systems, *N*-glycosylation often determines membrane expression level and addressing of polypeptides to the membrane (Varki et al., 2002). However, site-directed mutagenesis to delete the *N*-glycosylation site of human UT-B expressed in *Xenopus* oocytes demonstrated that the lack of *N*-glycosylation did not affect urea uptake when compared to the wild-type UT-B (Lucien et al., 2002). Consequently, further investigation of the role of *N*-glycosylation on UT-B function is needed.

Implications

Both *N*-glycosylated and non-glycosylated forms of urea transporter-B (UT-B) are expressed in the gastrointestinal tract, kidney, liver, and parotid salivary gland of lambs fed a forage-based diet. Greater expression of the *N*-glycosylated form of UT-B in the ventral rumen in response to daily supplementation with ruminally degradable protein suggests that UT-B may serve an excretory function for urea, rather than recycling of urea into the gastrointestinal tract. Nonetheless, further research into the physiological significance of *N*-glycosylation in the regulation of UT-B function is needed.

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Table 2. Effect of ruminal protein degradability and frequency of supplementation on the abundance of urea transporter-B protein (in arbitrary densitometry units) in the gastrointestinal tract and related tissues in lambs fed a forage-based diet.

	Treatment[1]					
Item	RDP-D	RDP-A	MIX-A	RUP-A	SEM	$P <$[2]
Reticulum						
32 kDa	2.495	2.207	2.314	2.072	0.405	0.90
47 kDa	0.460	0.384	0.349	0.348	0.085	0.76
Ratio[3]	5.406	6.617	6.631	6.568	1.071	0.81
Dorsal Rumen						
32 kDa	2.096	1.632	2.101	2.069	0.345	0.72
47 kDa	0.266	0.239	0.252	0.182	0.029	0.24
Ratio	8.717	6.834	8.427	6.834	2.154	0.41
Ventral Rumen						
32 kDa	2.780	1.902	1.790	1.317	0.515	0.30
47 kDa	1.085[a]	0.530[b]	0.358[b]	0.298[b]	0.166	0.029
Ratio	2.890	3.640	5.353	4.639	1.163	0.44
Small (Spiral) Colon						
32 kDa	0.563	0.685	0.751	0.502	0.237	0.85
47 kDa	3.564	3.595	3.315	2.867	0.375	0.52
Ratio	0.160	0.205	0.214	0.173	0.058	0.89
Large Colon						
32 kDa	0.105	0.069	0.042	0.038	0.026	0.29
47 kDa	1.883	2.013	2.162	1.383	0.245	0.18
Ratio	0.056	0.034	0.020	0.035	0.019	0.58
Cecum						
32 kDa	0.114	0.321	0.117	0.013	0.087	0.16
47 kDa	2.665	1.529	1.744	2.674	0.783	0.62
Ratio	0.040	0.207	0.054	0.007	0.058	0.14
Kidney						
32 kDa	0.038	0.028	0.044	0.028	0.018	0.87
47 kDa	3.452	3.153	3.090	2.726	0.575	0.85
Ratio	0.010	0.009	0.014	0.009	0.005	0.83
Liver						
32 kDa	8.157	8.513	8.563	8.513	1.841	0.99
47 kDa	0.739	0.739	0.648	0.284	0.361	0.72
Ratio	12.897	37.914	132.178	56.847	105.522	0.74
Parotid Salivary Gland						
32 kDa	0.087	0.081	0.091	0.079	0.029	0.99
47 kDa	1.669	1.669	1.683	1.793	0.225	0.90
Ratio[3]	0.046	0.050	0.052	0.043	0.017	0.98

[1]Treatments consisted of RDP-D = high ruminally degradable protein (RDP) fed daily; RDP-A = RDP fed on alternate days; RUP-A = high ruminally undegradable protein (RUP) fed on alternate days; MIX-A = 50:50 mixture of the RDP and RUP supplements fed on alternate days. .

[2]*P*-value of differences between treatments.

[3]Ratio = 32 kDa/47kDa.

[ab]Means with unlike superscripts within row are different ($P \leq 0.05$)

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

FEEDING VALUE OF CORN AND 'VALIER' BARLEY FOR FINISHING STEERS

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ABSTRACT: Eighty crossbred steers (average initial weight 436 ± 3.9 kg) were used to evaluate the performance, nutrient digestibility, and grain energy content of finishing diets based on corn or 'Valier' barley. Barley was dry rolled prior to being fed and diets were formulated to be isocaloric (2.04 Mcal/kg NE_m and 1.43 Mcal/kg NE_g) and isonitrogenous (2.6% N). Diets were formulated (DM basis) to contain 80% grain, 6% straw, 3% soybean oil, and 11% vitamin/mineral supplement. Steers were weighed at the beginning and end of the 98-d study. Diet, ort, and fecal samples were collected on d 41, 68, and 98. Acid insoluble ash was used as an internal marker to estimate fecal output and calculate apparent nutrient digestibility. Steers were slaughtered when 70% were visually estimated to grade Choice and carcass measurements were collected. Weight and carcass data were analyzed using the GLM procedure of SAS with pen as the experimental unit. Means were separated using LSMEANS when $P < 0.10$. Average daily gain did not differ ($P = 0.94$) between steers fed finishing diets based on corn and Valier, averaging 2.0 kg/d. Gain:feed did not differ ($P = 0.77$) between steers fed Valier- or corn-based finishing diets. Backfat was lower ($P = 0.02$) from carcasses of steers fed Valier compared to carcasses from steers fed corn (1.24 vs 1.09 cm). Ribeye area tended to be smaller ($P = 0.13$) from carcasses of steers fed Valier compared to carcasses of steers fed corn (80.3 vs 78.5 cm^2). All other carcass characteristics did not differ ($P > 0.27$) between treatments, averaging 341 kg hot carcass weight, 1.9% KPH, Yield Grade 3, and Choice- Quality Grade. Steers consuming Valier had higher ($P < 0.001$) DM and ADF intake; but lower ($P < 0.01$) N and starch intake compared to steers consuming corn. Dry matter and ADF digestibility were lower ($P < 0.001$) in steers fed Valier than steers fed corn; however, starch digestibility was greater ($P = 0.005$) in steers fed Valier compared to steers fed corn. Nitrogen digestibility did not differ ($P = 0.91$) between treatments. There was no difference ($P > 0.24$) in grain NE_m and NE_g between Valier and corn. These data suggest that Valier barley and corn have similar feeding values when fed as finishing diets in the feedlot.

Key Words: Barley, Corn, Finishing steers

Introduction

Starch digestion in the small intestine has been estimated to provide 42% more energy than starch digestion in the rumen (Owens et al., 1986). A barley variety with lower DMD could shift more of the starch digestion from the rumen to the small intestine, in effect making barley more like corn in site of digestion (Bowman et al., 2001). Valier is a new barley variety released by the Montana Agricultural Experiment Station with lower DM, starch, N and ADF digestibility compared to corn (Kincheloe et al., 2003). However, in other studies Valier had lower DM and ADF digestibility, but similar starch and N digestibility compared to corn (Grove et al., 2006a,b). Furthermore, energy values for Valier have been similar to (Kincheloe et al., 2003; Grove et al., 2006a) or less than corn (Grove et al., 2006b). The objective of the current study was to further evaluate the performance, digestibility, and energy value of corn and Valier barley using more pens per treatment.

Materials and Methods

Eighty crossbred steers (average weight 436 ± 3.9 kg) were assigned by weight to 16 pens in a completely randomized design. Steers were fed finishing rations based on corn or 'Valier' barley. Chemical composition of the grains is presented in Table 1. Valier was developed from crossing Lewis and Baroness for improved feed quality characteristics and released by MSU. Barley was dry rolled prior to being fed and diets were formulated to be isocaloric (2.04 Mcal/kg NE_m and 1.43 Mcal/kg NE_g) and isonitrogenous (2.6% N). Diets were formulated (DM basis) to contain 83% grain, 6% straw, 3% soybean oil, and 8% vitamin/mineral supplement. Animals were cared for under protocols approved by the Montana State University Animal Care and Use Committee.

Steers were weighed on 2 consecutive days at the beginning and end of the 98-d study. Steers were fed once daily at 0800 and were given ad libitum access to water. Steers were gradually brought up to ad libitum intake of their respective treatment diets over 28 d. Diet, ort, and fecal samples were collected from individual steers and composited by pen on d 41, 68, and 98. Diet and fecal samples were dried in a 60°C forced-air oven, ground through a Wiley mill (1-mm screen), and analyzed for DM (AOAC, 1999), N (Leco Corporation, St. Joseph, MI), ADF (Van Soest et al., 1991), starch (Megazyme, Sidney, Australia), and AIA (4N HCl method; Van Keulen and Young, 1977). Acid insoluble ash was used as an internal marker to estimate fecal output and calculate apparent nutrient digestibility. Grain energy content (NE_m and NE_g) was calculated based on steer average weight, DMI, and ADG using NRC (1984) equations.

Steers were slaughtered when 70% were visually estimated to grade Choice, and hot carcass weights were collected. All other carcass measurements were taken after

a 24-h chill. A USDA grader assigned quality grades and marbling scores.

Weight and carcass data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. Intake and digestibility data were analyzed as repeated measures using PROC MIXED with pen as the experimental unit. Least square means were separated using the Least Significant Difference method when $P < 0.10$.

Results and Discussion

Steer final weights and ADG did not differ ($P > 0.53$) between steers fed finishing diets based on corn and Valier (Table 2). Gain:feed was the same ($P = 0.77$) for steers fed finishing diets based on Valier compared to corn. Backfat thickness was lower ($P = 0.02$) from carcasses of steers fed Valier compared to carcasses from steers fed corn. Ribeye area had a tendency ($P = 0.13$) to be smaller from carcasses of steers fed finishing diets based on Valier barley. All other carcass characteristics did not differ ($P > 0.13$) between treatments. Kincheloe et al. (2003), Grove et al. (2006b), and Iversen et al. (2006) reported similar ADG by cattle consuming finishing diets based on corn and Valier barley. There was no difference in G:F for steers consuming Valier- and corn-based diets according to Kincheloe et al. (2003) and Grove et al. (2006a), but lower G:F in two other studies (Grove et al., 2006b; Iversen et al., 2006). Similar to our data, cattle fed barley based finishing diets had less backfat than cattle fed corn based finishing diets (Miller et al., 1996; Kincheloe et al., 2003). Iversen et al. (2006) reported that steers consuming Valier had a lower percent KPH; however all other carcass characteristics did not differ between treatments.

Steers consuming Valier had greater ($P = 0.001$) DM intake but lower ($P < 0.01$) N and starch intake compared to steers consuming corn. Acid detergent fiber intake was greater ($P = 0.003$) by steers consuming Valier compared to steers consuming corn. Similarly, Iversen et al. (2006) and Grove et al. (2006a) reported greater DMI by steers consuming Valier compared to corn; however, Kincheloe et al. (2003) reported no difference in DMI between steers consuming Valier- and corn-based finishing diets. Similar to our data, Iversen et al. (2006) reported lower N and starch intake, for steers fed Valier- compared to steers fed corn-based finishing diets; however, in contrast to our results, steers consuming Valier had lower ADF intake than steers consuming corn.

Dry matter and ADF digestibility were lower ($P < 0.009$) in steers fed Valier than steers fed corn; however, starch digestibility was higher ($P = 0.005$) by steers consuming Valier compared to steers consuming corn. There was no difference ($P = 0.91$) in N digestibility between diets. Similar to our data, Valier also had lower total tract DM digestibility yet similar starch digestibility compared to corn (Kincheloe et al., 2003; Grove et al., 2006 a; and Iversen et al., 2006). Agreeing with our results, Dion and Seoane (1992) and Boss and Bowman (1996) found higher apparent starch digestibility for barley diets compared to corn. In contrast, Iversen et al. (2006) reported lower N digestibility by steers consuming Valier compared to corn.

There was no difference ($P > 0.24$) in grain NE_m and NE_g content between Valier and corn.

Implications

Dry matter and ADF intake was higher; however, N and starch intake was lower for Valier- compared to corn-based finishing diets. Steers consuming Valier had higher starch digestibility and similar N digestibility compared to steers consuming corn, but lower DM and ADF digestion. This however, did not result in a difference in animal performance between treatments. These data suggest that Valier barley and corn have similar feeding values when fed as finishing diets in the feedlot.

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Table 1. Nutrient composition of corn and 'Valier' barley used in finishing diets fed to steers

Item	Corn	Valier
DM, %	84.7	93.9
N, %	1.33	2.42
ADF, %	2.72	3.82
Starch, %	73.1	56.2
ISDMD, % at 3 h	26.4	28.87
Particle size, um	-	1324
Bulk density, kg/hL	64.4	61.8

[a] ISDMD = In situ DM disappearance

Table 2. Animal performance and carcass characteristics of steers fed finishing diets based on corn or 'Valier' barley

Item	Corn	Valier	SE	*P*-value
Weight, kg				
Initial	435	437	1.3	0.15
Final	605	608	3.7	0.53
ADG, kg	2.02	2.03	0.052	0.94
FE, kg gain/100 kg feed	15.1	14.9	0.40	0.77
Carcass wt, kg	344	338	3.9	0.31
KPH fat, %	1.9	1.9	0.03	0.50
Fat thickness, cm	1.24	1.09	0.045	0.02
REA, cm^2	80.3	78.5	0.90	0.13
Marbling score	429	428	11.3	0.94
USDA quality grade[a]	11.9	11.9	0.10	1.0
USDA yield grade	3.0	2.9	0.07	0.27
Diet NE_m, Mcal/kg	1.98	2.04	0.040	0.41
Diet NE_g, Mcal/kg	1.33	1.4	0.04	0.41
Grain NE_m, Mcal/kg	2.06	2.16	0.060	0.24
Grain NE_g, Mcal/kg	1.4	1.5	0.050	0.40

[a] 11 = Select, 12 = $Choice^-$, 13 = $Choice^o$, 14 = $Choice^+$

Table 3. Nutrient intake and apparent digestibility by steers fed finishing diets based on corn or 'Valier' barley

	Treatment			
Item	Corn	Valier	SE	Pr > F
Intake				
DM, kg/d	12.7	13.9	0.18	0.001
N, g/d	310	291	0.005	0.010
Starch, kg/d	6.9	5.9	0.09	0.001
ADF, kg/d	1.3	1.5	0.04	0.003
Digestibility, %				
DM	75.3	69.9	1.30	0.009
N	69.3	69.6	1.60	0.914
Starch	91.9	94.7	0.60	0.005
ADF	49.1	11.6	3.9	0.001

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

USE OF METHYLGLYOXAL AS A TOOL IN PREDICTING RUMINAL NITROGEN STATUS

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ABSTRACT: A three year study was conducted to determine the effects of protein supplementation to cattle grazing dormant winter forage on ruminal production of methylglyoxal (MG). Methylglyoxal is a toxic α-ketoaldehyde produced by rumen microbes in response to excess glucose and insufficient nitrogen (N), as is often the case when ruminants graze dormant forage. Ruminal microbes produce MG through an alternate shunt of the glycolytic pathway. Six ruminally-cannulated mature cows (average BW = 638.33 ± 50 kg) were assigned to two treatments using a completely random experimental design. Treatments consisted of: 36% CP cottonseed meal supplement fed 3x / week (900 g $head^{-1}$ • $feeding^{-1}$) and no supplement (salt and mineral only). Dormant pastures grazed were typical of central NM winter forage (yr 1: 8.2% CP, 75.1% NDF; yr 2: 7.0% CP, 65.4% NDF and yr 3: 6.7% CP, 68.3% NDF; DM basis). Rumen fluid was collected every other week from mid-December to mid-February for a total of 5 sampling days and was analyzed for ammonia, pH, and MG. Treatment did not affect ruminal ammonia ($P = 0.94$), pH ($P = 0.96$), or MG ($P = 0.17$). Year (yr) study was conducted, influenced ammonia ($P < 0.04$), pH ($P < 0.01$), and MG ($P < 0.01$) concentrations. Ruminal pH values were different ($P < 0.01$) for yrs 1, 2, and 3 (5.89, 6.33, and 5.59 ± 0.096, respectively). Ruminal ammonia in yr1 was 57.0% higher ($P < 0.01$) than yr2 and 61.7% higher ($P < 0.01$) than yr3. Methylglyoxal was 55.6% higher ($P < 0.01$) in yr2 than yr1 and 48.8% higher ($P < 0.01$) in yr3 compared to yr1. Ruminal MG differed by day ($P < 0.01$) and was lower for collection 1 than 5 (0.87 vs. 2.27; $P < 0.01$). These data indicate that yearly forage quality is important for ruminal bacteria metabolism. In yr 1 carbohydrate and N were better balanced compared to yrs 2 and 3. This was supported by greater MG production in year 1 vs. yrs 2 and 3. Additionally, MG production differed by day which may be indicative of MG being a sensitive indicator of declining forage quality during the grazing season.

Keywords: Methylglyoxal, cattle, carbon:nitrogen ratio

Introduction

The ruminal ammonia pool is the most common method of assessing ruminal N availability in the ruminant model. This pool is variable in concentration, and susceptible to constant inputs and outputs of nitrogen. Because of this, the ammonia pool may not give an accurate nitrogen value in regards to the nitrogen needs of the ruminal microbes.

Methylglyoxal (MG) is a dicarbonyl compound (Staniszewsha and Nagaraj, 2005), more specifically, a toxic α-ketoaldehyde, which is produced as a by-product of glycolysis in most living organisms (Chaplen et al., 1996). Methylglyoxal is produced via the MG shunt of glycolysis as described by Russell (1998). Methylglyoxal production is a form of energy spilling utilized by the ruminal bacteria as a survival mechanism. Energy spilling is based around the ATP pool. When ATP levels exceed what is needed for anabolic reactions such a protein synthesis; or other microbial requirements, additional ATP is spilled and not utilized by the bacteria. When N is limiting, anabolic reactions are greatly reduced, thus ATP within the pool is more abundant and bacteria are more apt to spill energy (Ferguson et al., 1998; Bauchop and Elsden, 1960). Unfortunately, if glucose levels do not normalize, MG buildup will ultimately lead to bacterial lysis (Ferguson et al., 1998).

Ruminants may produce MG when fed high concentrate diets, or in the case of the current study, diets high in dormant forage with insufficient N. Both scenarios create conditions in the rumen with carbohydrate being in excess and inadequate protein (N) in relation to the needs of the microbial population. In addition to production of MG via glycolysis, this toxin may result from amino acid breakdown and/or metabolism of fatty acids in the tissues (Beisswenger et al, 2005).

The objective of this study was to determine if MG production could be used as a predictor of nutrient imbalance when cows grazed dormant range in New Mexico.

Materials and Methods

A three year study was conducted at the Corona Range and Livestock Research Center from December 2004 through February 2007. Six mature ruminally cannulated cows (average BW=638.33 ± 50kg) were randomly divided into two groups and assigned to one of two treatments. Treatments consisted of supplemented cows, fed a 36% CP cottonseed meal based supplement 3X•week (900g•$head^{-1}$•$feeding^{-1}$), or a non supplemented group that were not fed supplement in addition to the basal dormant forage diet. Treatment groups were placed in separate pastures of similar size and forage composition. Available forage for consumption was dormant and typical of central NM winter forage (yr 1 & 2: 8.2% CP, 75.1% NDF, and 40.9% ADF; yr 3: 6.7% CP, 68.3% NDF, 42.7% ADF; DM basis). Rumen fluid was collected from cows every other week from mid December to mid February, each year. Rumen fluid was analyzed for ammonia, pH, and MG, as well as VFA levels.

Ammonia samples were analyzed using the phenol–hypochlorite procedure of Broderick and Kang

(1980), adapted to a microtiter plate. Volatile fatty acid concentration was determined by gas chromatography (Star 3400, Varian, Walnut Creek, CA) utilizing the methods of May and Galyean, (1996). Methylglyoxal content was analyzed using HPLC (Agilent 1100 Series, Agilent Technologies, Santa Clara, CA) following the procedures of Lodge-Ivey et al. (2004). Methylglyoxal samples were derivatized with 6-hydroxy-2,4,5-triaminopyrimidine to produce 6-methylpterin, which was quantified using a fluorescence detector.

Statistical analysis was performed using the MIXED procedure of SAS. The model included year, treatment, and day of sample collection and all possible interactions. The error statement used was animal nested within treatment, and the covariance structure was compound symmetry. The observed level of significance was set at $P < 0.05$.

Results & Discussion

Ruminal pH was different each year of the study ($P < 0.01$). Average pH values for yr 1 (5.89 ± 0.09), yr 2 (6.33 ± 0.01) and yr 3 (5.59 ± 0.10) were lower than those of Mertens (1977), in which it was suggested fiber digestion is inhibited at a pH lower than 6.7. The reduced pH in the current study may be due to the cows consuming a dormant forage diet, with a slow rate of passage leading to acetate accumulation in the rumen, causing an increased organic acid load. The accumulation of acetate may be due to the reduced capacity of acetate to be absorbed across the rumen wall.

Ruminal ammonia, also displayed a difference by year ($P < 0.01$). Values obtained from ammonia analysis were below those reported by Satter and Slyter (1974). These authors reported that approximately 3mM of ammonia was required for maximal microbial protein synthesis. Our data indicates that only in yr 1 were ruminal ammonia levels adequate (3.18 ± 0.42mM) for microbial protein synthesis while ammonia levels measured in yrs 2 and 3 were less than favorable (1.37 ± 0.52mM and 1.22 ± 0.45mM, respectively). Ammonia in year 1 was 57.0% higher ($P = 0.0085$) than yr 2 and 61.7% higher ($P = 0.0022$) than yr 3.

Thus, supplementation 3x•week may not have been sufficient to correct the nutrient imbalance and provide adequate nitrogen for maximal microbial protein synthesis, hence, no treatment affect was observed for ammonia. Within year, ammonia samples were not different from the first through the last sample date ($P = 0.68$). Mean values of ammonia in yr 1 attest that, ammonia was supplied in great enough quantities to possibly satisfy the needs of the microbial population. Lack of change in ammonia values, gathered between yrs 2 and 3 reveal that N supplied by the protein supplement was rapidly utilized by the microbes, thus no changes amongst values were observed. Furthermore, although the rumen micro-flora utilized supplement provided N, there was not enough supplied to meet their requirements and for ammonia values to rise noticeably.

Analysis of total VFA concentration, which serves as an indicator of ruminal fermentation, showed no effect for treatment ($P = 0.94$) or year ($P = 0.98$). However, a day x year interaction was detected ($P < 0.01$). Total VFA concentration within yr 1 and 3, decreased from the first sample date to the last sample date approximately 59% and 67%. These results are comparable to those of McCollum (1983), who showed that VFA concentration tended to decline from late August through January. Year 2 was different however, with total VFA concentration increasing from the first to last sample day by roughly 75%. This could be due to the relatively mild, open winter and abnormal precipitation experienced during the second year. This anomaly may have actually increased feed quality and propionate production from the forage.

Methylglyoxal showed a difference for year ($P < 0.01$), with yr 1 being inconsistent with yr 2 ($P < 0.01$) and 3 ($P < 0.01$), however yr 2 and 3 were similar ($P = 0.32$). Average values of MG ranged from 0.65 mM–2.27 mM. Prior research by Russell (1993) indicated that 1.0 mM of MG inhibited growth and decreased viability in ruminal bacteria. Ruminal MG was 55.6% higher ($P < 0.01$) in yr 2 than yr 1 and 48.8% higher ($P < 0.01$) in yr 3 compared to yr 1. Methylglyoxal production differed depending on sample day ($P < 0.01$), no noticeable change occurred amongst sample days 1 through 4 ($P > 0.10$), but sample day 5 was different from all other sample days ($P < 0.01$).

It is possible that the results obtained during the duration of this experiment, were in part due to precipitation patterns. Year 1 was a dry, droughty year, not uncommon of central NM. Crude protein levels in yr 1 were greater (8.2%) than in yrs 2 and 3 (6.7%). It appears that the nutrient composition of the forage in yr 1 better fit the needs of the rumen bacteria than in yrs 2 or 3. This is indicated by lower methylglyoxal levels produced (0.65 ± 0.12mM) in yr 1.

Also noted, was the fact that MG production was low in yr 1, while ammonia concentrations were approximately 5x greater. Years 2 and 3 saw slightly elevated MG levels over recorded values for ammonia. This further attests that when protein was supplemented and a nutrient balance was established, MG production was minimal. Conversely, when forage quality was low such as in yrs 2 and 3 the amount of protein supplemented was not enough to meet microbial N requirements and correct the nutrient imbalance.

Ammonia values were not different across sample day, and suggest no change in diet quality. Methylglyoxal values suggest otherwise. The difference associated with MG for sample day suggest that as the winter progressed and forage quality decreased, that possibly nutrient imbalance broadened. From this potentially broadened imbalance of protein and carbohydrate, MG production increased. Change in MG by sample date is testament that MG may be more sensitive in measuring ruminal microbial available N than ammonia alone.

It is necessary to maintain a healthy, functional bacterial population in the rumen for an efficient rumen environment and maximal performance of the host animal. The need to keep an ever watchful eye on energy:protein balance, is crucial in alleviation of MG production. Strategic supplementation of protein may be essential for cows grazing dormant range, in order to maximize bacterial

potential, and improve overall nutritional status of the animal.

Implications

These results indicate MG production is associated with a nutrient imbalance of carbohydrate and protein. Further research is necessary to determine the origin of MG in the ruminant model. More information regarding the conditions conducive to MG production and MG's improved sensitivity, may potentially lead to development of a cost effective, producer friendly method of MG quantification. This would be advantageous to the producer, offering yet another tool to aid in diet and supplement formulation, which could augment their bottom line.

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Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

IN SITU RATES OF INSOLUBLE MACRO-MINERAL RELEASE FROM ALFALFA AND TROPICAL GRASSES, AND RELATIONSHIPS WITH DRY MATTER DISAPPEARANCE

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Introduction

In most tropical areas of the world, particularly in undeveloped countries, the primary source of nutrition for cattle and other ruminants is forage, which is harvested by the animal as live material, as opposed to feed which is harvested by the farmer and provided to the animal. In these areas it is simply not economically practical to provide any large degree of supplementation to the animals. A similar situation can be found in Hawaii. Although it is well-developed, because of its geographical isolation the importation and delivery of supplements to the animals is often considered impractical.

This is in contrast to more well-developed, temperate regions of the world where supplements can be produced locally or imported at lower cost. This inability to supplement is compounded by the fact that tropical grasses are higher in fiber and lower in protein and other nutrients than temperate grasses, and nutrients present are often less available to the animal than in temperate grasses. It has been well-documented that animals on tropical grasses typically underperform animals on temperate grasses (Kaiser and O'Neill, 1975; Minson and McLeod, 1970; Stobbs, 1971).

In other warm-climate areas, mineral deficiencies have been blamed for poor production of beef cattle (McDowell, 1985). There are extensive records in the literature documenting deficiencies, as well as toxicities, of minerals in both soils and plants (McDowell, 1997, 1985). Therefore, it is important to be aware of the mineral status of the animals. Often, only soil and/or plant levels of minerals are assessed in making a decision to supplement, and the level of a mineral in the soil or plant does not necessarily reflect what is available to the animal. Additionally, mineral supplements are often expensive and could lead to excess excretion and/or poor utilization by the animal. Unnecessary supplementation can have a dramatic impact on both economic returns and environmental sustainability, in addition to leading to toxicities. To both ensure adequate nutrition and eliminate unnecessary supplementation, it is necessary to know the levels of minerals present in the forages being grazed and the bioavailability to the animal.

Previous *in situ* trials show that there are differences in rate and extent of mineral release in the rumen between forages (Emanuele and Staples, 1990; Emanuele et al., 1991). Therefore, it is dangerous to draw general conclusions from limited studies and it becomes necessary to determine the rate and extent to which minerals are released from each individual forage in question.

It is also of interest how the rate of overall dry matter disappearance tracks with the rate of macromineral release in the rumen. It may be the case that dry matter disappearance is much more rapid and complete than mineral release.

The objectives of this research were to determine the levels and rates of insoluble macrominerals released from tropical forages in the rumen, and their relationship to DM disappearance.

Materials/methods

Forage and digesta residue samples from previously conducted *in situ* rumen digestion studies were analyzed for macromineral (Ca, P, Mg, K, Na, and S) levels.

Animals

Fistulated steers from the Mealani Research Station on the Big Island of Hawaii were used for these studies. All trials to assess the various grasses used either 2 or 3 steers and most of the studies were conducted at the Waialee Livestock Research Farm on Oahu. All animals were kept on an alfalfa hay/cube diet. Prior to the trials, a trace mineral salt mix was available, but this was removed during the trials.

Grasses

Seven grasses were analyzed in this study, including kikuyu grass (*Pennisetum clandestinum*), fresh and hay, California grass (*Brachiaria mutica*) hay, guinea grass (*Panicum maximum*) hay, green panic grass (*Panicum maximum* var. *trichoglume*) hay, napier grass (*Pennisetum purpureum*), pangola grass (*Digitaria eriantha*), fresh and hay, and pearl millet (*Pennisetum glaucum*). Alfalfa (*Medicago sativa*) was also analyzed. The grasses and alfalfa were harvested with a sickle bar mower and both the fresh and dried hay samples were chopped so that average particle length was <16mm.

Incubation procedure

Enough sample of each feed to provide for 10-15 g of dry matter was inserted into a nylon bag. Each feed was run in duplicate per steer. The duplicate bags were clamped together then inserted into the steer and tied to a 1 kg stainless steel weight. The bags were incubated for 0, 4, 8, 12, 24, 36, 48, 72, and 96 hours in the rumen. Only the 0, 12, 24, 48, and 96 h samples were assayed for mineral content. Bags were rinsed several times with warm water and frozen upon removal from the rumen. At time of analysis samples were thawed, dried in a forced draft oven (50oC), and ground through a 1mm stainless steel screen in a Thomas Wiley mill.

Mineral Analysis

Samples were assayed for mineral content using inductively coupled plasma-emission spectroscopy at the Louisiana State University Soil Test and Plant Analysis Lab. Duplicates for each animal were combined for mineral analysis.

Calculations and Statistical Analysis

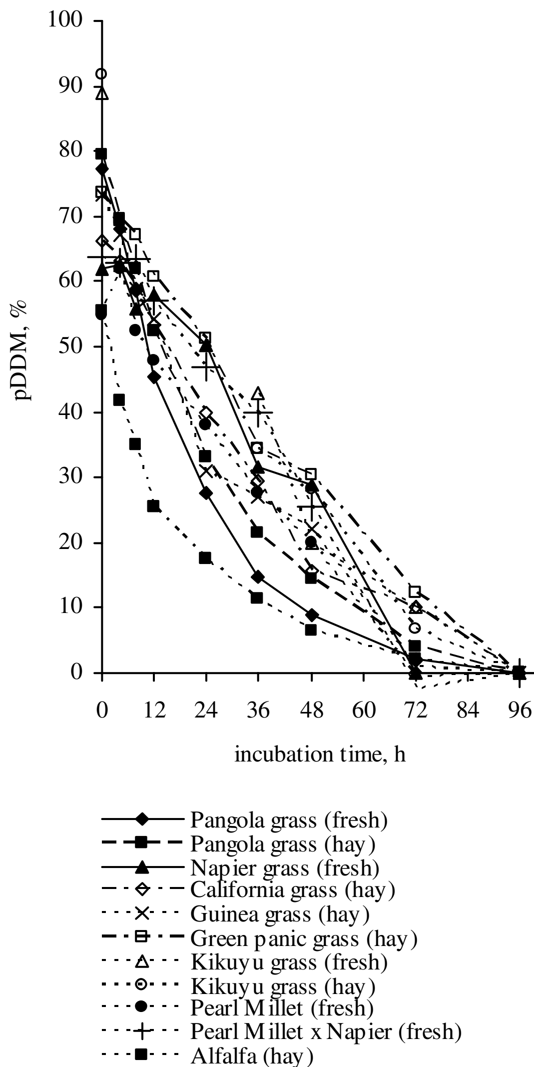
The fractional rate of disappearance was calculated from the slope of the equation ($y=k_Dx+c$) relating the natural logarithm of the proportion of mineral remaining in the bag (y) to time of incubation (x), as demonstrated by Rooke et al. (1983). Treatment effects were evaluated by ANOVA and treatment means with compared by Duncan's multiple range test. Statistics were done using SAS 9.1.

Results

Dry matter disappearance

Figure 1 shows the rate of dry matter disappearance of the feeds. All the feeds displayed similar patterns of dry matter disappearance. Alfalfa hay shows a faster initial rate of dry matter digestion than the grasses; by 48 h only 6% of

Figure 1. Dry matter disappearance of forages.



potential digestible dry matter (**pDDM**) remains. Dry matter was released faster than magnesium and sodium, at the same rate as sulfur and calcium, and more slowly than phosphorus and potassium ($p \leq 0.05$).

Mineral Release

The pattern of mineral release of the different grasses is shown in Figure 2.

Calcium. There is little difference in the pattern of calcium release of the feeds. With the exception of alfalfa (51% release), there was little calcium release at 12 h, and a small amount of calcium appears to have been sorbed (percent recoveries range from 88% in pangola hay to 130% in napier grass). From this point there is a steady decrease in calcium recoveries until 96 hours (percent recoveries range from 14% in alfalfa hay to 49% in napier grass).

Phosphorus. There is an obvious difference in the patterns of phosphorous release in the test feeds. Napier grass and california grass hay sorbed phosphorous (96 h recoveries of 132 and 165%, respectively), while guinea grass hay, pearl millet, and pearl millet x napier exhibited very little phosphorous release (97-101% recovery after 96 hours). The other feeds exhibited varying levels of phosphorous release (96 h recoveries ranged from 15% in pangola grass hay to 45% for green panic hay), with the majority of the phosphorous released by these feeds being released within 12 h.

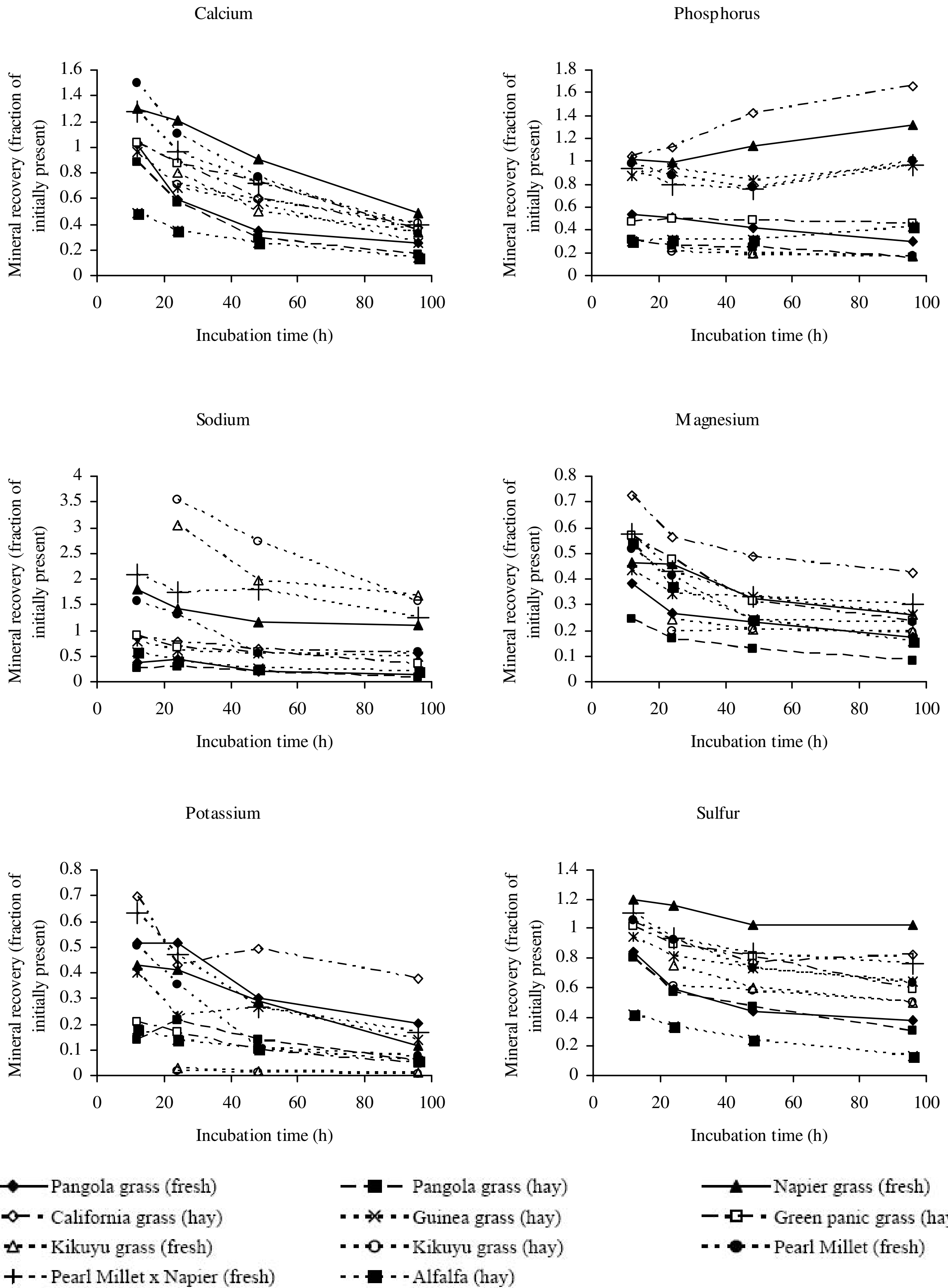
Magnesium. Magnesium, like calcium, showed little difference in the pattern of release between feeds. None of the feeds demonstrated any sorption of magnesium, and, after 96 hours, magnesium recovery ranged from 8% in kikuyu grass hay to 42% in california grass hay.

Potassium. There is more variation over time and within feed in the release of potassium from the test feeds than there is among the other macrominerals. Pangola grass hay sorbed some potassium from 12 to 24 h (an increase of 14 to 22%), while california hay and guinea hay sorbed potassium from 24 to 48 h (43 to 49% and 23 to 27%, respectively). Overall, however, after 96 h all the feeds had released most of the insoluble potassium present at the beginning of the incubation (96 hour recoveries range from 1% in kikuyu hay to 48% in california grass hay). Of particular note is the rapidity with which kikuyu released its potassium; at 36 hours recovery was only 2 and 3%, for hay and fresh grass, respectively.

Sodium. Kikuyu grass (both hay and fresh), napier grass, and pearl millet x napier sorbed large quantities of sodium. Fresh kikuyu and kikuyu hay in particular had 36 h recoveries of 303 and 352%, respectively, while napier grass and pearl millet x napier had a 12 h recovery of 179 and 209%, respectively. All four of these grasses released sodium from that point on, resulting in 96 h recoveries of 168, 156, 111, and 123% for fresh kikuyu, kikuyu hay napier grass, and pearl millet x napier, respectively. The other five feeds released sodium readily and evenly across the incubation, resulting in 96 h recoveries ranging from 8% in pangola hay to 54% in california grass hay.

Sulfur. Napier grass sorbed sulfur as well (12 h recovery of 119%) but released to near initial levels at 96 h (recovery of 102%). California grass hay, green panic hay, and pearl millet x napier also initially sorbed some sulfur (12 h recoveries of 105, 102, and 110%, respectively), but released sulfur from there. Alfalfa released sulfur particularly well, probably owing to high levels of digestible protein. The other feeds released sulfur to varying degrees, with 96 h recoveries ranging from 30% for pangola hay to 82% for california grass hay.

Figure 2. Mineral recovery after ruminal incubation as a fraction of initial insoluble mineral in sample at 0, 12, 24, 48, and 96 h.



Fractional rate of disappearance

Table 1 shows the fractional rate of disappearance ($\mathbf{k_D}$) of minerals and DM for the 11 forages looked at in this paper. Across all feeds, calcium, phosphorus, and potassium had significantly greater fractional rates of disappearance than did DM. The other minerals did not differ significantly from DM.

Discussion

The data presented here make it evident that different feeds vary in their ability to sorb/release the various minerals assayed. Furthermore, within an individual feed, different minerals are released differently.

Playne et al., (1978) looked at the release of minerals from four tropical hays, and found differences between the hays in minerals released, as was found here. However, the rank order of least released to most released mineral is different. Playne et al. found that P<Ca<Na<S<Mg<K, whereas the data here suggest a ranking of Na<Mg<S<Ca<P<K. With the exception of potassium, these data are almost the inverse of those results obtained by Playne et al. The findings in this paper, however, match up well with Rooke et al. (1983) who looked at the release of minerals from grass silages.

Calcium was sorbed in the early stages of the incubation by some of the grasses, although in relatively small quantities. Ibrahim, et al. (1998) also found that calcium was sorbed by certain feeds that are low in calcium. They found that guinea grass and rice bran had calcium recoveries of 315 and 455%, respectively, after rinsing in water.

Phosphorous was sorbed by napier grass and california grass, as well. Of the grasses that had a net release of phosphorous after 96 h, only four of the grasses released greater than 70% of the initially insoluble phosphorous.

In the same study, Ibrahim, et al, (1998) found that, in grasses, 55-78% of the magnesium was released during ruminal incubation. The data obtained in this study ranged from 58-96%, and are roughly in line with the data obtained by the above authors. Potassium was released to a similar extent as magnesium. Half of the feeds released more then 70% of potassium after 48 h. However, within 96 h, only california grass hay released less than 80% of the total insoluble potassium.

Sodium was not as thoroughly released as other minerals and there was a large amount of sorption by napier grass and kikuyu grass (both fresh and hay). Only pangola grass (fresh and hay) released more than 80% of the insoluble sodium, and of the remaining grasses, only guinea grass hay released more than 50%.

Sulfur was sorbed by napier grass and small quantities early in the incubation by kikuyu and green panic hay. The remaining grasses released 18-70% of the insoluble sulfur after 96 h of incubation. There does not appear to be a discernible pattern of mineral release among the feeds and minerals tested, and, therefore, it may be necessary to test a given grass and/or method of processing individually in order to accurately determine the extent to which a given mineral will be released.

Fractional rate of disappearance of minerals is much lower than that found by Rooke et al. (1983), which is not surprising given the nature of the feeds involved. They also found no significant difference between minerals in the fractional rate of disappearance of the slowly released (initially insoluble) fraction.

Incomplete washing of samples may have lead to bacterial contamination of the samples assayed via retention on particulate matter in the rumen. There is also the matter of elemental flux in steers during rumination. Because these were live animals, the recycling of mineral elements via the saliva could not be controlled.

The data presented here are not the entire picture as far as mineral availability to livestock is concerned. This paper concerns itself only with the insoluble portion of the minerals, and, in most feeds, the largest part of the minerals are immediately soluble in the rumen. Also, this paper does not consider the impact of abomasal digestion on mineral release and further investigation will be necessary to determine the complete picture of mineral availability of tropical grasses.

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Table 1. Fractional rate of disappearance of DM and macrominerals.

Forage	Fractional rate of disappearance (k_D), %/h						
	DM	Ca	P	Mg	K	Na	S
Pangola grass (fresh)	1.29 ± 0.06[d]	1.55 ± 0.04[c]	1.46 ± 0.11[d]	1.03 ± 0.14[d]	1.47 ± 0.18[ab]	1.81 ± 0.16[de]	1.04 ± 0.11[e]
Pangola grass (hay)	1.36 ± 0.09[d]	2.00 ± 0.07[d]	2.13 ± 0.16[e]	1.49 ± 0.09[e]	2.35 ± 0.33[bc]	2.34 ± 0.33[e]	1.20 ± 0.08[e]
Napier grass (fresh)	0.49 ± 0.03[a]	0.92 ± 0.07[a]	1.16 ± 0.07[bc]	0.34 ± 0.22[ab]	1.78 ± 0.22[abc]	0.18 ± 0.14[ab]	0.07 ± 0.04[a]
California grass (hay)	0.73 ± 0.01[b]	1.13 ± 0.05[ab]	0.79 ± 0.06[a]	0.56 ± 0.02[a]	0.84 ± 0.17[a]	0.63 ± 0.09[bc]	0.27 ± 0.07[ab]
Guinea grass (hay)	0.79 ± 0.06[b]	1.45 ± 0.08[c]	1.06 ± 0.12[b]	0.01 ± 0.05[b]	1.67 ± 0.83[abc]	0.66 ± 0.16[bc]	0.45 ± 0.06[bc]
Green panic grass (hay)	0.88 ± 0.04[bc]	1.16 ± 0.06[b]	1.33 ± 0.03[bcd]	0.55 ± 0.23[c]	2.66 ± 0.36[c]	1.12 ± 0.20[c]	0.57 ± 0.07[cd]
Kikuyu grass (fresh)	0.85 ± 0.04[bc]	1.18 ± 0.06[b]	1.60 ± 0.05[d]	1.72 ± 0.04[e]	4.29 ± 0.10[d]	0.38 ± 0.07[a]	0.73 ± 0.05[d]
Kikuyu grass (hay)	0.73 ± 0.02[b]	0.95 ± 0.08[ab]	1.58 ± 0.06[d]	1.75 ± 0.06[e]	4.67 ± 0.14[d]	0.25 ± 0.18[a]	0.70 ± 0.02[d]
Pearl millet (fresh)	0.82 ± 0.08[b]	1.45 ± 0.13[c]	1.36 ± 0.09[cd]	0.01 ± 0.08[b]	2.54 ± 0.18[bc]	0.91 ± 019[c]	0.56 ± 0.06[cd]
Pearl millet x napier	0.49 ± 0.04[a]	1.14 ± 0.03[ab]	1.08 ± 0.04[b]	0.03 ± 0.19[b]	1.74 ± 0.13[abc]	0.18 ± 0.32[ab]	0.35 ± 0.07[bc]
Alfalfa (hay)	1.01 ± 0.01[c]	1.45 ± 0.11[c]	1.37 ± 0.05[cd]	0.11 ± 0.45[b]	2.01 ± 0.28[bc]	1.20 ± 0.22[cd]	1.43 ± 0.03[f]

*Data in the same column with different superscripts are significantly different ($p \leq 0.05$).

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

THE EFFECTS OF SAFFLOWER AND VITAMIN E ON THE FEEDLOT PERFORMANCE OF RAMS DIVERGENTLY SELECTED FOR LAMBING RATE

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ABSTRACT: Sixty-eight Rambouillet ram lambs (average weight of 32 kg ± 5.94 kg and average age of 156 d ± 5 d) were used in a 2 x 2 factorial arrangement of treatments to determine the effect of energy source and level of Vitamin E on feedlot performance. Rams were weighed after an 18 h shrink and randomly assigned to one of 12 feedlot pens. Each pen was randomly assigned to 1 of 4 diet treatment combinations in a completely randomized design. Pen was the experimental unit with either 5 or 6 lambs in each pen. Energy treatments were either a 6% oil ration using whole safflower seeds (**SS**) or a starch based isocaloric and isonitrogenous control (**SC**) combined with either 400 IU (**VE**) or 0 IU (**VC**) of supplemental Vitamin E. All rams started on a 70% roughage, 30% energy concentrate diet. Rams had ad libitum access to water. Salt and mineral were included in both the VE and VC supplements. Diets were pelleted, hand mixed, and placed in self feeders allowing rams ad libitum access to their respective diets. Over an 18 d period, the amount of concentrate in the diets was increased until a finishing diet with 1.79 NEm and 1.16 NEg was achieved. Rams received the finishing diet for 61 d. Rams were weighed at the beginning and end of the trial, as well as when transitioning from the step-up period to the finishing diet. This allowed for the analysis of DMI, ADG, total kg gained, and G:F within the step-up, finishing periods, and for the entire study. No interactions were detected ($P >$ 0.10) between the S and V treatments. Step-up, finishing and entire study ADG, gain, and DMI, and step-up and finishing period G:F did not differ ($P >$ 0.10) for the main effects of energy source or level of Vitamin E. Entire study G:F was less ($P < 0.05$) for both SS compared to SC and VE compared to VC. Neither safflower oil nor supplemental Vitamin E enhanced feedlot lamb performance.

Introduction

Saturated fatty acids are believed to contribute to heart disease (American Heart Association, 2007), which has caused consumers to demand a healthier product that is lower in saturated fat. Therefore, the need for products containing appropriate levels of unsaturated fatty acids has increased. One way to achieve this is by increasing the levels of CLA precursors fed to ruminant animals, resulting in meat products with higher levels of CLA, coupled with an increase in the PUFA of meat (Kott et al., 2003).

Wang and Jones (2004) reported both an increase in PUFA and a decrease in the body fat mass of animals when supplemented with CLA. Also, the anticancer potential of CLA suggests there are benefits of incorporating foods high in CLA into the diet (Kelly, 2001). Recent research (Bolte et al., 2002; Kott et al., 2003; Boles et al., 2005) has focused on using safflower seeds to increase the amounts of PUFA and CLA.

Unfortunately, unsaturated fatty acids are more susceptible to lipid oxidation than SFA, because their double bond structure is more vulnerable to free radical attack (Wiegand et al., 2002). Lipid oxidation indirectly causes changes in surface color, which causes a lack of consumer appeal (Wulf et al., 1995). A possible solution to lipid oxidation is the incorporation of Vitamin E into the diet of animals. Vitamin E, an antioxidant, has been shown to slow the conversion of oxymyoglobin, or normal meat color, to a less desirable metmyoglobin (Turner et al., 2002).

The benefits of CLA to carcass quality have been shown, but little research is available that has investigated the effect on feedlot performance when combining both a product that will increase CLA levels while also increasing Vitamin E. Thus, the objective of this study was to determine the effects of safflower seeds and Vitamin E on feedlot performance.

Materials and Methods

All animal procedures were approved by the Montana State University Institutional Animal Care and Use Committee (AA-041). Lambs were born in April and May at the Red Bluff Research Ranch near Norris, Montana. Lambs grazed on native range with their dams until being weaned in late August at an average age of 128 (± 5) d. Following weaning, sixty-eight Rambouillet ram lambs from three different lines of a long term selection project (Schoenian and Burfening, 1990; Burfening et al., 1993)were randomly selected for use in this study

and moved to the Fort Ellis Sheep facilities near Bozeman, Montana.

Feedlot Procedures: After an 18 hour shrink, lambs were weighed and then assigned to one of twelve feedlot pens in a manner that standardized average pen weight. There were either five or six lambs per pen. Each pen was randomly assigned to a 2 x 2 factorial arrangement of four diet treatments in a completely randomized design. This provided for three pens per treatment combination. Energy treatments were either a 6% oil ration using whole safflower seeds (**SS**) or a starch based isocaloric and isonitrogenous control (**SC**) combined with either 400 IU (**VE**) or 0 IU (**VC**) of supplemental Vitamin E. All rams were started on a 70% roughage, 30% energy concentrate diet for 5-d which allowed rams to acclimate to the trial. Rams had ad libitum access to water. Salt and minerals were included in both the VE and VC supplements. Pelleted diets were hand mixed and placed in self feeders. Rams had ad libitum access to their respective diets. Over an 18 d period, the amount of concentrate in the diets was increased until a finishing diet with 1.79 NEm and 1.16 NEg was achieved. Rams received the finishing diet for 61 d. Random grab samples of the diets were taken after each adjustment of the diets and submitted for laboratory analysis (Table 1). If needed at the end of each feed level, feed refusals were removed, weighed, and recorded. During the finishing period feed bunks were monitored daily and more of the finishing period diet was added to keep bunks constantly full. Random grab samples of the diets were taken during the course of the finishing period. At the conclusion of the finishing period, feed refusals were removed, weighed, and recorded. These refusals were later dried and then used for calculating DMI. Lambs were weighed at the conclusion of the step-up period and at the end of the finishing period.

Statistical Analyses: Data from this project were analyzed using the GLM procedures of SAS (SAS Inst., Inc., Cary, NC). The variables analyzed were total gain, ADG, G:F, and DMI. All variables were analyzed over the entire trial, during the step-up period, and during the finishing period. The experimental unit was pen. Included in the model were the effects of energy source, level of supplemental Vitamin E, and the interaction between energy source and Vitamin E. Line was found to be a non-significant source of variation in preliminary analyses (Kelley, unpublished data) and therefore was omitted from further analyses. Significance level was set at an alpha of 0.10. A covariate of initial weight was used.

Results and Discussion

Total gain and ADG did not differ ($P > 0.11$) between either sources of energy or levels of Vitamin E during the entire study or the step-up or finishing period (Table 2). Results from this trial were supported by Boles et al. (2005), who reported that the level of safflower oil in the diet, tested at 0, 3, or 6% on an as-fed basis, did not alter the ADG of lambs. Furthermore, Bolte et al. (2002) found that the daily gain of lambs didn't differ when comparing lambs fed a control diet, high-oleate, or high-linoleate safflower seeds. Hristov et al. (2005) found that ADG was not affected by feeding either high-oleate or high-linoleate safflower. However, Kott et al. (2003) found that lambs on a safflower diet had higher ADG than lambs on a control diet. Similar to this trial, the trial for Kott et al. (2003) was conducted at MSU Fort Ellis Sheep Research Center and both fed isocaloric and isonitrogenous diets. The study methods of that trial differed from the methods of this trial in that they fed lambs twice daily and adjusted the amount fed based on refusals. Macit et al. (2003) and Lauzurica et al. (2005) reported that Vitamin E supplementation had no effect on ADG. Also, Vitamin E supplementation did not affect the ADG of heifers (Montgomery et al., 2005) or Holstein and crossbred steers (Arnold et al., 1992; Arnold et al., 1993). However, Wulf et al. (1995) found that lambs receiving the highest level of Vitamin E (1000 IU) gained less than lambs receiving an intermediate level of Vitamin E (500 IU).

Gain:feed did not differ ($P > 0.10$) between either energy source or levels of Vitamin E during both the step-up and finishing periods. However, it was lower ($P < 0.05$) for both energy source and level of Vitamin E for G:F over the entire trial. (Table 2). Unlike this trial, where safflower fed lambs had a lower G:F than control lambs, Kott et al. (2003) reported that G:F was greater for lambs fed safflower compared to lambs fed a control diet. Other studies found no differences in G:F, such as Boles et al. (2005), who discovered the concentration of safflower oil in the diet, being 0, 3, or 6%, did not alter G:F. Also, Bolte et al. (2002) reported that feed efficiency did not differ between lambs being fed a control diet, high-oleate, or high-linoleate safflower seeds. Hristov et al. (2005) found that feed efficiency, measured as a gain to feed ratio, was not affected by oil type, being either high-oleate or high-linoleate safflower. In contrast to this trial, Lauzurica et al. (2005) reported that the feed efficiency of lambs was not affected by Vitamin E supplementation. The inclusion of Vitamin E in the diet did not affect the gain to feed ratio of heifers

(Montgomery et al., 2005) or of Holstein steers (Arnold et al., 1993).

DMI didn't differ ($P > 0.51$) between either energy source or levels of Vitamin E during the entire study or the step-up or finishing period (Table 2). Boles et al. (2005) found that the concentration of safflower oil in the diet did not cause differences in DMI. Kott et al (2003) and Van Wagoner et al. (2001) also found that safflower had no effect on daily DMI. The DMI was not affected by supplementation with either linoleic acid or oleic acid safflower oil in a study on finishing cattle by Hristov et al. (2005). However, Mir et al. (1999) reported a difference in DMI, with lambs being treated with safflower having a lower DMI than lambs receiving a control diet. Lauzurica et al. (2005) found that feed intake was not affected by Vitamin E supplementation. The level of Vitamin E concentration did not alter the daily DMI of lambs (Turner et al., 2002). Also, Montgomery et al. (2005) demonstrated no effect by the addition of Vitamin E to the diet of steers on a finishing diet.

Implications

The addition of safflower seed oil and/or Vitamin E to a feedlot diet did not positively impact feedlot lamb performance. In fact, they caused a decrease in feed efficiency over the entire feeding trial.

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Table 1. Analysis of feed fed to lambs during feedlot trial, DM basis

	Alfalfa Pellets	Safflower Seeds	Barley Grain	Vitamin E Supplement	Vitamin Control Supplement
DM (%)	90.74	93.42	90.07	86.09	86.50
CP (%)	20.2	20.4	14.0	22.4	22.3
CF (%)	2.05	45.2	1.98	3.19	2.63
TDN (%)	67.9	127	86.2	74.6	73.9
Vit. E (IU)	--	190	--	402.57	--

Table 2. Estimates of Least Squares Mean for the feedlot performance of Rambouillet ram lambs

	Treatments					*P*-value[2]		
	SSVE[1]	SSVC	SCVE	SCVC	SE	S	V	S x V
Total Trial Gain[3]	19.5	22.7	22.8	23.5	1.11	0.11	0.11	0.29
Finish Period Gain	13.9	14.2	15.5	17.0	1.32	0.12	0.50	0.68
Step-up Period Gain	5.7	8.6	7.3	6.5	1.02	0.81	0.33	0.11
Total Trial ADG[4]	0.25	0.29	0.29	0.30	0.27	0.11	0.11	0.29
Finish Period ADG	0.23	0.23	0.26	0.28	0.02	0.12	0.50	0.68
Step-up Period ADG	0.32	0.48	0.40	0.36	0.06	0.81	0.33	0.11
Total Trial G:F	0.062	0.071	0.072	0.073	0.00	0.03	0.05	0.14
Finish Period G:F	0.200	0.205	0.226	0.248	0.02	0.10	0.48	0.70
Step-up Period G:F	0.023	0.034	0.029	0.026	0.01	0.84	0.37	0.11
Total Trial DMI[5]	1.7	1.8	1.8	1.8	0.04	0.77	0.71	0.77
Finish Period DMI	1.8	1.8	1.8	1.9	0.05	0.62	0.64	0.99
Step-up Period DMI	1.6	1.7	1.7	1.6	0.05	0.51	0.88	0.31

[1] SSVE = Safflower seed and Vitamin E, SSVC = Safflower seed and Vitamin control, SCVE = Safflower control and Vitamin E, SCVC= Safflower control and Vitamin control
[2] P-value for the effect: S= energy source; V = level of vitamin E; S x V = energy source and level of vitamin E interaction
[3] Gain measured in kg
[4] ADG measured in kg . d^{-1}
[5] DMI measured as kg. d^{-1}

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECT OF FEEDING FREQUENCY ON FEEDLOT STEER PERFORMANCE

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ABSTRACT: Two hundred seventy crossbred yearling steers (318.37 ± 7.05 kg initial BW) were utilized at the Southeastern Colorado Research Center to determine the effect of feeding frequency (once vs. twice vs. three times a d) on performance and carcass characteristics. Steers were used in a previous receiving trial and were re-randomized for this feeding frequency trial upon initiation of the finishing phase. Steers were stratified by BW within previous receiving trial and randomly assigned to pens. Pens were then randomly assigned to one of three treatment groups: once daily feeding, twice daily feeding, or three times a day feeding. Steers were fed a standard high concentrate steamed flaked corn finishing ration for 170 d. Pen served as the experimental unit and cattle were harvested at a constant days on feed. Steers were individually weighed at the initiation and termination of the trial and pen or individual weights were obtained approximately every 42 d. Average daily gain was similar for steers fed once or twice per d. However, ADG ($P < 0.03$) and ADFI ($P < 0.04$) were greater in steers fed three times a d as compared to once or twice daily feeding. Feed efficiency was similar for all 3 treatment groups. Steers fed three times per d had a greater ($P < 0.01$) HCW than steers fed once or twice per d. No differences were detected between the treatment groups for USDA quality or yield grade. These data indicate similar performance between feeding once or twice a d; however, feeding three times a day increased ADG, ADFI, and HCW.

Key Words: Feeding Frequency, Feedlot Management, Performance, Steers

Introduction

In most large feedlots, cattle are fed more than once a day. Feeding more often has the assumed benefit of keeping feed fresh, reducing overeating, and improving performance as feed trucks may stimulate the cattle to eat (Schwartzkopf-Genswein, K. 2000). Daily feed intake is favorably related to the health and profitability of feedlot cattle. By determining what effects feeding methods have on intake, producers can determine how many times per day steers should be fed in order to achieve maximum profitability by keeping labor, machine, and feed costs to a minimum.

In a study conducted in Iowa, cattle fed once daily at consistent morning times had higher ADG and better feed efficiencies than cattle fed once in the afternoon or twice daily (Delehant et al., 1996). Moreover, ADFI was similar between feeding frequencies. Cattle fed once daily in the morning had higher dressing percentages and quality grades, larger loineye areas, and less backfat than cattle fed twice daily.

The objective of the present study was to determine what effect feeding once, twice, or three times per day had on overall performance and carcass characteristics of finishing feedlot steers at the Southeastern Colorado Research Center (SECRC).

Materials and Methods

Two hundred seventy crossbred yearling steers (318.37 ± 7.05 kg initial BW) were used in this experiment. Steers were from a previous receiving study at SECRC in Lamar, CO. Upon initiation of this trial (d -1), all steers were weighed, assigned a breed type code, implanted with 200 mg progesterone and 20 mg estradiol; Vet Life, Des Moines, IA, and given an electronic identification tag. Initial data were sorted by weight and all steers greater or less than two standard deviations from the mean initial body weight were removed from the experiment. The lightest steers were then removed to obtain 270 head.

The trial steers were ranked by weight and assigned a random number using the random number function in Microsoft Excel. The lightest 135 steers were assigned using the random number within a successive set of five steers to one of five replicates numbered 1-5. The lowest random number within each set of five was assigned to replicate one while the highest random number within each set was assigned to replicate five. This process was repeated until all 135 light steers were assigned to a replicate. Next, the heaviest 135 steers were assigned additional replicates that were numbered 6-10 using the same process as used for replicates 1-5. The steers were sorted by replicate and breed type and using the random number were assigned to one of three treatment groups: once daily feeding, twice daily feeding, or three times a day feeding. On trial day 0, trial steers were returned through the chute, weighed, tagged with a visual tag, and sorted into one of thirty pens of nine head each.

All steers were fed a finishing diet of steam flaked corn grain, a roughage source, and a urea and limestone based vitamin and mineral supplement. Diets were formulated to meet or exceed all nutrient requirements for finishing steers (NRC, 1996). Feed calls were determined daily prior to the morning feeding. The once a day treatment was fed 100% of their ration starting at 8:00 am, the twice a day steers were fed 60% of their total ration starting at 7:30 am and the remaining 40% of their

ration starting at 1:00 pm, the three times a day steers were fed 34% of their ration starting at 7:00 am, 33% of their ration starting at 10:00 am, and the remaining 33% of their ration starting at 2:00 pm. Orts were collected, weighed, and recorded throughout the trial as feed became spoiled or on weigh days.

Steers were housed in pens measuring 6.10 x 18.29 m and a single automatic waterer was shared between every two pens. Steers were fed in fence-line 3.66 m long concrete bunks (0.31 m/hd) which had a 3.66 m wide 6.10 m long concrete apron adjacent to the bunk to provide a solid area to stand on while eating. Steers were weighed individually on day -1, day 0, day 47 and day 169. Steers were pen weighed on day 83 and day 126.

Steers were harvested after 170 days on feed. On the shipping date, steers were transported approximately 170 miles to a commercial abattoir, and harvested using humane procedures. Trained personnel matched ear tag with carcass identification tag on the day of harvest. USDA carcass grade data were obtained from the harvest plant carcass data sheets.

Statistical Analysis. Statistical analysis of data was preformed using the mixed model procedure of SAS (2003). The model included the fixed effects of treatment, time, treatment x time interactions where appropriate, and initial weight as a covariate to account for the light and heavy weight blocks. Random effects were pen within treatment. Treatment and treatment x period interactions were considered to be significant if $P < 0.05$. Differences among means were separated using linear and quadratic contrasts. All frequency data were tested for significance using chi-square tests, and within-class variances were compared using *F*-tests.

Results and Discussion

Performance. The effects of feeding frequency on performance characteristics are shown in Table 1. All steers had similar initial BW, however, linear effects of feeding frequency on finished weight were significant ($P < 0.05$). Average daily gain ($P < 0.03$) and ADFI ($P < 0.04$) increased linearly with increased feeding frequency.

Gibson (1981) reported similar results, as ADG increased as frequency was increased. Putnam et al. (1961) reported that greater feeding frequency increased ADG in Angus heifer calves.

Feed efficiency was found to be similar for all treatment groups ($P < 0.99$). Goonewardene et al. (1995) reported no differences in ADG or feed efficiency for feedlot steers fed once, twice, or three times per day. However, this contrasts with a summary of findings compiled by Gibson (1981) where improvements in feed efficiency were observed with increased feeding frequency.

A study conducted by Pritchard et. al. (2003) revealed the importance of feed delivery time, as cattle fed once daily in the late afternoon as compared to early morning feeding produced greater daily production efficiencies. Additionally, this study showed that once daily afternoon feeding had similar performance results as twice daily feeding. Therefore it may be that the three times a day feeding treatment had greater performance results than the once or twice a day feeding treatments because they had a greater availability of feed in front of them in the early morning and later afternoon because they were fed first and last of each daily feeding.

Increased feeding frequency has a positive impact on digestibility as greater feeding frequencies have shown to result in increased numbers of protozoa (McAllen, 1987). Increasing the feeding frequency has shown to yield more stable rumen kinetics which has resulted in higher ADG's (Ruiz and Mowat, 1987).

Carcass Characteristics. Effects of feeding frequency on carcass weights and dressing percentage are shown in Table 1. Hot carcass weight for the steers increased linearly ($P < 0.03$) and quadratically ($P < 0.05$) with increased feeding frequency suggesting that HCW was greater for steers fed three times daily as compared with once or twice daily steers.

Thirty four percent of the steers graded USDA Choice and all steers averaged a USDA Select quality grade (Table 2). Average yield grade was 3.29 (Table 3) with no differences between treatment groups. There was a greater percentage of steers in treatment three with abscessed livers (Table 4).

Implications

Results of this study suggest that feeding frequency had a major impact on steer performance at SECRC. Average daily feed intake and ADG were greater for the steers fed three times a day vs. those steers fed once or twice per day. Increased feeding frequencies might result in higher intakes and gains. However, if managed properly, once a day feeding could result in greater end profits. Once daily feeding would reduce labor and equipment operation costs at the Southeast Colorado Research Center and perhaps for other feeding operations. However, at large feedyards if the same number of loads and equipment hours are needed to haul enough feed for the cattle, labor and equipment costs may not necessarily be reduced by once daily feeding.

Acknowledgements: Thanks to Dr. Kristy Dorton and Sandra Gruber for their help with the statistical analysis of the data. Thanks to the staff at the SECRC for their help in conducting and collecting data during this study.

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Table 1. Effects of feeding frequency on performance of finishing steers.*

Trait [a]	Treatment [b]			SEM	Trt P <	Contrast P ≤	
	1	2	3			Linear	Quadratic
Initial weight, kg	317.53	319.09	318.49	7.05	0.99	0.92	0.90
Final weight, kg	592.96	593.37	604.44	3.49	0.05	0.03	0.22
HCW, kg	362.05	360.70	370.88	2.26	0.01	0.01	0.05
Dressing percentage [c]	61.05	60.80	61.37	0.21	0.18	0.29	0.12
ADG, kg	1.63	1.64	1.71	0.02	0.03	0.02	0.22
ADFI, kg DM	9.24	9.27	9.67	0.13	0.04	0.02	0.24
FE	0.18	0.18	0.18	0.003	0.99	1.00	0.95

* A covariate of in-weight was used in SAS analysis
[a] Abbreviations used: ADG = average daily gain (kg/hd/d); ADFI =average daily feed intake (kg/hd/d); FE = feed efficiency (kgs gained/intake).
[b] 1= fed once daily, 2 = fed twice daily, 3= fed three times daily.
[c] Final weights were reduced by 4% to represent a standard industry shrink.

Table 2. Percentage of carcasses within each treatment that qualify for each of four quality grade marketing categories.

Quality grade marketing category [b,c]	Treatment [a]		
	1	2	3
USDA Prime	0.0	0.0	0.0
USDA Choice	31.3	34.5	36.1
USDA Select	63.9	58.3	60.2
USDA Standard	2.4	7.1	3.6
USDA Cutter	1.2	0.0	0.0
USDA Utility	1.2	0.0	0.0

[a] 1= fed once daily, 2 = fed twice daily, 3= fed three times daily.
[b] Quality grade marketing category determined by expert marketing score: USDA Prime = Slightly Abundant^0 to Abundant^{99}; USDA Choice = Small^0 to Moderate^{99}; USDA Select = Slight^0 to Slight^{99}; USDA Standard = Traces^0 to Traces^{99}; USDA Cutter and Utility = ungraded.
[c] $P>\chi^2$ had an overall value of 0.56

Table 3. Percentage of carcasses within each treatment classified into each of five USDA yield grades.

USDA Yield grade [b]	Treatment [a]		
	1	2	3
USDA 1	3.6	4.8	1.2
USDA 2	27.7	27.4	34.9
USDA 3	45.8	45.2	41.0
USDA 4	19.3	19.1	20.5
USDA 5	3.6	3.6	2.4

[a] 1= fed once daily, 2 = fed twice daily, 3= fed three times daily.
[b] $P>\chi^2$ had an overall value of 0.92

Table 4. Percentage of carcasses within treatment assigned to one of four liver scores.

	Treatment [a]		
Liver Score	1	2	3
Liver score 0	91.0	95.9	84.0
Liver score A-	7.7	2.7	10.1
Liver score A	0.0	0.0	2.9
Liver score A +	1.3	1.4	2.9

[a] 1= fed once daily, 2 = fed twice daily, 3= fed three times daily.

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECT OF WATER QUALITY ON HOLSTEIN CALF PERFORMANCE

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ABSTRACT. Water is an essential nutrient in dairy cattle, but its availability and quality has been drastically affected. The effect of water quality on the pre-weaning performance of Holstein calves was evaluated. Thirty Holstein calves with 40 +/-0.94 kg initial average body weight, were randomly assigned at calving time to two experimental groups: Well Water (WW, 6 females and 9 males) drinking water and water utilized to prepare milk replacer came from farm well, and Inverse Osmosis Water (IOW, 7 females and 8 males) where water from well was processed by inverse osmosis. Calves were fed during the first 3 d after calving with colostrum, and a commercial milk replacer (4 L/d), as well as an starter commercial concentrate (ad libitum) from 7 until 56 d of age. Water quality was analyzed monthly; drinking water and DMI were determined daily and individually; dry matter digestibility, crude protein digestibility and digestible protein intake were determined at the end of the experiment; average daily gain and body weight were measured weekly. Drinking water intake, DMI, and animal performance were analyzed by PROC MIXED of SAS. Digestibility data were analyzed using the PROC GLM of SAS. Total dissolved solids and bacteriologic count from water were reduced 93% (1,469 +/-75 vs. 107 +/-31 ppm) and 98% (1,506 +/-1296 vs. 29 +/-39 ufc/100 ml), respectively, by IOW. Water intake was not different (3,554 vs. 3,088 ml; P>0.05), but DMI was 26% increased (676 vs. 500 g/d; P<0.007) in calves of IOW. Dry matter and crude protein digestibility, as well as digestible protein intake were not affected (74.8%, 65.1%, and 0.169 kg, respectively). Daily gain and body weight were improved 22.7% (0.43 vs. 0.33 kg/d; P<0.02) and 10% (64.3 vs. 58.6 kg; P<0.0006), respectively, in calves of IOW. It was concluded that processing low quality water by inverse osmosis removes most undesirable compounds and improves animal performance during pre-weaning phase.

KEYWORDS: Water Quality, Inverse Osmosis, Holstein Calves.

Introduction

Water is an essential nutrient in dairy cattle, but its importance has been commonly forgotten in dairy systems (Hole et al., 2006). Intake of low quality water affects animal health, growth and development, and decrease milk yield (Looper and Waldner, 2002). Water quality has been extensively revised, but there is scarce information regarding water quality and its impact on animal performance (NRC, 2001), particularly in young animals.

The "Comarca Lagunera" is one of the most important dairy areas in México. However, water in this region has a high concentration of total dissolved solids and heavy metals (Wong et al., 2005). Because the negative impact of low quality water is greater in young animals it is convenient to provide dairy producers with alternate technology for improving water quality. Inverse osmosis has shown to markedly reduce total dissolved solids, heavy metals, and microbiologic count (Miller, 2003).

The objective was to evaluate Holstein calves performance during the pre-weaning phase consuming inverse osmosis treated water.

Materials and Methods

The study was conducted at the dairy production unit "18 de Julio" of the Universidad Autónoma de Chapingo, located in Bermejillo, Durango, México. Thirty Holstein calves with 40 +/-0.94 kg initial average body weight, were randomly assigned at calving time to two experimental groups: Well Water (WW, 6 females and 9 males), where drinking water and water utilized to prepare milk replacer came from farm well, and Inverse Osmosis Water (IOW, 7 females and 8 males) where water from well was processed by inverse osmosis.

Calves were fed during the first 3 d after calving with colostrum, and a commercial milk replacer (4 L/d), as well as a starter commercial concentrate (ad libitum) from 7 until 56 d of age.

Water quality (chemistry and bacteria) was analyzed monthly by the SIMAS Laboratory of Torreón, Coahuila, México. Water intake and DMI were determined daily and individually. At the end of the experiment, DM and CP digestibility was determined using indigestible FDA as an internal marker (Penning and Johnson, 1983; Huhtanen et al., 1994). Digestible protein intake was estimated by CP intake and CP digestibility coefficient. Average daily gain and body weight were measured weekly.

Drinking water intake, DMI, and animal performance were analyzed by PROC MIXED of SAS (2005). Digestibility data were analyzed using the PROC GLM of SAS (2005).

Results and Discussion

Water total dissolved solids were reduced 93% (1,469 +/-75 vs. 107 +/-31 ppm) by inverse osmosis treatment of

water (Table 1). This was associated to the removal of most bicarbonates, chlorides and sulfates during water filtering. Total dissolved solids in both water sources were within the allowed salinity limits for dairy cattle (Waldner and Looper, 2002).

Water pH (Table 1) was reduced in water treated by inverse osmosis compared to farm well water (6.6 vs. 7.0, respectively), because salinity was decreased. Water pH values were acceptable, as desirable pH water range is from 6.0 to 8.0 (Waldner and Looper, 2002).

Water nitrate concentration (Table 1) was reduced by inverse osmosis treatment (117 +/-3 vs. 20 +/-0.4 ppm). These water nitrate concentration values are considered with no harmful effects for calves, since a nitrate concentration from 45 to 132 ppm is safe (Waldner and Looper, 2002).

Even though filtering water by inverse osmosis reduced total coliform bacteria (Table 1) by 98% (1,506 +/-1,296 vs. 29 +/-39 ufc/100 ml) and fecal coliform bacteria by 98.6% (1,301 +/-1,210 vs. 18 +/-21.5 ufc/100 ml), cleaned water could affect health and calf performance, since total and fecal coliform counts should be less than 10/L (Waldner and Looper, 2002).

Water intake (Table 2) was not different between treatments. However, calves of WW had a slightly reduction of 13% in water intake compared to calves of IOW (3,088 vs. 3,554 ml; P>0.5). This response probably was associated to the lower salinity of water treated by inverse osmosis, as most of total dissolved solids were removed. This result is in agreement with Solomon et al. (1995) which reported a reduction of 8.5% in water intake of lactating dairy cows drinking salty water (1,479 ppm) vs. desalinated water (441 ppm).

An increase of 26% in DMI (Table 2) was observed for calves of IOW compared to calves of WW (676 vs. 500 g/d; P<0.007). However, other experiments did not show this response when water quality was enhanced by decreasing salinity in lactating dairy cows (Solomon et al., 1995), or by reducing water sulfate content in growing steers (Patterson, 2003).

Dry matter and crude protein digestibility (Table 2) were not affected by improving water quality. However, calves of WW had a slightly higher (P>0.05) digestibility of DM and CP. These results are consistent with Utley et al. (1970) which reported that nutrient digestibility is increased when water intake is limited or restricted.

Intake of digestible CP (Table 2) was not affected by treatment, but calves of IOW had an increase of 9.5% (0.157 vs. 0.172 kg; P>0.05). This was associated with a higher DMI, as CP digestibility was slightly lower in IOW.

The ADG of calves (Table 2) of IOW was higher (P<0.05) compared to calves of WW (0.43 vs. 0.33 kg/d, respectively). Patterson et al. (2002) reported an ADG increase of 40% in growing steers, drinking water with 400 vs 3,100 ppm of sulfates. Also, lactating dairy cows drinking water with 4,41.6 vs 1,479.9 mg/L of salts improved milk yield by 2.1 kg (Solomon et al., 1995) Probably, the improve on ADG by calves of IOW can be explained by the higher DMI, which did not affect digestibility, allowing an improvement in digestible nutrients intake.

Final live weight (Table 2) was higher (P<0.006) in calves of IOW (64.3 vs. 58.6 kg), as a consequence of higher ADG.

Implications

Inverse osmosis treatment enhanced chemical and microbiological parameters of water, allowing an improvement on performance of Holstein calves during the pre-weaning phase.

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Table 1. Chemistry and microbiology of water

Item	WW[1]	IOW[1]
pH	7.0 ± 0	6.6 ± 0
Bicarbonates (ppm)	196 ± 6	34 ± 8
Chlorides (ppm)	115 ± 3	6 ± 3
Sulfates (ppm)	853 ± 13	24 ± 10
Nitrates (ppm)	117 ± 3	20 ± 0.4
Total dissolved solids (ppm)	1,469 ± 75	107 ± 31
Total coliform (ufc/100ml)	1,506 ±1,296	29 ± 39
Fecal coliform (ufc/100 ml)	1,301± 1,210	18 ± 21.5

[1] WW= well water, IOW= inverse osmosis water

All values are an average from three monthly analysis

Table 2. Effect of water quality on animal performance and digestibility

Item	WW[1]	IOW[1]
Water intake (ml/d)	3,088 ± 268[a]	3,554 ± 250[a]
Dry matter intake (kg/d)	500 ± 44[b]	676 ± 42[a]
Average daily gain (kg/d)	0.33 ± 0.03[b]	0.43 ± 0.02[a]
Live weight at 56 d (kg)	58.6 ± 1.2[b]	64.3 ± 1.1[a]
	Digestibility (DM %)	
Dry matter	77.2 ± 1.9[a]	73.0 ± 1.9[a]
Crude protein	68.9 ±3.2[a]	61.8 ± 3.2[a]

[1] WW= well water, IOW= inverse osmosis water

[a,b] means in the same parameter followed by different superscripts are significantly different ($P<0.05$)

Proceedings, Western Section, American Society of Animal Science

Vol. 58, 2007

EFFECTS OF CONJUGATED TANNINS ON FORAGE ENSILING AND *IN VITRO* RUMINAL FERMENTATION

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ABSTRACT: Alfalfa is widely used in rations of dairy cows in the Western United States. Protein in alfalfa is highly degradable in the rumen; in addition alfalfa protein can be degraded via the ensiling process. Condensed tannins have the ability to bind to protein forming a complex that resists ruminal degradation. Pecan shells contain 1% tannins and the internal material contains 26.4%. In 1999, NM and West TX produced about 31.7 million kg of in-shell pecan of which 55% was waste which is a potential feedstuff for ruminants. Information on the effects of condensed tannins from pecan shells on digestibility of protein and amino acids is limited. In this study, pecan shells (PS) and tannic acid (TA) were supplied at 2% PS, 3% PS, 2% TA, and 3% TA (DM) to *in vitro* fermentation of high quality alfalfa (HQ), low quality alfalfa (LQ), alfalfa silage (AS), soybean meal (SBM), and corn. Also, alfalfa was ensiled with 5% PS, 10% PS, and 3% TA. Samples were incubated for 0, 24, and 48h. Treatments had no effects (P>0.05) on pH at end of fermentation. Addition of PS and TA to HQ, LQ, and AS had no effect (P>0.05) on ADF digestibility and concentrations of acetate, butyrate, propionate, valerate, total VFA, total isoacids or acetate: propionate ratio of in vitro buffer concentrations. Addition of PS and TA to HQ, LQ, and AS lowered (P<0.01) CP of residual forage after incubation, IVDMD (P<0.01), NH_4 (P<0.05), isobutyrate (P<0.02), isovalerate (P<0.02), and lactate (P<0.01) concentrations. Adding PS and TA to SBM and corn lowered NH_4 concentrations (P<0.01), IVDMD (P< 0.01) and CP (P<0.05) of the residual forage, also altered acetate, butyrate, isovalerate, and propionate concentrations (P<0.05). Ensiling alfalfa with PS and TA lowered (P<0.05*)* NH_4 concentrations and CP but had no effect (P>0.05) on IVDMD of silage and did not change concentrations of total or individual VFA or acetate: propionate. Adding PS and TA altered in vitro fermentations of HQ, LQ, AS, SBM, and corn. Ensiling alfalfa with PS and TA changed the fermentation characteristics of nitrogen fractions of the silage.

Keywords: tannins, pecan shell, rumen fermentation

Introduction

One way to increase animal performance is feeding a diet that meets the needs of the animal. In ruminants, protein is second only to energy in importance for achieving high performance. Unfortunately, most dietary protein is degraded in the rumen which can reduce its nutritive value by producing excess ammonia nitrogen which is not utilized by the animal and is excreted in urine. Forages provide ruminant animals with protein needed to meet its requirements. Rapid protein degradation in alfalfa is considered one of the limiting nutritional factors. Efficient forage protein utilization occurs when rumen ammonia does not accumulate. When alfalfa is ensiled, plant proteases degrade 50% of alfalfa protein and microorganisms degrade 50% of remaining protein (Brodrick et al., 1990). A potential method to improve nitrogen efficiency is to reduce urinary nitrogen loss. High rate of alfalfa protein being converted to ammonia during silage fermentation results in a reduction of protein and an increase in NPN. This can cause excess rumen ammonia in relation to microbial growth rate (Vagnoni and Brodrick, 1997). Tannin binds protein forming a tannin-protein complex. This complex is slowly degraded in the rumen, and goes to the small intestine as rumen undegradable protein (Hagerman and Butler, 1981; Siebert et al., 1996). Pecan contains condensed tannins that are found in hulls. Pecan shells contain 1% tannins and hulls contain 26.4% tannins (Woodroof, 1967). Therefore a study was conducted to determine the effects of tannins either from pecan shells (PS) or tannic acid (TA) on forage ensiling and *in vitro* ruminal fermentation of several common dairy feedstuff.

Materials and Methods

Rumen fluid was obtained from a 500 kg Angus cow fed *ad libitum* high quality alfalfa hay diet and fitted with ruminal cannula. Rumen fluid was incubated in a water bath at 39ºC for 30 min with continuous bubbling of CO_2 allowing the feed particles to rise to the top of the flask. Particle free rumen fluid was added (ratio 1:5 by volume) to a buffer that was prepared as described by Russell and Martin (1984). Forty mL of each salt was mixed with 0.60 g cestein hydrochloride, 1 mL of 1% resazorine solution and 875 mL of deionized water. Media was autoclaved, cooled under CO_2 bubbling, and 100 mL of 8% Na_2CO_3 (boiled under CO_2) was added. Fifty mL of the total solution were anaerobically added to each bottle containing 0.5 g of feed substrate. Bottles were incubated in a water bath at 39ºC for 0 h, 24 h, and 48 h. All feed substrates were dried and ground to pass through 2 mm. Substrate samples were analyzed for dry matter, organic matter, crude protein, neutral detergent fiber and acid detergent fiber. Pecan shells were analyzed for N and condensed tannins (May and Galyean, 1996). After all fermentations pH was measured directly.

Experiment I. Alfalfa silage (AS) and high quality alfalfa (HQ) were prepared as described above and either pecan shells (PS) (0%, 1%, 2%, 3%, and 4%) or tannic acid (TA) (0%, 1%, 2%, 3%, and 4%) added. Samples were incubated for 0 h and 24 h and centrifuged (Sorvall Refrigerated Centrifuge Model RT 6000, Kendro

Laboratory Products, Newtown, CT; 10,000 x g for 15 min at 4ºC) and stored for ammonia analysis.

Experiment II. Five substrates, Alfalfa silage (AS), high quality alfalfa (HQ), low quality alfalfa (LQ), corn, and soybean meal (SBM) were prepared as previously described and incubated for 0 h, 24 h, and 48 h. Pecan shells (PS) or tannic acid (TA) were added at 0%, 2%, and 3% of substrate weight. After incubation, samples were centrifuged. Supernatant was transferred to polypropylene tubes and stored at -20ºC for later. Supernatant was analyzed for ammonia following a procedure modified for micro analysis as described by Brodrick and Kang, (1980), using 96 wells microplate reader (MRX HD, Dynex Laboratories, Chantilly, VA). Supernatant was analyzed for volatile fatty acids (VFA) by gas chromatography (Star 3400. Varian. Walnut Creek. CA). Supernatant was prepared for VFA analysis by mixing 500 µL of supernatant with 100 µL of metaphsophoric acid (May and Galyean, 1996). In vitro dry matter digestibility (IVDMD) was determined by filtering the residue through a Buckner funnel, with #1 Whaterman filter paper dried and the difference in weight (before incubation and after incubation) was calculated to estimate IVDMD. The residue was then analyzed for N and crude protein was estimated.

Experiment III. Alfalfa silage (AS) samples were previously ensiled by packing in laboratory scale bucket silos with no additive, 5% PS, 10% PS, and 3% TA. After removal from the silo, samples were dried and in vitro fermentations were performed with no additional TA or PS. Samples were incubated for 0 h, 24 h, and 48 h. Samples were analyzed for pH, VFA, IVDMD, and ammonia

Results and Discussion

Experiment I. Tannin doses (0-4% DM) were within a range in which condensed tannins are hypothesized to promote ruminal escape protein (Barry et al., 1986). Alfalfa silage and HQ treated with PS and TA had no effect on ammonia concentrations ($P > 0.05$). The pH averaged 6.7 ± 0.08 for HQ and AS after 24 h of fermentation. Adding PS and TA did not reduce ($P > 0.05$) ammonia concentration. However, numerically lower values were observed for 2 and 3% PS and TA. Therefore, these concentrations were used in experiment 2. Lower ammonia concentrations can indicate less protein was degraded in the rumen or increases utilization of ammonia pool by rumen microbes.

Experiment II. The addition of TA (2 and 3%) to HQ decreased ($P < 0.05$) ammonia concentrations compared to control at 24 h and 48 h, and addition of PS was similar to control. For AS samples at 24 h, 3% PS and 3% TA increased ($P < 0.05$) ammonia concentrations and 2% PS increased ammonia concentrations at 48 h (Table 1). Addition of 3% TA to SBM decreased ($P < 0.05$) ammonia concentrations at 24 h and 48 h . Ammonia concentrations in corn fermentations were similar ($P > 0.05$) among treatments at 0, 24, and 48 h of fermentation (Table 1). Lowering ammonia concentrations with PS and TA could indicate tannins binding to protein forming an insoluble complex, decreasing protein degradability. Barry et al., (1986) reported that tannin-protein complex resist ruminal degradation and dissociated in the small intestine. The IVDMD decreased ($P < 0.05$) with addition of 3% PS and 3% TA for 24 h and 48 h fermentation of LQ compared to control. The IVDMD decreased ($P < 0.05$) with 3% TA for HQ at 0 h fermentation. For AS, IVDMD decreased ($P < 0.05$) at 3% TA for both 24 h and 48 h fermentations compared to control. The SBM IVDMD decreased ($P < 0.05$) with 2% TA, 3% TA, and 3% PS at 24 h and 48 h compared to control (Table 1). Miller and Ehlke (1994) reported that condensed tannins reduced ruminal protein degradation with little reduction in dry matter digestibility. Tannins may decrease IVDMD because tannins are firmly bound to cell wall and cell protein forming a less digestible complex and decreasing digestibility (Reed, 1995). Addition of PS and TA to fermentation of HQ, LQ, and AS had a variety of effects on CP of the undigested fraction of forage ($P < 0.01$). The 48 h LQ fermentations were lower ($P < 0.05$) in CP for the 3% PS treatment. The CP at 24 h fermentation of HQ was higher ($P < 0.05$) with 2% Ta and 3% TA compared to control. In 24 h fermentations of AS, addition of 2% PS lowered ($P < 0.05$) CP while CP increased ($P < 0.05$) in 48 h fermentation of AS with 2 and 3% TA (Table 1). Tannins protect protein from enzyme hydrolysis by binding to the protein or free amino acids and rendering them inaccessible for enzyme hydrolysis (Hagerman and Butler, 1981; Barry, 1989). In the current experiment, addition of TA or PS reduced in vitro degradation of SBM. This agrees with data presented by Makkar et al., (1995).

Experiment III. At 24 h of fermentation of AS, 10% PS and 3% Ta had lower ($P < 0.05$) ammonia concentrations than the control. At 48 h, the 10% PS treatments had the lowest ($P < 0.05$) ammonia concentrations and the 5% PS had the highest ($P < 0.05$) concentration (Table 2). At 48 h of fermentation the 5% PS and 10% PS were lower ($P < 0.05$) in CP while 3% TA was not different from the control. Ensiling alfalfa with PS and TA had no effects on IVDMD and did not change ($P > 0.05$) concentrations of total or individual VFA and acetate:propionate ratio (Table 2).

Implications

Addition of PS and TA to in vitro ruminal fermentation of forage and concentrate, even at low levels used in this study, altered ruminal fermentations. Changes in protein degradation, VFA concentrations, and IVDMD were seen. Addition of PS and TA to feed may reduce protein degradation in the silo or rumen. This reduction may be accompanied by a reduction in the dry matter and fiber digestibility when PS and TA are added to the diet. Tannins could be fed at low concentrations; this will protect protein from degradation while having minor effects on microbial fermentations. Pecan shells can be used as feed additives without adversely affecting ruminal fermentation or digesta kinetics. Further research is needed to define the use of pecan shells as a tannin source in feeding ruminant animals.

Table 1. Effect of pecan shell (PS) and tannic acid (TA) on ammonia concentrations (NH_4, mM), in vitro dry matter digestibility (IVDMD %), and crude protein (CP %) of *In Vitro* mixed culture fermentations of forages and concentrates.

	Time[1]															
	0 h					24 h					48 h					
Sample[2]	Con[3]	2% PS	3% PS	2% TA	3% TA	Con	2% PS	3% PS	2% TA	3% TA	Con	2% PS	3% PS	2% TA	3% TA	SE(n=2)
LQ																
NH_4	4.85	4.80	4.87	4.66	4.73	5.34	5.59	5.77	5.26	6.01	5.33	5.48	5.69	4.73	5.20	0.54
CP%	2.49	2.59	2.50	2.46	2.42	3.19	3.28	3.81	3.29	3.20	3.76^{a}	3.42^{ab}	2.57^{b}	3.67^{ab}	3.58^{ab}	0.40
IVDMD%	37.19	37.83	37.43	37.63	38.90	42.50^{a}	40.54^{abc}	36.14^{bc}	41.14^{ab}	35.35^{c}	55.87^{a}	51.55^{ab}	49.84^{b}	50.59^{ab}	43.93^{c}	1.96
HQ																
NH_4	4.97	5.15	4.66	4.88	4.95	12.93^{a}	13.30^{a}	12.23^{a}	10.00^{b}	9.46^{b}	15.27^{a}	14.63^{a}	14.47^{a}	12.48^{b}	12.62^{b}	0.54
CP%	6.81^{c}	7.21^{bc}	7.54^{bc}	8.28^{ab}	8.91^{a}	5.47^{b}	5.47^{b}	5.39^{b}	7.50^{a}	7.71^{a}	3.87^{b}	4.63^{b}	4.64^{b}	7.50^{a}	7.35^{a}	.040
IVDMD%	60.47^{a}	56.94^{a}	59.98^{a}	56.70^{ab}	53.30^{b}	60.55^{a}	60.10^{ab}	55.32^{ab}	54.93^{b}	55.33^{ab}	62.08^{a}	60.60^{ab}	56.60^{b}	56.18^{b}	56.60^{b}	1.96
AS																
NH_4	4.56	4.89	4.38	5.08	4.72	11.10^{bc}	12.45^{ab}	13.17^{a}	10.42^{c}	13.14^{a}	13.87^{bc}	15.78^{a}	14.94^{ab}	14.58^{abc}	13.31^{c}	0.054
Crude Protein%	6.92	6.76	6.44	7.00	6.47	8.46^{a}	7.14^{b}	7.76^{ab}	8.27^{a}	8.25^{ab}	5.43^{b}	4.85^{b}	5.01^{b}	7.05^{a}	7.38^{a}	0.40
IVDMD%	52.54	50.47	50.97	51.81	53.95	54.63^{a}	52.32^{ab}	50.31^{ab}	54.66^{a}	48.78^{b}	62.15^{a}	60.17^{ab}	58.73^{ab}	58.28^{ab}	56.57^{b}	1.96
SBM																
NH_4	4.53	4.38	4.56	4.40	4.33	14.94^{a}	14.23^{ab}	15.31^{a}	13.57^{ab}	12.82^{b}	39.26^{a}	38.11^{a}	37.57^{a}	37.78^{a}	29.77^{b}	0.67
IVDMD%	64.15^{a}	62.93^{a}	62.78^{a}	55.81^{b}	54.77^{b}	85.29^{a}	82.03^{ab}	79.21^{bc}	76.22^{c}	70.46^{d}	94.63^{a}	92.12^{ab}	89.31^{b}	87.68^{bc}	83.78^{c}	1.67
Corn																
NH_4	3.99	4.55	4.33	4.19	4.61	4.10	4.25	4.08	4.38	3.03	5.06	5.19	4.65	4.31	3.87	0.67
IVDMD%	25.17	26.35	27.39	24.98	22.93	85.56^{a}	83.34^{ab}	82.56^{ab}	78.75^{bc}	75.48^{c}	90.22^{a}	87.27^{ab}	85.50^{b}	85.60^{ab}	83.37^{b}	1.67

[1]Data were collected after 3 periods of fermentation: 0 h, 24 h, and 48 h.
[2]Five substrates were used: low quality alfalfa (LQ), high quality alfalfa(HQ), alfalfa silage (AS), soybean meal (SBM) and corn.
[3]Treatments were control, 2% PS, 3% PS, 2% TA, and 3% TA.
[abcd]Means within a row with different subscript differ ($P < 0.05$).

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Table 2. Effects of Pecan shell (PS) and tannic acid (TA) ensiled with alfalfa on alfalfa silage (AS) ammonia concentrations (NH_4, mM), crude protein (CP), and in vitro dry matter digestibility (IVDMD).

	Time[1]												
	0 h				24 h				48 h				
Sample[2]	Con[3]	5% PS	10% PS	3% TA	Con	5% PS	10% PS	3% TA	Con	5% PS	10% PS	3% TA	SE (n=2)
AS													
NH_4	5.46	5.68	5.62	5.64	12.34[a]	11.94[ab]	11.29[b]	11.62[b]	13.48[b]	14.44[a]	12.71[c]	13.69[b]	0.23
CP	6.64	6.21	5.99	6.07	7.41	5.83	5.90	7.68	8.60[a]	5.84[b]	5.90[b]	7.83[ab]	0.75
IVDMD	51.39	49.32	48.02	49.22	62.68	59.81	57.18	58.22	57.80	61.45	60.54	60.22	1.56

[1]Dtata were collected after 3 periods of fermentation: 0 h, 24 h, and 48 h.
[2]One substrate was used: Alfalfa silage (AS).
[3]Treatments were control, 5% PS, 10% PS, and 3% TA.
[abc]Means within a row with different subscript differ ($P < 0.05$).

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American Society of Animal Science - Western Section
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